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Per- and polyfluoroalkyl substances (PFAS) in Australia: Current levels and estimated population reference values for selected compounds

L.M.L. Toms[a], J. Bräunig[b], S. Vijayasarathy[b], S. Phillips[a], P. Hobson[c], L.L. Aylward[b,d,*], M.D. Kirk[e], J.F. Mueller[b]

[a] School of Public Health and Social Work, And Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, QLD, Australia
[b] Queensland Alliance for Environmental Health Sciences (QAEHS), University of Queensland, Brisbane, QLD, Australia
[c] Sullivan Nicolaides Pathology, QLD, Australia
[d] Summit Toxicology, LLP, Falls Church, VA, USA
[e] Research School of Population Health, College of Health and Medicine, Australian National University, Canberra, ACT, Australia

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ABSTRACT

Background: Increased public awareness of PFAS contamination in Australia has resulted in serum biomonitoring efforts in individuals in potentially affected communities. However, population-based reference values for assessing whether individual results exceed the typical range in the Australian general population are not currently available.

Objective: Estimate population upper bound reference values based on updated serum PFAS concentrations in pooled samples from southeast Queensland, Australia and population variation observed in the US National Health and Nutrition Examination Survey (NHANES) datasets.

Methods: We calculated ratios of 95th percentile to arithmetic mean (P95:AM ratios) using data from the NHANES 2013–14 and 2015–16 cycle samples for frequently detected PFASs: PFOA, linear and branched PFOS, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), and perfluorohexanesulfonate (PFHxS). We estimated Australian age-specific means for PFAS using pooled serum samples collected in 2014–15 and 2016–17. We used the P95:AM ratios to estimate 95th percentile concentrations in the Australian population based on the results of the 2016–17 pooled samples.

Results and conclusions: P95:AM ratios for each PFAS were similar across NHANES cycle and age group, so overall compound-specific ratios were estimated for PFOA (2.1), PFNA (2.4), PFDA (2.7), PFHxS (2.7), and linear (2.4) and summed PFOS (2.3). Australian mean PFAS concentrations continued previously reported declining trends. The estimated P95 values can be used as preliminary substitutes for more rigorous population reference values to identify samples with clearly elevated serum PFAS concentrations in Australian biomonitoring efforts. Given uncertainties and variability inherent in this evaluation, the estimated P95 values should be interpreted with caution. Mean and estimated P95 serum PFAS concentrations in Australia should continue to be monitored to document declining trends in population serum concentrations.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are a current focus of biomonitoring studies in populations around the world. Some of these compounds are biologically and environmentally persistent, and are globally distributed. The use and manufacture of selected PFASs has been restricted or eliminated, i.e. perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), but other PFASs including PFAA precursors continue to be manufactured or used (UNEP, 2009; 2015; Wang et al., 2017). Biomonitoring is being conducted in a variety of contexts, including in the course of ongoing national monitoring programs (for example, US National Health and Nutrition Examination Survey [NHANES] and Canadian Health Measures Survey), as exposure biomarkers in epidemiological studies, and for evaluation and investigation in communities with suspected elevated exposures through environmental contamination or from other sources (Bräunig et al., 2017; CDC, 2013–2014; Li et al., 2018; Rotander et al., 2015; Steenland et al., 2009; Tremblay et al., 2007).

Concentrations of various PFASs in human serum can vary widely by country and with age (Jian et al., 2018; Toms et al., 2014). In

* Corresponding author. Summit Toxicology, LLP. 6343 Carolyn Drive, Falls Church, VA, 22044, USA.
E-mail address: laylward@summittoxicology.com (L.L. Aylward).

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addition, because of the phase out of selected legacy PFASs in some countries as well as changing patterns of uses by compound, human serum concentrations for many PFASs are changing over time (Eriksson et al., 2017; Haug et al., 2009; Toms et al., 2014). Thus, establishment of relevant reference values for understanding whether individual serum measurements are elevated above levels typical in a given population requires ongoing updates in a local context. Such reference values are useful for evaluating whether measurements in individuals are higher than usually expected in the general population (Angerer et al., 2011) and are important when studies of communities with concerns regarding local contamination are conducted.

Beginning in 2002, a series of biomonitoring studies have been conducted in Australia using pooled surplus pathology serum samples. Chemicals examined in these studies include polychlorinated dibenzo-p-dioxins and related compounds, polychlorinated biphenyls, polybrominated diphenylethers and related chemicals, and selected PFASs (Drage et al., 2019; Harden et al., 2007; Kärrman et al., 2006; Toms et al. 2009, 2014). Selected PFASs including PFOA and PFOS were measured in serum pools periodically beginning in 2002 for adults and older children, and in 2006 for children ages 0–4 years old (Toms et al., 2009). Analyses of these two compounds over the period from 2002 to 2011 demonstrated significant declines in the pooled sample concentrations, with PFOA and PFOS declining by more than 50 percent over this time period (Toms et al., 2014).

Additional results for PFOA and PFOS, as well as a range of other PFASs, are now available from serum pools collected in 2014–2015 and 2016–2017 in southeast Queensland, Australia. Analysis of serum pools provides estimates of the arithmetic mean concentrations for each age group and analyte at the time of collection of the included serum samples. The advantages of pooling include large sample volumes, savings on analytical costs and reduced time of recruitment (Heffernan et al., 2014), and, when implemented over time, a consistent basis for assessing trends. However, these pooled samples do not provide direct information on variation in individual sample concentrations in the population, although variation in the pooled sample concentrations has been proposed for use in evaluation of underlying population variation (Caudill, 2010). In particular, pooled samples do not allow direct estimation of a population upper bound reference value such as a 95th percentile.

In Australia, as in other countries around the world, exposure to PFAS compounds is ubiquitous due to the presence of PFASs in the environment, in foods and food contact materials, and in various materials used in consumer products. In addition, many Australian communities have experienced PFAS contamination of groundwater and soil as a result of the extensive use of PFAS-containing fire-fighting foams by both civilian and military facilities (http://www.defence.gov.au/Environment/PFAS/). In response to concerns over the contamination, PFAS concentrations in blood have been measured in individuals in several communities in order to determine whether there has been elevated exposure in these communities (Australian National University). Thus, there is a need to establish or estimate age- and compound-specific population reference values (usually set around the 95th percentile; Angerer et al., 2011) representing the upper bound of general population serum levels in the absence of such point source exposures for use as benchmarks to identify concentrations indicating elevated exposures.

