Environment Protection Authority

Per and polyfluorinated alkyl substances (PFAS) in the marine environment

Preliminary ecological findings

EPA South Australia

Per and polyfluorinated alkyl substances (PFAS) in the marine environment – Preliminary ecological findings

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Non-technical summary

Bottlenose dolphins live in and around heavy industry in the Port River and Barker Inlet, and as a population they are thriving. Water quality management in the past decade has improved the condition of the environment and the dolphin population has increased over the same time.

However, the potential long-term uptake of chemicals known as PFAS (per and polyfluorinated alkyl substances) has raised questions about whether bottlenose dolphins are taking up these chemicals and whether the environment is able to cope.

The EPA surveyed PFAS in dolphins, fish and water in the Port River and Barker Inlet. Findings were compared to other locations in South Australia, interstate and overseas. We found some dolphins have the highest levels of PFAS in the world because of their close association with heavily industrialised locations. All types of fish sampled were safe to eat, but the type of fish made a large difference to how much PFAS were accumulated. Water sampling pointed to locations where PFAS are coming into the Port River and Barker Inlet and management is now looking at how to address this.

While dolphins have high levels of PFAS, there is nothing to say that they are unhealthy because of it. The Port River and Barker Inlet dolphins are flourishing, with the last decade having the highest numbers of dolphins seen in the area since records began. Even though we can detect many chemicals in the environment, we have confidence that the environment is improving over time. Notwithstanding this, work is needed to understand how the environment can cope with emerging chemicals including PFAS, and how they can be managed for the future.

Technical summary

Per- and polyfluorinated alkyl substances (PFAS) are man-made industrial and household substances used extensively in Australia and worldwide since the 1960s in a range of applications including non-stick cookware, stain repellents, food packaging and firefighting foams. Perfluorooctansulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexanesulfonate (PFHxS) are typically the most common of the PFAS chemicals. These chemicals are stable and do not readily breakdown. They bioaccumulate in biota and they biomagnify, increasing in concentration in higher predators. Over the last 10 years or so, the realisation of their chemical properties and potential for entry into the environment has resulted in increased concerns regarding contamination in the environment and the potential for ecological impacts, particularly to long-lived predators.

The EPA has undertaken a number of surveys to assess the risk from PFAS to the marine environment, particularly focusing on high-risk locations such as the Port River.

Southern Australian Bottlenose dolphins (*Tursiops* sp) were sampled for PFAS. Dolphins that reside in heavily industrialised regions particularly the Swan River (Western Australia) and to a lesser extent the Port River (South Australia) had some of the highest PFOS concentrations found in marine mammals worldwide. Despite this, the Port River dolphin population has increased over the last 30 years suggesting that the population is not affected by these high concentrations, but further work is needed to confirm this.

Commonly targeted recreational fish and invertebrates from the inner Port and North Arm region were sampled. In all fish samples, the liver was found to be the site of PFAS storage with lower concentrations in the frames, and the fillets having the lowest concentration. While small traces of PFOS were found in the flesh of fish and invertebrates, advice from SA Health stated that they were all safe to eat, even for people who eat large amounts of fish. Salmon (*Arripis truttacea*) were found to have the highest concentrations of all fish species tested, which may be related to their prey selection and biology.

Water sampling indicated that there are numerous locations where PFAS are entering the Port River and Barker inlet, with the highest concentrations in the stormwater wetlands throughout Gillman. The waters around the inner port shipping terminals (Berths M and N and Dock 1) were also elevated compared to the rest of the system. Based on knowledge of possible sources, these findings were not unexpected.

1 Introduction

Per- and polyfluorinated alkyl substances (PFAS) are a group of synthetic industrial chemicals which have been found in the environment. There are a large number of chemicals in the PFAS category, although perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexane sulfonate (PFHxS) are the most significant. These are surfactant chemicals, being very water soluble and similar to detergents in some respects, although these are also extremely resistant to degradation in the environment.

PFAS have been produced throughout the world for over 50 years and used extensively in a wide range of applications. As a result of their use and their extreme persistence in the environment, PFAS have been detected in waters, groundwater, sediments and a wide range of living organisms around the world and in Australia. In the marine environment, PFAS have been detected in oceans throughout the world.

PFOS and PFOA have been shown to be acutely toxic to fish and invertebrates in both short- and long-term tests. The point where toxicity starts is variable for different organisms. For example, a no observed effect concentrations at 0.25 mg/L for the mysid shrimp *Mysidopsis bahia* (Yamashita *et al* 2005), while reproductive toxicity has been observed in higher animals including rats between 0.58–1.07 mg/kg body weight (Lau *et al* 2004). The human health effects from exposure to PFAS are inconclusive and clear adverse human health effects has not yet been established (enHealth 2016a).

Some PFAS bioaccumulate and biomagnify within marine food webs. Bioaccumulation is the uptake of a chemical into an organism from the environment (water, sediment, etc), while biomagnification is the process where a chemical's concentration will increase higher in the food web, resulting in the highest concentrations in top predators. PFOS, PFOA and PFHxS will biomagnify and result in the highest concentrations in top predators, particularly marine mammals (Houde *et al* 2011).

PFAS have been observed in marine wildlife with high concentrations such as seals, polar bears, and dolphins, and it is also present in the human population (Houde *et al* 2011). Numerous factors govern the biomagnification potential of a chemical to a particular species. The chemical's properties, the position in the food web, prey sources and variety, age and exposure to multiple pollutant sources all change contaminant concentrations in any particular species, and then subtle differences between animals contribute to variability even in the same species, in similar locations (Houde *et al* 2006b).

It is important to note that presence of a synthetic chemical in an organism is not necessarily a sign or cause of an impact from that chemical. The evaluation of impact from a chemical, particularly on wild animals, is a very complex process subject to numerous confounding factors and considerations. It should also be stressed that contaminant concentration in an animal's tissues does not necessarily relate to its toxicity.

In Australia, PFAS (mainly PFOS) have historically been used in fire-fighting foams to extinguish class B liquid fires. This is known to have been used throughout airports and military airbases primarily for training activities, resulting in contamination in locations such as Williamtown in northern New South Wales (NSW), Oakey in Queensland and Edinburgh in South Australia (SA). While fire-fighting foams are likely to be the dominant source, there are other industrial applications for PFAS including the Scotchgard™ range of products used for their stain repelling properties on carpets, leather and other textiles. PFAS (mainly PFOA) are also used in high performance coatings on fabrics and metal surfaces and is known to have been used in non-stick cookware and electronic components (Benford *et al* 2008).

In 2000, the main global producer of PFAS chemicals voluntarily stopped their manufacture due to concerns about their persistence in the environment and long-term health and environmental impacts. In Australia, the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) recommended that PFOS and related PFAS should continue to be restricted to only essential uses where less hazardous alternatives are unavailable. The use of PFAS chemicals has greatly reduced with PFOS containing fire-fighting foams largely phased out.

The *Stockholm Convention on Persistent Organic Pollutants* is an International environmental treaty that aims to eliminate or restrict the production and use of certain chemicals. PFOS and PFOA are persistent, bioaccumulative and toxic and in 2009, PFOS was added to Annex B of the Stockholm Convention thereby restricting its use. PFOA is currently proposed for listing under the convention.

This report is prepared as a preliminary evaluation of the occurrence of PFAS in the marine environment in South Australia and attempts to put the observed levels into context and discuss areas in need of further work. The report is aimed for the general public and as such tries to avoid the use of complex chemical and toxicological terminology by referring to appendices and linking to external documents where required. Unfortunately, not all complex terminology can be avoided and a glossary of terms is provided. The aims of this work are to evaluate the risk to the marine environment from PFAS around the Port River and Barker Inlet system and highlight areas in need of further work.

The work has three parts:

1 Analysis of PFAS in dolphin liver samples from southern Australia indicating biomagnification and attempting to put levels observed into context of other regions in southern Australia and the world.

2 A coarse water sampling snapshot to investigate whether areas had measurably higher concentrations of PFAS, potentially indicating possible sources of entry to the environment in need of further investigation.

3 A preliminary survey of PFAS in common fish and invertebrates (mussels and crabs) to evaluate PFAS concentrations with respect to human health risks and ecological risk to the fish and higher marine predators from consumption of the fish.