Previous analysis of data from several population-representative biomonitoring surveys for a variety of persistent compounds demonstrated that while absolute concentrations of such chemicals varied in different populations and over time, the degree of variation across individuals in the underlying populations in specific age groups was relatively similar (Aylward et al., 2014). Thus, examination of population-based datasets from populations outside Australia may provide insight into the relationship between age-specific upper bound values (such as the 95th percentile) and the arithmetic mean for PFASs of interest.

This manuscript presents the results of the 2014–15 and 2016–17 pooled sample biomonitoring from Australia for an expanded group of PFASs including PFOA, perfluorononanoic acid (PFNA) perfluorodecanoic acid (PFDA), perfluorohexanesulfonate (PFHxS), and both linear and branched PFOS. In addition, estimated age-specific population 95th percentiles for each compound in Australia are presented for these compounds based on an analysis of the population variation in the corresponding age-specific data from the most recent cycles of the US National Health and Nutrition Examination Survey (NHANES), similar to a previous effort (Aylward et al., 2014). Specifically, we characterized the variation in population serum concentrations of PFASs by estimating the ratio of the 95th percentile (P95) to the arithmetic mean (AM) in each age group for each analyte in the US NHANES dataset (CDC, 2013–2014, 2015–2016). If population variation in concentration is assumed to be similar between the US and Australian populations, then this ratio can be used to approximate age-specific reference upper bound values for each age group in the Australian population based on the pooled sample concentrations.

An analysis of P95:AM ratios was previously conducted on NHANES data from 1999 to 2000 and 2009–2010 for PFOS and PFOA as well as other persistent organic compounds (Aylward et al., 2014). The current effort extends this analysis to NHANES datasets from the 2013–14 and 2015-16 NHANES cycles and covers additional PFAS analytes. Finally, we compare the most recent Australian pooled sample concentrations to the arithmetic mean concentrations from the corresponding age groups in contemporaneous NHANES datasets.

2. Materials and methods

2.1. Serum pools

Serum pools were assembled from archived surplus sera collected in 2014–15 and 2016–17 from a community pathology laboratory in southeast Queensland, Australia. Each pool was composed of up to 1 ml of serum each from 100 individuals with equal volumes taken from each participant for a specified pool. Four pools were constructed for each of six age groups at each time period. The age groups were as follows: 0–4, 5–15, 16–30, 31–45, 46–60, and ≥60 years of age. Thus, for each two-year sampling period, 2400 individual serum samples were grouped into 24 pools, 4 pools per age group, with males and females pooled separately. Ethics approval was gained through the University of Queensland Ethics Committee (2013000317).

2.2. Analysis of PFAS

A total of 30 PFASs were analyzed in this study using the isotope dilution method. Native PFAS standards used included perfluoroalkylcarboxylic acids (PFCAs), perfluoroalkylsulfonates (PFSAs), fluorotelomer sulfonates (FTSs), perfluorooctanesulfonamido acetic acids (FOSAAs), perfluorooctanesulfonamides (FOSAs) and perfluorooctanesulfonamidoethanols (FOSEs). A mix of isotopically labelled standards used included $^{13}C_4$-PFBA, $^{13}C_3$-PFPeA, $^{13}C_2$-PFHxA, $^{13}C_4$-PFHpA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{18}O_2$-PFHxS, $^{13}C_4$-PFOS and $^{13}C_2$-6:2FTS. Analytes were quantified via isotope dilution. Where isotopically-labelled standards were unavailable a surrogate internal standard was used for quantitation or analytes were quantified using external calibration. Labelled instrument performance internal standards $^{13}C_8$- PFOA and $^{13}C_8$-PFOS were used for recovery calculation. All standards were purchased from Wellington Laboratories (Guelph, Ontario, Canada). A full list of analytical standards used can be found in the Supplementary Information (Table S1). Solvents and reagents used were of analytical grade.

Serum samples were extracted using modifications to previously published protocols (Eriksson et al., 2017; Rotander et al., 2015). An aliquot of 1 ml of serum was added to a 15 ml Eppendorf tube and spiked with mass labelled internal standards and vortexed. Proteins

were precipitated by addition of 7.5 ml acetonitrile and PFASs were extracted by 15 min ultrasonication, followed by centrifugation. The supernatant was filtered (Phenomenex RC membrane 0.2 µm syringe filter) and evaporated to 0.2 ml under a gentle stream of nitrogen. Ammonium acetate in water (5 mM) was added to the sample to a final volume of 0.5 ml, recovery standards; ^{13}C8-PFOS and ^{13}C8-PFOA were added to the vial before analysis.

PFASs were analyzed using high performance liquid chromatography (Nexera HPLC, Shimadzu Corp., Kyoto Japan) coupled to a tandem mass spectrometer (QTrap 5500 AB-Sciex, Concord, Ontario, Canada) operating in negative electrospray ionization mode and multiple reaction monitoring (MRM) mode. A volume of 5 µL was injected onto a Gemini NX C18 column (50 × 2 mm, 3 µm, 110 Å, Phenomenex, Lane Cove, Australia) held at a constant temperature of 50 °C, and separation of PFASs was achieved by gradient elution from the column using mobile phases 10% (A) and 90% (B) methanol, respectively, with 5 mM ammonium acetate. A pre-column (C18, 50 × 4.6 mm, 5 µm, Phenomenex, Lane Cove, Australia) was installed between the solvent reservoirs and the injector to trap and delay the background of PFASs originating from the HPLC system. Identification and confirmation of peaks was done using retention times and comparing the ratios of MRM transition area between the samples and the calibration standards in the same batch of analysis. Quantification was conducted using labelled PFASs. Calibration standards were made up in 500 µL (200 µl methanol and 300 µL 5 mM ammonium acetate in water). The concentration range of the eight prepared calibration standards was 0.1–100 µg/L (0.1; 0.4; 1; 4; 10; 20; 40; 60; 80; 100). Data acquisition and processing was carried out using Analyst® TF 1.6 and MultiQuant™ software (Sciex).

2.3. Quality control

Laboratory blanks (MilliQ water) and standard reference material (SRM; NIST SRM, 1957) were extracted and analyzed in parallel with each batch of samples. Batches included inter-batch replicates which generally showed %CV of ≤16% except for PFBA with 27%. The method limit of quantification (LOQ) was calculated by multiplying the SD obtained from injecting the lowest calibration standard seven times by 10. Percentage average recovery ranged from 80 to 94.

2.4. Quantification of linear and total PFOS

Total PFOS was quantified in this study using linear PFOS standard, by integrating both linear and branched PFOS isomers as one peak. In human serum samples, comparison of total PFOS results for SRM 1957 compared to literature values (Riddell et al., 2009), suggests that quantification of total PFOS (branched plus linear isomers) against the linear standard produces viable results. In this study we quantified an average of 17.8 ng/ml (n = 2) for SRM 1957 compared to 17.9 ng/ml by Riddell et al., (2009) using a mix of linear and branched isomers. However, as all isomers of PFOS using the current method were quantified against linear PFOS (m/z 499 > 99), the results have an associated uncertainty factor of unknown magnitude due to a known difference in MS/MS response factors for structural isomers (Riddell et al., 2009).

2.5. Statistical analysis of the NHANES datasets

Serum PFAS data from the US were downloaded from the NHANES website for two cycles, covering the years 2013–2014 and 2015–2016, the two most recent cycles available (CDC, 2013–2014, 2015–2016). Summary statistics by age groups corresponding to those used in the Australian pooled samples were generated, including frequency of detection, arithmetic means (AM) and standard errors, and the 95th percentile (P95). Means and corresponding P95:AM ratios were not calculated if the detection frequency for a given age group and compound was lower than 60%. Non-detected concentrations were imputed with limit of quantitation divided by the square root of 2 for the calculation of the mean P95:AM ratios and confidence intervals on the P95:AM ratios were calculated using the bootstrapping procedure described previously (Aylward et al., 2014). Briefly, the data for a given age group was resampled with replacement 1000 times. Summary statistics including the AM, P95, and P95:AM ratio were calculated using the survey weights for each iteration, and the central tendency and 95% confidence intervals for the P95:AM ratio were derived from the 1000 trials.

Limited data were available from NHANES for the youngest age groups. In general, chemicals are measured in sera only for ages 12 and above in the NHANES program. However, a supplemental analysis was conducted on surplus sera from NHANES 2013–2014 for ages 3 to 11 in order to provide reference data for the US (Ye et al., 2018). These data allow calculation of P95:AM ratios for the ages 3–4 and 5–15 based on the 2013-14 NHANES cycle. For the 2015–16, no data for individuals under age 12 were available, so no P95:AM calculations for ages 0 to 4 were made based on the NHANES 2015–16 cycle data. Also, in the 2015–16 cycle of NHANES, no individuals younger than 12 years were included, so the P95:AM ratio for ages 5–15 years in that cycle was based only on data for individuals ages 12–15 years.

P95:AM ratios were calculated, plotted, and compared across age groups and NHANES cycles for those analytes with sufficient detection frequencies. The P95:AM ratios for each PFAS were assessed visually to identify any clear trends by age group or time period. Based on this evaluation, the P95:AM ratios across all age groups and cycles of NHANES data were averaged for each PFAS compound to estimate an average P95:AM ratio. The most recent age-specific Australian pooled sample concentrations for each PFAS were multiplied by the PFAS-specific ratio to generate current age-specific estimates of the population 95th percentiles for selected PFAS compounds.

3. Results

Five PFASs were detected consistently in the Australian pooled samples: linear PFOA, PFNA, PFDA, PFHxS, and PFOS. Linear PFOS was measured directly, and total PFOS was also measured as discussed in the Methods section. The difference in the two measurements is accounted for by branched PFOS, but the specific branched PFOS species were not directly quantified. Other PFASs were detected and quantified infrequently or not detected at all in the Australian pooled samples. Specifically, PFBA was detected in all but one pool in 2014–15 samples, but about half of these detects were below the limit of quantitation (LOQ) of 0.08 ng/ml. In the 2016-17 samples, PFBA was not detectable in more than half of the pools. PFHxA was generally not detected in either year (LOQ = 0.09 ng/ml), while PFHpA was detected in more than half of the pools in both years but often below the LOQ (0.06 ng/ml). PFUnDA was detectable in most 2014–15 pools, but fell below the LOQ of 0.09 ng/ml in many samples in the 2016–17 pools. The remaining PFASs were generally not detected (LOQs between 0.05 ng/ml and 0.56 ng/ml, Table S1). The average Australian pool concentrations by age group for the frequently-detected PFASs are reported in Table 1.

NHANES datasets for 2013–14 and 2015–16 provide data on up to 15 PFASs, depending on the age group and cycle. Six PFASs were detected in greater than 60% of the individuals in nearly all of the age groups in the two NHANES cycles: linear PFOA, PFNA, PFDA, PFHxS, and linear and monomethyl branched PFOS. Selected summary statistics are reported by age group for these cycles in Supplementary materials (Table S2). The mean concentrations by age group for six frequently detected compounds measured in both the US NHANES dataset (2015–16 cycle) and in the Australian pooled analyses (2014–15 and 2016–17 collections) are plotted in Fig. 1. In general, when comparing the US and Australian data from 2015, the US means are slightly lower in each age group. The exceptions are for PFNA and linear PFOS in the older age groups, where the US NHANES means are somewhat higher

Table 1
Age group-specific arithmetic mean and standard deviation of pool PFAS concentrations and estimated age-specific population 95th percentile (P95) values based on Australian pooled biomonitoring samples from 2016 to 17.