2 Methods

Water

Marine water samples (n = 46) were collected from the Port River and Barker Inlet system in South Australia on 4 February 2016 (Figure 1). Sites varied in depth between 0.5–14.5m deep. A pre-rinsed 1-L Niskin bottle sampled 150 mL of water from approximately 1 m above the sediment at each site. If the site was less than 1 m deep, the sample was taken at approximately 0.5 m above the sediment.

Stormwater wetland water samples (n = 11) were taken from standing water downstream from the inlet of nine stormwater wetland systems that discharge into the Port River and Barker Inlet system. At each location a 150-mL grab sample of water was taken 0.5 m from the surface using a sampling rod.

All samples were taken on a day of dodge tide resulting in very little tidal movement to reduce variability from water between sites. Duplicate samples were taken from 5% of sites and travel blank, pre-rinse blank and post-rinse blank samples were collected and to ensure quality control. All samples were kept in the dark and on ice until transport to the laboratory.

Fish

Laboratory studies of fish have shown uptake of PFAS from water via the gills and from the diet are important routes of exposure (Houde *et al* 2006b). The physical properties of PFAS result in the chemicals binding to blood proteins in an exposed animal and accumulate in the liver, rather than stored in fat tissues like more well-known legacy persistent organic pollutants, such as polychlorinated biphenyls (PCBs). For this reason, where possible, samples were taken from the liver or similar organ to evaluate ecological risk and allow comparison to literature. Additionally, PFAS samples were sampled from the edible portion of the animal to allow comparison to interim human health guidance documentation.

Popular recreational targeted fish species (Salmon: *Arripis truttaceus* n = 12, Bream: *Acanthopagrus butcheri* n = 3, King George Whiting: *Sillaginodes punctata* n = 3) were caught using rod and reel from the Inner Port River and North Arm, and West Lakes on 4 and 5 February 2016. Drop nets were used to capture blue swimmer crabs (*Portunus armatus n = 10*) and blue mussels (*Mytilus* sp. n = 16) were collected by hand from marina infrastructure on the same days. This report also uses data collected from a 2012 survey of bream (n = 6) collected in 2012 from the Port River, Patawalonga Lake and West Lakes using a Fyke net.

2012

Large (30–45 cm) fish were filleted (skin on), and fillets and frames (including liver) separated. At each location 2–3 fish were pooled to increase the representativeness of the sampling while maintaining an efficient program. All fish were dissected within 12 hours of capture, samples wrapped in aluminium foil, labelled and frozen until laboratory analysis for PFAS analysis.

2016

Fish were filleted and samples were obtained for livers (where size permitted), fillets (skin on) and the frames (remaining body parts (eg head, skeleton and gills), edible portions of crab and the hepato-stomach extracted, and mussels opened and meat extracted. Whiting livers were unable to be confidently extracted due to the small size of the animals. Where animal numbers were sufficient, samples were composited into 2–3 fish per sample and 8 mussels per sample. All fish and invertebrates were dissected within 12 hours of capture, samples wrapped in aluminium foil, labelled and frozen until laboratory analysis for PFAS analysis. Comparisons between 2012 and 2016 frame samples were not undertaken due to the difference in dissection techniques.

Dolphins

A total of 44 dolphin (*Tursiops* sp.) livers were sampled from the South Australian Museum's marine mammal sample bank, archives at Murdoch University in Western Australia, the Taronga Conservation Society in New South Wales and the Marine Conservation Program and Animal Health Laboratory in the Department of Primary Industries, Parks, Water and Environment in Tasmania. Sample archives from other states in Australia were requested but no samples were provided due to either lack of adequate samples, sample size or preservation method. Where possible *Tursiops aduncus* species was preferred, however in many cases detailed identification between *T. aduncus* and *T. truncatus* was not undertaken or *T. aduncus* samples were unavailable.

Dolphin liver samples were selected from animals based on their known or inferred proximity (whether near or far) to industrialisation using location of stranding and life history information if available. Additionally, animals were sampled to ensure a spread of sizes and in the cases of sexually mature adults, a preference was made for males to be sampled to reduce any reduction in PFAS observed in female animals through transfer to calves.

Approximately 5 grams of liver tissue were subsampled from larger liver samples taken during post mortem examination of *Tursiops* sp. at the time of recovery. Morphological information and known life histories were compiled by the relevant authorities. Samples were catalogued, wrapped in alfoil and frozen until laboratory analysis for PFAS analysis.