Age Group	*P95:AM Ratio*[a]:	Serum Concentrations, ng/ml					
		PFOA	PFNA	PFDA	PFHxS	linear PFOS	tot-PFOS
		2.1	*2.4*	*2.7*	*2.7*	*2.4*	*2.3*
1–4 yrs	Mean (SD)[b]	2.72 (0.45)	0.52 (0.08)	0.26 (0.05)	1.28 (0.42)	2.49 (1.42)	3.14 (1.47)
	Est. P95[c]	***5.7***	***1.3***	***0.7***	***3.4***	***6.0***	***7.2***
5–15 yrs	Mean (SD)	1.92 (0.19)	0.38 (0.05)	0.24 (0.05)	1.55 (0.14)	2.39 (0.27)	3.01 (0.35)
	Est. P95	***4.0***	***0.9***	***0.6***	***4.2***	***5.7***	***6.9***
16–30 yrs	Mean (SD)	1.92 (0.3)	0.46 (0.06)	0.26 (0.07)	1.94 (0.39)	2.65 (0.42)	3.46 (0.56)
	Est. P95	***4.0***	***1.1***	***0.7***	***5.3***	***6.4***	***8.0***
31–45 yrs	Mean (SD)	1.85 (0.32)	0.46 (0.02)	0.25 (0.04)	1.87 (0.96)	3.52 (1.06)	4.52 (1.45)
	Est. P95	***3.9***	***1.1***	***0.7***	***5.1***	***8.5***	***10.4***
46–60 yrs	Mean (SD)	1.84 (0.26)	0.47 (0.05)	0.27 (0.01)	2.11 (1.06)	4.45 (1.36)	5.71 (1.89)
	Est. P95	***3.9***	***1.1***	***0.7***	***5.7***	***10.7***	***13.1***
> 60 yrs	Mean (SD)	2.5 (0.21)	0.56 (0.06)	0.27 (0.05)	2.28 (0.29)	5.48 (0.69)	7.26 (0.7)
	Est. P95	***5.3***	***1.4***	***0.7***	***6.2***	***13.2***	***16.7***

[a] Estimated from NHANES datasets; see text and Fig. 2.
[b] N = 4 pools per age group.
[c] Estimated by multiplying the P95:AM ratio derived from the NHANES datasets by the arithmetic mean concentration of the age group-specific pools.

than the corresponding mean Australian pool concentrations in 2015. Because of the difference in study designs between the Australian and US NHANES programs, no formal statistics were applied in these comparisons.

The pattern of relative concentrations among the frequently detected PFASs was the same in the NHANES and Australian sampling. In both populations, PFOS demonstrated the highest concentrations, and these concentrations were dominated by linear PFOS. PFOA was present at the next highest concentrations, followed by PFHxS and PFNA. PFDA, while consistently detectable in the Australian pooled samples, was present at much lower concentrations. With respect to patterns with age, concentrations of PFOS, and to some degree, PFHxS, increased with age. No discernible trend with age was observed for PFNA or PFDA. Concentrations of PFOA follow a U-shaped pattern in the Australian datasets, with the youngest and oldest age groups having the highest concentrations, and the other four groups showing lower concentrations.

The P95:AM ratios and 95% CIs calculated for each age group for each of the six frequently-detected compounds based on the NHANES 2013–14 and 2015-16 datasets are presented in Fig. 2. NHANES 1999–2000 cycle data for PFOA and PFOS are also presented based on a previous assessment (Aylward et al., 2014). The 95% CIs on the ratios are somewhat skewed, reflecting the influence of upper tail of the distribution of measured concentrations in the NHANES datasets. While there were some differences between ratios for various age groups, no general patterns with age or consistent disparities by age groups or across NHANES cycles were observed. Thus, the average ratios across age groups were also plotted on the figures, with the overall average for each compound indicated. For the other PFASs, detection frequencies were below 60% in all age groups. As a result, a reliable AM and P95:AM ratio could not be calculated for these compounds.

In order to estimate current age group-specific P95 concentrations for the Australian population for these six compounds, the mean Australian pool concentrations were multiplied by the average P95:AM ratio for each compound. Table 1 gives age- and compound-specific pool means and standard deviations as well as the estimated P95 values for each age group based on the Australian 2016–17 pooled data.

4. Discussion

The PFAS pooled serum data and estimated age-specific P95 values for frequently-detected PFAS compounds presented here represent an updated assessment of exposure levels in the general Australian population. The ratios and estimated P95 values generated here can be used as preliminary estimated population upper bound reference values. Such values have utility for public health agencies, researchers, and others involved in the collection and interpretation of PFAS biomonitoring data in Australia for evaluation of whether measured concentrations in individuals clearly exceed the typical range of concentrations in the Australian general population. Until such time as a more extensive dataset based on comprehensive population representative sampling in Australia is available, this is a practical approach with acknowledged uncertainties. These pooled samples were collected in one urban region of Australia and therefore represent the population of that region. However, a previous assessment indicated no meaningful differences in exposure levels across geographic regions of Australia (Kärrman et al., 2006). Thus, we cautiously use the results from these samples as indicators of body burden across Australia.

The average pool PFAS concentrations from sampling during 2014–15 and 2016–17 in Australia continue the available data on trends in average PFOS and PFOA serum concentrations in Australia (Toms et al., 2014). Over the decade since 2007, concentrations of PFOA and PFOS in the 0–4 year age group have declined by 60 and 73%, respectively. Over the same time period, concentrations of PFOA and PFOS in pooled samples from ages 5–15 years have declined by 71 and 82%, respectively. These patterns are also observable in pooled sample results for PFOA and PFOS from individuals ages 16 years and older (Fig. 3). Concentrations of PFHxS in pooled samples from adults do not display a clear pattern over this time period. Concentrations of PFNA in Australian pooled samples collected from individuals ages 16 and older displayed a more complicated picture (Fig. 3). Concentrations rose from 2002 through the 2008–09 collection period, then declined through the 2014–15 collection period (Fig. 3). Concentrations in 2016–17 were similar to the previous cycle. PFAS concentrations in the 2016-17 Australian pooled samples remain slightly higher than the corresponding age-specific concentrations in the US NHANES 2015-16 survey data for most compounds, with the exception of total PFOS in the older age groups (Fig. 1). However, the differences have narrowed compared to earlier time periods (Kärrman et al., 2006).

The trends in concentrations by age for the PFASs in the Australian pooled samples are similar to those seen in previous evaluations. Of particular interest is the U-shaped trend observed for PFOA, with higher concentrations observed in the youngest and oldest age groups compared to the intermediate age groups. This pattern was also observed in earlier cycles of the Australian pooled sampling (Eriksson et al., 2017) and a similar pattern is seen in the NHANES 2013–2014 dataset which

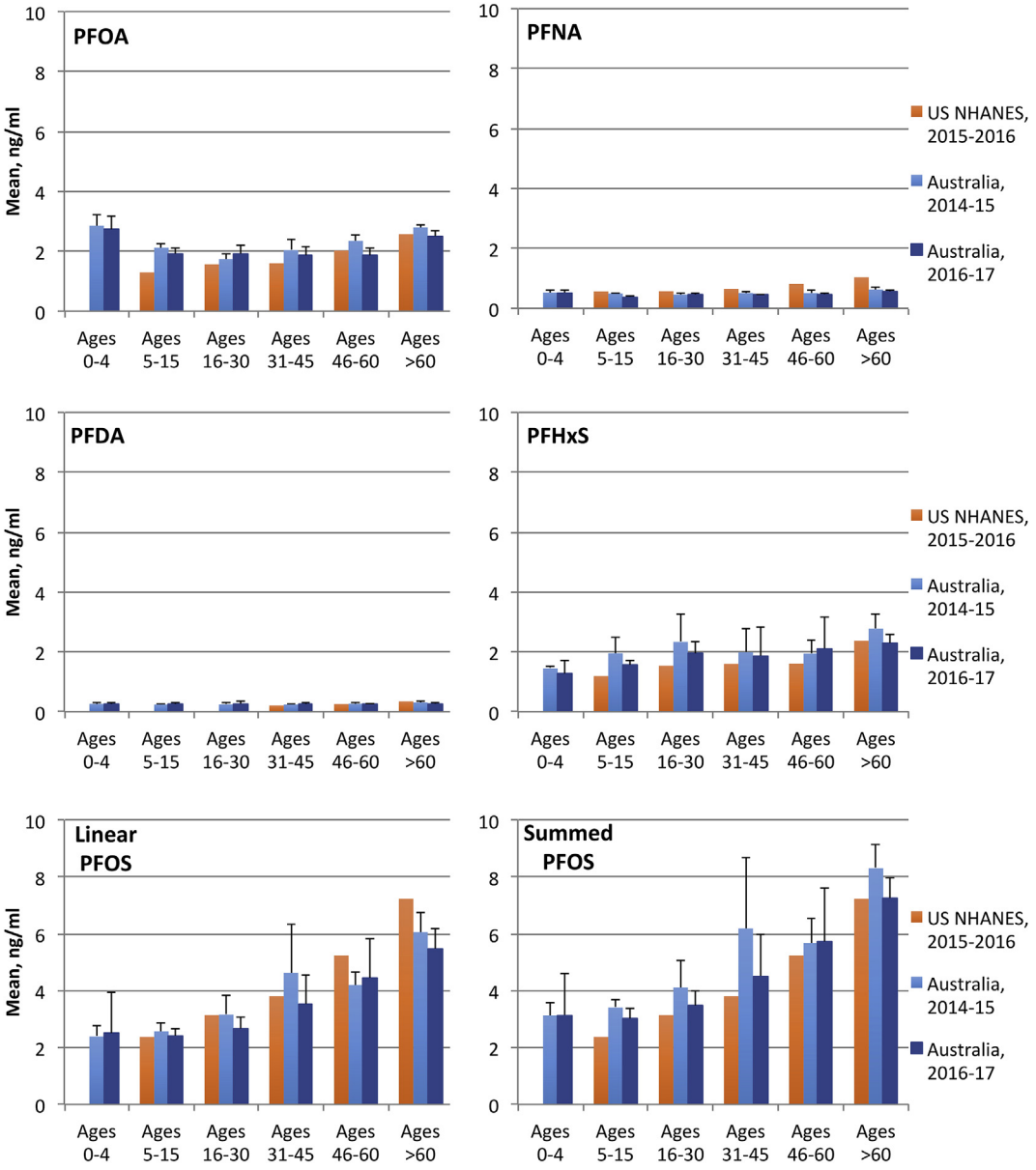

Fig. 1. Mean concentrations by age group for frequently detected PFASs from NHANES 2015–2016, Australian 2014–15 pools, and Australian 2016–17 pools. Note that NHANES data are not available for under age 12, so the NHANES data for the 5 to 15 age group contains data only for ages 12–15. Error bars are one standard deviation from the mean of four pools in each age group in the Australian sampling data. Corresponding error bars could not be plotted for NHANES data due to the reliance on individual rather than pooled samples in the NHANES survey.

includes children as young as 3 (Supplementary Materials, Table S2). This pattern suggests that children ages 0 to 4 experience higher exposures, perhaps via intake from breast milk, higher intake in foods per kg bodyweight, or greater environmental contact rates (for example, via incidental ingestion of house dust) (Winkens et al., 2017). The lower concentrations in the next age group may reflect growth dilution, although estimated body burdens (total amount of compound in the body) may remain unchanged or even increase in children (Koponen et al., 2018). The higher concentrations in the oldest age group could be due to a number of factors, including potentially lower renal function and elimination of PFOA (Gekle, 2017). It might be expected that these factors would also result in a U-shaped pattern with age for other PFASs; however, we did not observe this pattern.

Gyllenhammar et al. (2018) investigated the relationship between accumulation of selected PFASs in infants and breastfeeding. The ratio of infant serum concentrations to maternal serum concentrations decreased as chain length increased, with ratios of 2.8:1 for PFHpA and PFOA declining to ratios less than 1:1 for PFUnDA and longer chain compounds. Cariou et al. (2015) found that while the concentrations of PFASs in breast milk were 20–150-fold lower than in maternal serum, the breast milk:maternal serum ratio was higher for PFOA than for PFOS or PFHxS. These factors could contribute to the observable peak in the youngest age group for PFOA compared to other PFASs.