Laboratory analyses

Laboratory analysis for PFAS in water, fish and invertebrate and dolphin livers were undertaken at the National Measurement Institute. Detailed laboratory methods are described in Appendix 1.

Table 1 Acronyms used for per and polyfluorinated alkyl substances

Acronym	Full name
PFDoA	Perfluoro-*n*-dodecanoic acid
PFHxS	Perfluoro-*n*-hexane sulfonate
PFBS	Perfluoro-*n*-butane sulfonate
PFOS	Perfluoro-*n*-octane sulfonate
6:2 FTS	C2H4 perfluoro octane sulfonate
8:2 FTS	C2H4 perfluoro decane sulfonate
PFHxA	Perfluoro-*n*-hexanoic acid
PFHpA	Perfluoro-*n*-heptanoic acid
PFOA	Perfluoro-*n*-octanoic acid
PFNA	Perfluoro-*n*-nonanoic acid
PFDA	Perfluoro-*n*-decanoic acid
PFUdA	Perfluoro-*n*-undecanoic acid

3 Results

Water

PFOS, PFHxS and PFOA were the most commonly detected PFAS with measurable concentrations detected in 92%, 82% and 66% of samples respectively. However PFNA (perfluorononanoic acid), PFBuA (perfuorobutanoic acid), PFPeA (perfluoropentanoic acid) and 8:2 FTS (fluorotelomer sulfonate) were not detected in any sample. PFAS constituents were strongly correlated suggesting close association between chemicals (Appendix 2: Table 2).

PFOS was the main PFAS chemical found in samples comprising between 38–62% total PFAS detected. Outer Harbor was the only region that PFOS did not dominate with PFHxA comprising 51%. It is noted that this region only had three samples. PFHxS was typically the second largest PFAS component detected in water with between 16–38% of the total PFAS. PFOA was not a large component typically comprising 6–14% with a maximum of 26% in the stormwater wetlands.

Using profiles of similarities in chemical composition (Clarke *et al* 2008), five main groups were observed in the water sampling (Figure 1):

1 Water from the stormwater wetlands had significantly higher PFAS than all receiving environments (●)

2 The tidal creeks around North Arm were different to the rest of the sites (◆)

3 The terminal berths around the Inner Harbour, including berths M and N (fuel berths) and Dock One were different to other sites (▼)

4 The North Arm, Inner Port River and Barker Inlet were also grouped together as being similar (▲)

5 The outer section of the river, including Pelican Point, Osborne and Outer Harbor were the lowest concentrations found and grouped as being similar in composition (■).

Figure 1 indicates multiple entry points for PFAS into the Port River, namely the stormwater wetlands (which points to urban stormwater pollution from catchments) and also the Inner Port Berths, which operate the fuel import terminals and the adjacent fuel storage facilities at Birkenhead. This finding demonstrates that PFAS pollution is widespread, with potential need for multiple coordinated management responses. The elevated results from the tidal creeks shows that PFAS are likely to be passing through the wetlands and into the tidal creeks such as North Arm creek which is to be expected given the high water solubility of PFAS chemicals. The detection of 6:2 FTS suggests more recent pollution because it is a major constituent in the modern products that have replaced PFOS in firefighting foams and the metal-plating industries in the mid to late 2000s.

It should be noted that the results of the water sampling only indicate the PFAS concentrations in the Port River–Barker Inlet system and nearby stormwater wetlands on 4 February 2016. There are many factors that may change these results over time.

PFOS has been measured in other industrialised marine waters. The Port River and Barker Inlet PFOS concentrations are lower than those found in similarly developed and enclosed water bodies such as the Parramatta River (Sydney Harbour) and Tokyo Bay. However they are higher than Guanabara Bay in Rio de Janeiro (Brazil) and the more open waters of coastal Hong Kong and Korea (Appendix 3: Table 6).

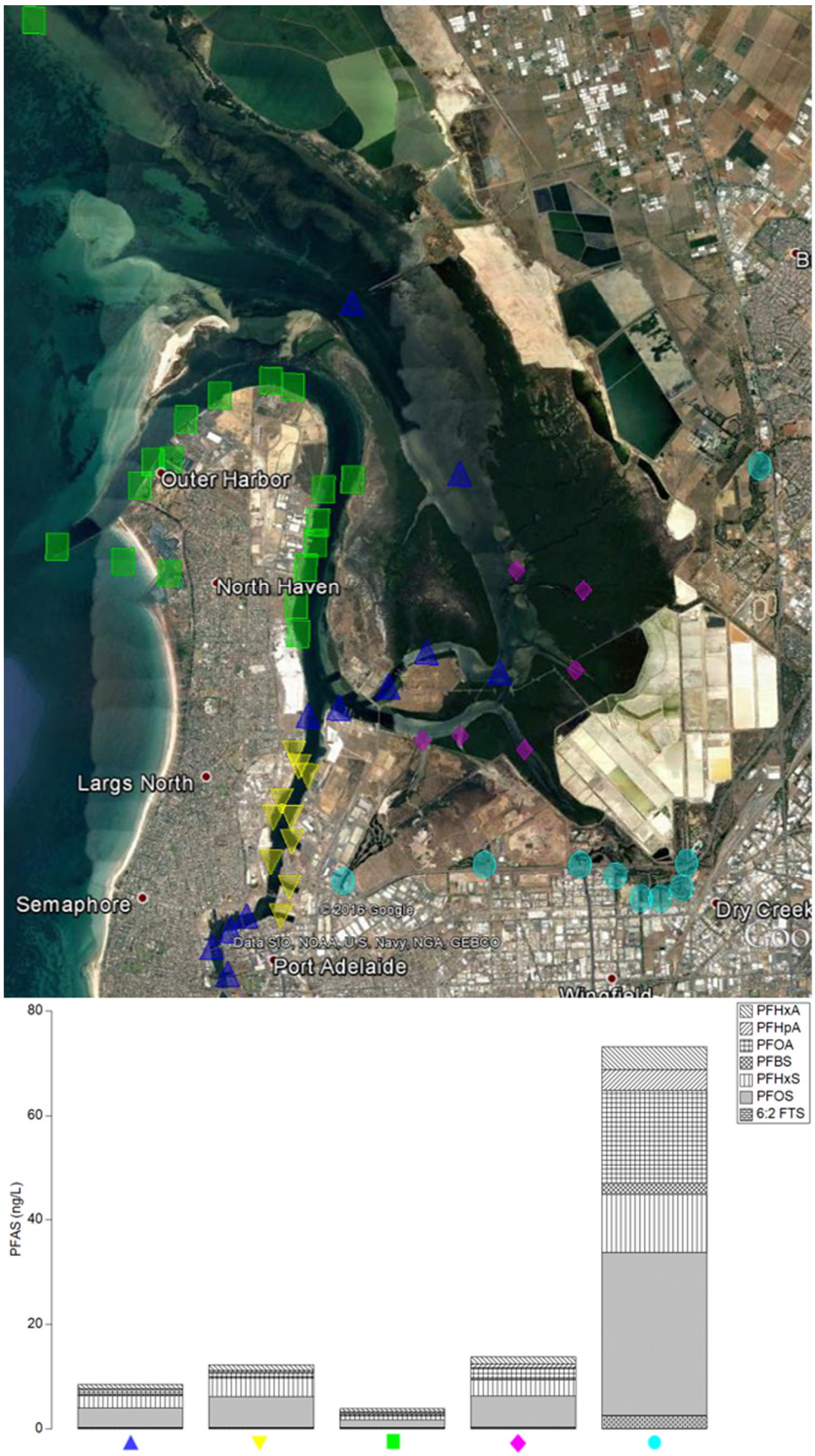

Figure 1 **Port River and Barker inlet PFAS (PFHxA, PFHpA, PFOA, PFBS, PFOS, 6:2 FTS) concentration (ng/L) and composition on 4 February 2016. Map colours represent SIMPROF groupings based on PFAS similarity profiles (Appendix 2: Figure 5).**

Fish

The fish fillets contained the lowest proportion of PFAS in all fish sampled, while the livers had the highest concentrations. PFOS was the most frequent and the largest proportion of PFAS detected in all organs, in all species and from all locations. PFDoA was the second most frequent while PFOA was only detected in one sample (Port River salmon liver) in trace amounts. PFHxA, PFHpA, PFNA, 8:2 and 6:2 FTS were not detected in any samples. This is consistent with literature on the chemistry of PFAS bioaccumulation and biomagnification.