The P95:AM ratios calculated here from the US NHANES datasets are a measure of the degree of population exposure heterogeneity of each of these PFASs in the general US population. This heterogeneity likely represents the composite effects of several underlying factors including both variation in external exposure rates (over time and across individuals) and variation in intrinsic elimination rates among individuals in the population. Previous analyses suggested that degree of variation represented by the P95:AM ratios for selected persistent organic compounds was similar in studies from populations in Spain, Canada, the US, Germany, and Belgium, even when average concentrations differed substantially (Aylward et al., 2014). This provides

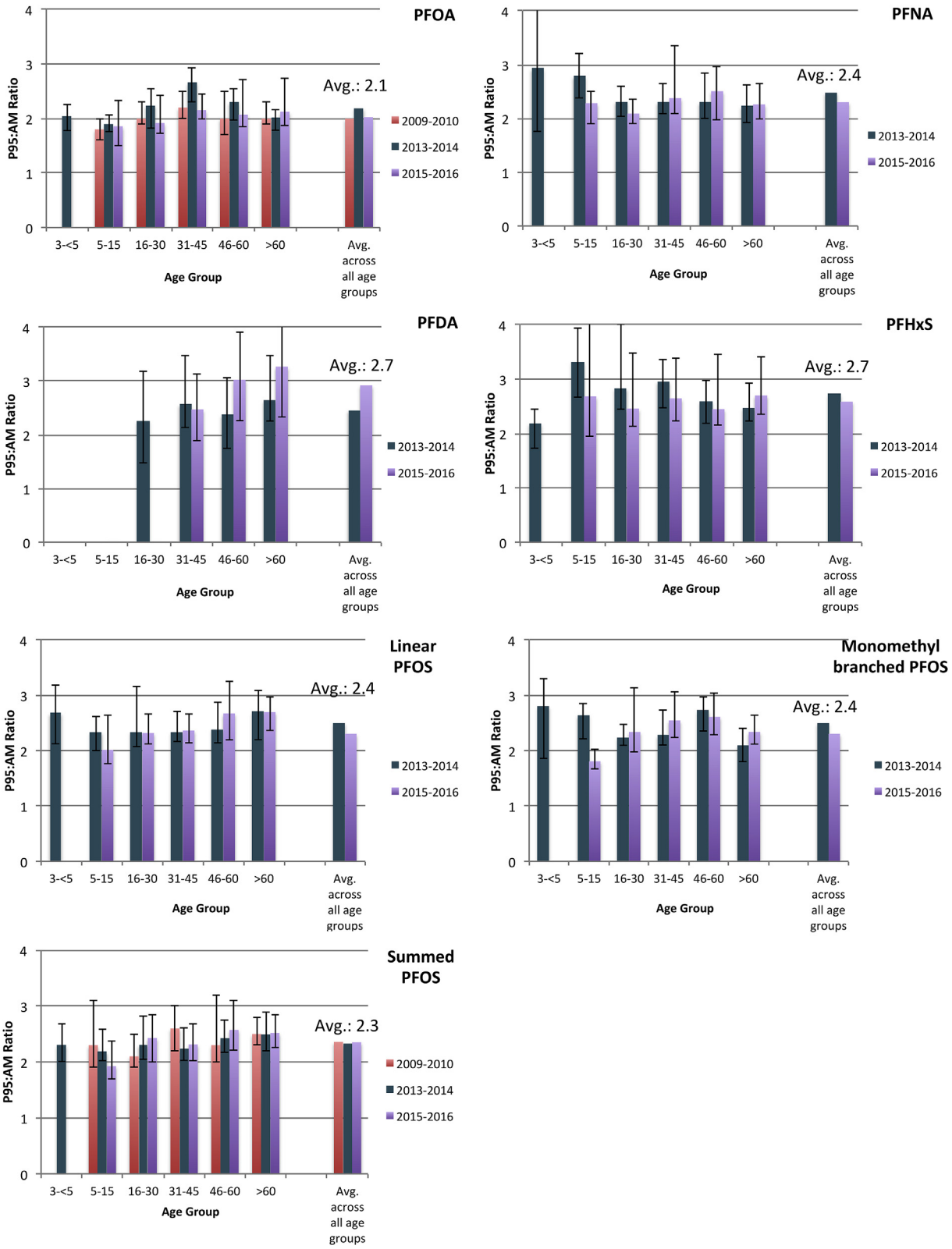

Fig. 2. Ratio of the 95th percentile to the arithmetic mean and 95% confidence intervals on the ratio by age group for the frequently detected PFASs from US NHANES cycles from 2009 to 2010 (PFOA and total PFOS only), 2013–2014, and 2015–2016.

some confidence in the general approach and the use of these ratios for estimating 95th percentiles in the Australian population.

The approach used here does not explicitly assume any specific shape for the underlying distribution of concentrations, but rather calculates an empirical ratio between a defined upper percentile and the arithmetic mean in the NHANES datasets. The approach does assume that this ratio can be extrapolated from the NHANES population to the Australian population, even though mean concentrations are somewhat different. As discussed in previous similar analyses, this is consistent with an underlying lognormal distribution (Aylward et al.,