For brevity and clarity, all further discussion regarding PFAS in fish will cover PFOS only because most published studies involving these compounds have focused on PFOS. Also, the relative proportions of the various PFAS compounds were very constant, as indicated by the strong Pearson correlation coefficients, and therefore this is unlikely to alter conclusions in any way (Appendix 2: Table 3).

Food safety

The EPA requested SA Health to provide advice regarding the application of guidelines or standards for human consumption of seafood from containing PFAS. SA Health advised that the Environmental Health Standing Committee (enHealth) released interim guidance values for human health exposure of PFAS in June 2016 (enHealth 2016b). They noted that there were limited data, especially about the health effects from consuming food containing PFOS or PFOA.

The interim guidance values adopted are based on the European Food Safety Authority (EFSA) guidance values, and are intended for use in site investigations in Australia pending the development of final guidance value recommendations by Food Standards Australia New Zealand (FSANZ). The EFSA has established a tolerable daily intake (TDI) for PFOS and PFOA at 150 ng/kg body weight per day for PFOS and 1,500 ng/kg body weight per day for PFOA (Benford *et al* 2008).

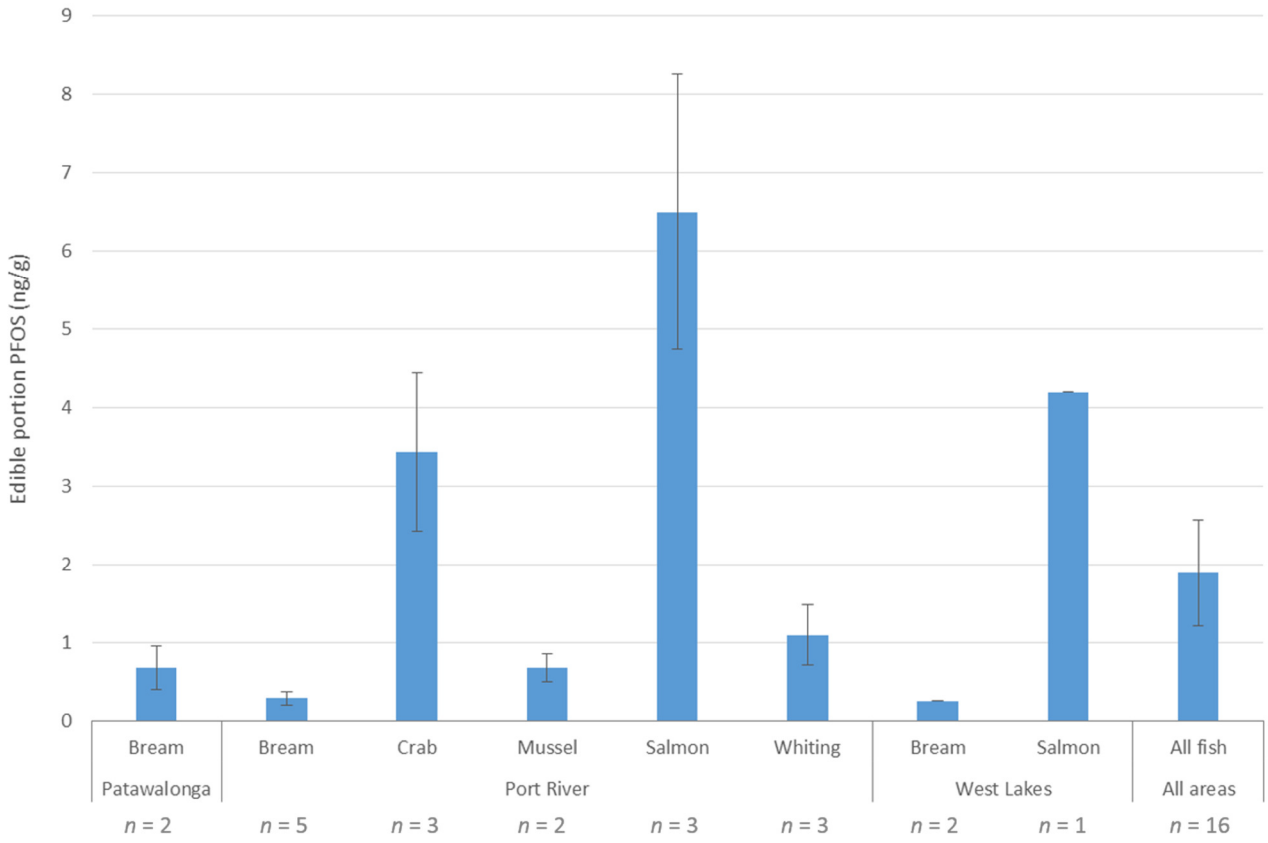

Figure 2 **Average PFOS concentration (ng/g wet weight) for fish and invertebrates from the Patawalonga, Port River and West Lakes. Error bars represent standard errors, *n* = sample size for each species and location.**

Figure 2 shows higher PFOS concentrations in the edible flesh of salmon and crabs than other species tested, particularly bream, which were the lowest. The large standard error bars in Figure 2 shows that there was considerable variability in PFOS concentration even within species caught from similar areas. PFOA was not detected in any edible flesh sample throughout the entire survey so comparisons against interim guidelines values were not undertaken for this compound.