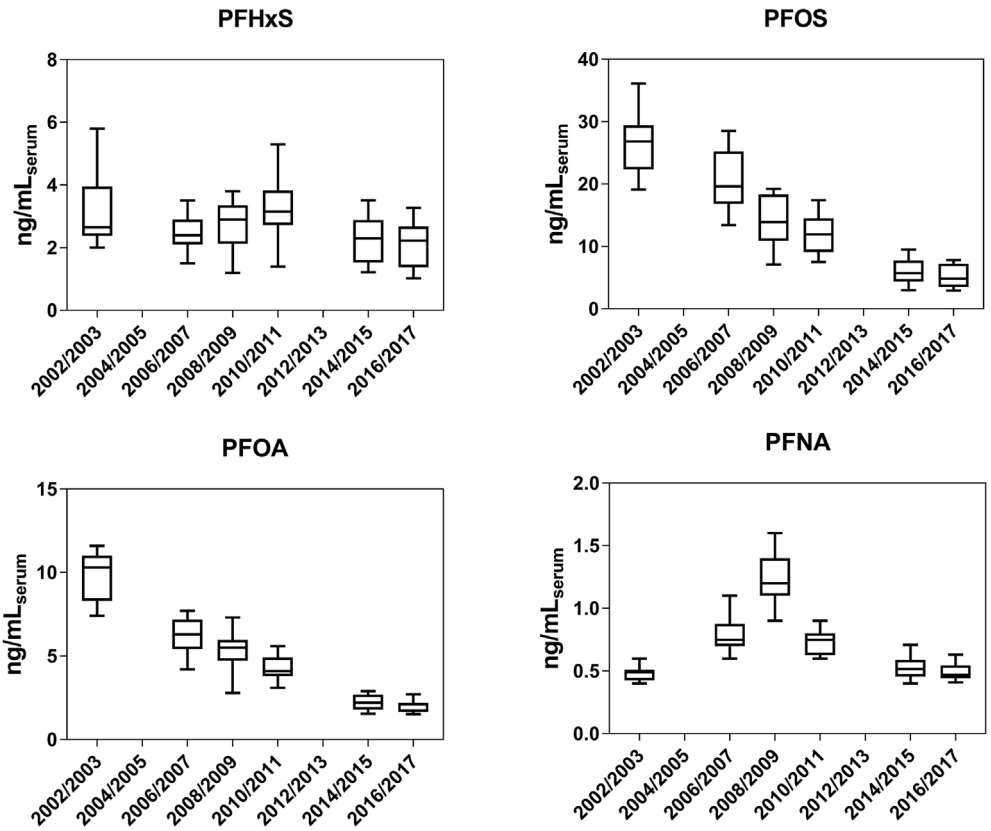

Fig. 3. Concentrations of PFOA, PFNA, PFHxS, and PFOS in Australian pooled samples 2002–2017, all pools ages 16 and above. Boxes represent the interquartile range of the pools, with the median represented by a horizontal line. Whiskers extend to the 5th and 95th percentiles.

2014). However, to the extent that upper percentile distributions are more skewed than predicted by a lognormal distribution, the approach used in this analysis captures that empirical population heterogeneity as well. There is clearly uncertainty associated with this approach. The coefficient of variation on these ratios was generally in the range of about 20 percent. Similarly, the coefficient of variation on the mean of the age-specific pool concentrations was in the range of 10–30 percent. As a result, the estimated P95 values in Table 1 should not be regarded as bright lines delineating elevated and non-elevated sample concentrations. However, the estimated P95 values can at least serve as one benchmark for tentative identification of samples that may indicate elevated exposures beyond the typical range anticipated in the general population in Australia.

The mean pooled serum PFAS concentrations and estimated P95 values presented here are indicators of exposures in the general Australian population. This study was not designed and is not able to estimate whether or how much health risk may be related to these exposures. The ability of scientists to interpret measured serum concentrations of PFASs in a health risk context is limited. Recently, the German Human Biomonitoring Commission considered available studies in human populations examining associations between health outcomes or clinical indicators and plasma PFAS concentrations in human populations (Apel et al., 2017; HBM-Kommission, 2016; HBM-Kommission, 2018). Based on these studies, they identified guideline values, designated as HBM I, for PFOA and for PFOS of 2 ng/ml and 5 ng/ml, respectively. The HBM I values represent “the concentration of a substance in human biological material at which and below which, according to the current knowledge and assessment by the HBM Commission, there is no risk of adverse health effects, and, consequently, no need for action” (Apel et al., 2017). Apel et al. (2017) state that levels above the HBM I indicate the need for exposure monitoring and, where practical, actions to reduce exposure. The mean Australian pool concentrations for PFOA and PFOS in the 2016–17 pools were very similar to the HBM I values. However, the estimated P95 values exceed the HBM I values and are likely to do so for another decade even if current declining trends continue. Biological responses and health outcomes potentially influenced by PFAS exposure, as well as the levels of exposure associated with any outcomes, remain the topic of substantial research. Further, a comprehensive exposure and risk assessment for the Australian population would require substantial additional data and analysis on exposure sources and pathways, biological responses and health outcomes, susceptible subpopulations, and other aspects. Thus, interpretations of the measured and estimated serum concentrations using the German HBM I values should be made cautiously. However, continued actions to monitor exposures and practical actions to identify and reduce exposure sources in the general population appear to be warranted.

In light of the continuing declining concentrations of these compounds in the Australian population, P95 values should be re-estimated when the next cycle of pooled sampling is conducted in Australia, and these estimates should be replaced with robust population P95 values based on broader population-representative biomonitoring studies in Australia if and when such studies become available. Given the stability of the estimated ratios across various cycles of the NHANES datasets, there is no reason to expect that the P95:AM ratios in NHANES would be markedly different in the next few years from those observed here. Based on this, the same ratios presented here can likely be used to estimate P95 values when new Australian pooled samples are analyzed. Furthermore, the range of compound-specific ratios was relatively narrow, ranging from 2.1:1 to 2.7:1. A generic ratio of approximately 2.5 could be applied cautiously to pooled concentration means to estimate reference values for other PFASs, with the understanding of the substantial uncertainty associated with this further extrapolation.

Acknowledgments and Conflict of Interest Statement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.03.004.

References

Angerer, J., Aylward, L.L., Hays, S.M., Heinzow, B., Wilhelm, M., 2011. Human biomonitoring assessment values: approaches and data requirements. Int. J. Hyg Environ. Health 214 (5), 348–360.

Apel, P., Angerer, J., Wilhelm, M., Kolossa-Gehring, M., 2017. New HBM values for emerging substances, inventory of reference and HBM values in force, and working principles of the German Human Biomonitoring Commission. Int. J. Hyg Environ. Health 220 (2 Pt A), 152–166.

Australian National University, PFAS Epidemiological Study.

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

Bräunig, J., Baduel, C., Heffernan, A., Rotander, A., Donaldson, E., Mueller, J.F., 2017. Fate and redistribution of perfluoroalkyl acids through AFFF-impacted groundwater. Sci. Total Environ. 596–597, 360–368.

Cariou, R., Veyrand, B., Yamada, A., Berrebi, A., Zalko, D., Durand, S., Pollono, C., Marchand, P., Leblanc, J.-C., Antignac, J.-P., Le Bizec, B., 2015. Perfluoroalkyl acid (PFAA) levels and profiles in breast milk, maternal and cord serum of French women and their newborns. Environ. Int. 84, 71–81.