Food consumption data for all ages (2+) and for children aged 2–6 from the National Nutrition and Physical Activity Survey (NNPAS) component of the Australian Health Survey 2011–13 was used to determine consumption volumes for the general population and from high consumers of seafood. It is standard practice to consider children (2–6 years \) as a separate category due to the relatively higher amount of food consumed per kilogram of body weight compared to adults (NSW Government 2015b).

Fish fillet data ('all fish' average) from the targeted recreational fishing areas were compared to the interim enHealth guideline values to establish whether fish and crustaceans were safe for human consumption. A person from the general population would need to consume approximately 5.6 kg of fillets caught from the target areas every day to exceed the PFOS interim TDI. If targeting only salmon (*Arripis truttaceus*), then a person eating 1.8 kg of salmon fillets per day, from the Port River, would exceed the TDI for PFOS. The estimates are based on whole-of-Australia fish consumption estimates and are approximate only. Similarly, children aged from 2–6 years would need to consume over 1.6 kg of any fish fillets from the target areas every day or 438 g of salmon fillets from the Port River to exceed the interim PFOS TDI.

FSANZ, the principle standards setting agency for food standards in Australia, is, at the time of publishing was undertaking an in-depth health risk assessment associated with PFAS in food. Once complete, estimates of risks associated with PFOS intake from fish consumption may require further examination.

Ecological assessment

Figure 3 shows that there is large variability between species tested, particularly between salmon, bream and whiting. Age-length relationships suggest that the whiting would have been less than two years old (McGarvey and Fowler 2002), salmon were estimated to be over two years old (Cappo 1987), and the bream were a mixture of three and over 20-year-olds (Morison *et al* 1998). This suggests that age or size is unlikely to be a driving factor in PFOS accumulation. Prey and species-specific biology (pelagic, demersal, benthic) are likely to contribute to the large differences seen between PFAS concentrations between species.