Caudill, S.P., 2010. Characterizing populations of individuals using pooled samples. J. Expo. Sci. Environ. Epidemiol. 20, 29–37.

CDC, 2013-2014. Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey (NHANES) Data. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Hyattsville, MD, pp. 2013–2014. https://wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/Overview.aspx?BeginYear=2013.

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

Drage, D.S., Harden, F.A., Jeffery, T., Mueller, J.F., Hobson, P., Toms, L.-M.L., 2019. Human biomonitoring in Australian children: brominated flame retardants decrease from 2006 to 2015. Environ. Int. 122, 363–368.

Eriksson, U., Mueller, J.F., Toms, L.M.L., Hobson, P., Kärrman, A., 2017. Temporal Trends of PFSAs, PFCAs and Selected Precursors in Australian Serum from 2002 to 2013. Environ. Pollut. 220 (Part A), 168–177.

Gekle, M., 2017. Kidney and aging - A narrative review. Exp. Gerontol. 87 (Part B), 153–157.

Gyllenhammar, I., Benskin, J.P., Sandblom, O., Berger, U., Ahrens, L., Lignell, S., Wiberg, K., Glynn, A., 2018. Perfluoroalkyl acids (PFAAs) in serum from 2–4-month-old infants: influence of maternal serum concentration, gestational age, breast-feeding, and contaminated drinking water. Environ. Sci. Technol. 52 (12), 7101–7110.

Harden, F., Toms, L., Paepke, O., Ryan, J., Müller, J., 2007. Evaluation of age, gender and regional concentration differences for dioxin-like chemicals in the Australian population. Chemosphere 67 (9), S318–S324.

Haug, L.S., Thomsen, C., Becher, G., 2009. Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. Environ. Sci. Technol. 43 (6), 2131–2136.

HBM-Kommission, 2016. HBM I values for perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in blood plasma statement of the German human biomonitoring commission (HBM commission). Bundesgesundheitsblatt - Gesundheitsforsch. - Gesundheitsschutz 59, 1364.

HBM-Kommission, 2018. [HBM-I values for perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in blood plasma - statement of the German human biomonitoring commission (HBM commission)]. Bundesgesundheitsblatt - Gesundheitsforsch. - Gesundheitsschutz 61 (4), 474–487.

Heffernan, A.L., Aylward, L.L., Toms, L.-M.L., Sly, P.D., Macleod, M., Mueller, J.F., 2014. Pooled biological specimens for human biomonitoring of environmental chemicals: opportunities and limitations. J. Expo. Sci. Environ. Epidemiol. 24 (3), 225.

Jian, J.-M., Chen, D., Han, F.-J., Guo, Y., Zeng, L., Lu, X., Wang, F., 2018. A short review on human exposure to and tissue distribution of per- and polyfluoroalkyl substances (PFASs). Sci. Total Environ. 636, 1058–1069.

Kärrman, A., Mueller, J.F., van Bavel, B., Harden, F., Toms, L.-M.L., Lindström, G., 2006. Levels of 12 perfluorinated chemicals in pooled Australian serum, collected 2002−2003, in relation to age, gender, and region. Environ. Sci. Technol. 40 (12), 3742–3748.

Koponen, J., Winkens, K., Airaksinen, R., Berger, U., Vestergren, R., Cousins, I.T., Karvonen, A.M., Pekkanen, J., Kiviranta, H., 2018. Longitudinal trends of per- and polyfluoroalkyl substances in children's serum. Environ. Int. 121, 591–599.

Li, Y., Fletcher, T., Mucs, D., Scott, K., Lindh, C.H., Tallving, P., Jakobsson, K., 2018. Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. Occup. Environ. Med. 75 (1), 46–51.

Riddell, N., Arsenault, G., Benskin, J.P., Chittim, B., Martin, J.W., McAlees, A., McCrindle, R., 2009. Branched perfluorooctane sulfonate isomer quantification and characterization in blood serum samples by HPLC/ESI-MS(/MS). Environ. Sci. Technol. 43 (20), 7902–7908.

Rotander, A., Toms, L.-M.L., Aylward, L., Kay, M., Mueller, J.F., 2015. Elevated levels of PFOS and PFHxS in firefighters exposed to aqueous film forming foam (AFFF). Environ. Int. 82, 28–34.

Steenland, K., Tinker, S., Frisbee, S., Ducatman, A., Vaccarino, V., 2009. Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. Am. J. Epidemiol. 170 (10), 1268–1278.

Toms, L.-M.L., Calafat, A.M., Kato, K., Thompson, J., Harden, F., Hobson, P., Sjödin, A., Mueller, J.F., 2009. Polyfluoroalkyl chemicals in pooled blood serum from infants, children, and adults in Australia. Environ. Sci. Technol. 43 (11), 4194–4199.

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

Tremblay, M., Wolfson, M., Connor, S.G., 2007. Canadian Health Measures Survey: rationale, background and overview. Health Rep. 18, 7–20.

UNEP, 2009. Listing of Perfluorooctane Sulfonic Acid, its Salts and Perfluorooctane Sulfonyl Fluoride. SC-4/17.

UNEP, 2015. Proposal to List Pentadecafluorooctanoic Acid (CAS No: 335-67-1, PFOA, Perfluorooctanoic Acid), its Salts and PFOA-Related Compounds in Annexes A, B And/or C to the Stockholm Convention on Persistent Organic Pollutants. UNEP/POPS/POPRC.11/5.

Wang, Z., DeWitt, J.C., Higgins, C.P., Cousins, I.T., 2017. A never-ending story of per- and polyfluoroalkyl substances (PFASs)? Environ. Sci. Technol. 51 (5), 2508–2518.

Winkens, K., Vestergren, R., Berger, U., Cousins, I.T., 2017. Early life exposure to per- and polyfluoroalkyl substances (PFASs): a critical review. Emerging Contaminants 3 (2), 55–68.

Ye, X., Kato, K., Wong, L.-Y., Jia, T., Kalathil, A., Latremouille, J., Calafat, A.M., 2018. Per- and polyfluoroalkyl substances in sera from children 3 to 11 years of age participating in the National Health and Nutrition Examination Survey 2013–2014. Int. J. Hyg Environ. Health 221 (1), 9–16.