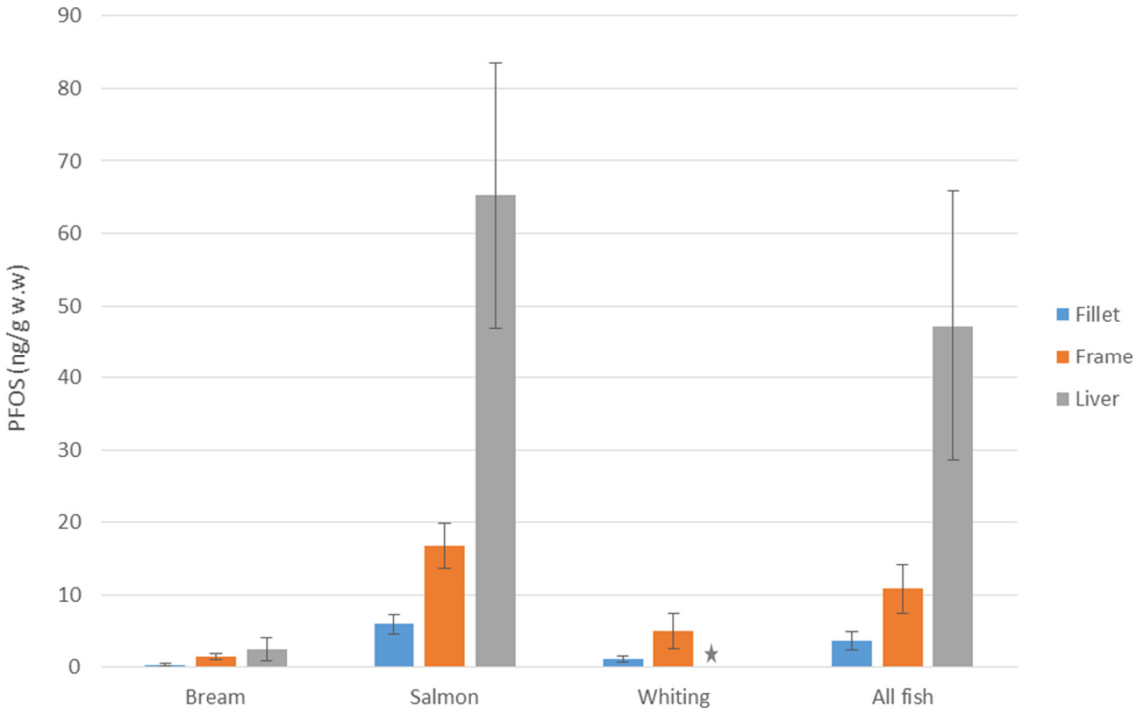

Figure 3 **Fish fillet, frame and liver PFOS concentration (ng/g wet weight) pooled for all locations. Error bars represent standard error. ★ denotes whiting livers were unable to be extracted.**

The large difference between species makes comparisons to other surveys difficult, however a broad comparison to adult sea mullet sampled in the Parramatta River shows fish liver and muscle (fillet) PFOS concentrations from South Australia were broadly similar to those from Sydney Harbour (Appendix 3: Table 7). However, both NSW and SA fish liver concentrations were higher than from Guanabara Bay in Brazil (Appendix 3: Table 8).

Mussels are filter-feeding bivalves that siphon significant volumes of water and typically accumulate contaminants, a characteristic that has seen them used extensively in pollution monitoring (O'Connor 1998). Nevertheless, this survey found mussels (n = 2) were relatively low in PFAS compared to other species, even though they were taken from areas with elevated PFAS during the water survey (within the Inner Port terminal berth group of sites ▼ Figure 1). This could suggest bivalves have a specific ability to regulate PFOS or perhaps have less proteinaceous material than fish (where PFOS accumulation predominates). This finding is consistent with bivalves sampled from Williamtown and Parramatta River in NSW, and Guanabara Bay in Brazil (Appendix 3: Table 9).

Bottlenose dolphins in South Australia

The Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) is a common bottlenose dolphin throughout Australia. In southeastern Australia, *T. aduncus* inhabit inshore areas and show a high degree of site fidelity and may belong to small subpopulations (Hale 1997, Möller *et al* 2001). Their site fidelity and inshore distribution may result in increased risk from land-based pollution (Hale 1997).

PFOS represented 94% of the PFAS chemicals detected in the South Australian dolphins livers. PFHxS comprised 2.4% while the remaining PFAS chemicals were all less than 1% of the total load respectively. This is consistent with results from *Tursiops* sp. from Florida (Fair *et al* 2012).

The Port River was the only region to show a significant difference between the age of the dolphins, with juveniles having significantly higher PFOS concentrations than adults. There is evidence that PFAS chemicals are passed on from the mother to the calf during pregnancy and lactation (Houde *et al* 2006a) and it is likely that the smaller body size of the juveniles contributes to the significantly higher PFAS concentration per gram of liver.

However this was not a consistent pattern across regions. One dolphin sampled was likely to be days old. During the post-mortem examination, a sample of stomach contents were collected and sampled for PFAS to indicate contaminant transfer from the mother's milk. The results showed PFOS of 890 ng/g (0.890 mg/kg) in the stomach contents, which is more than 15 times less (adjusted for dolphin weight) than an approximation of an acute oral dose in neonatal rats (Yahia *et al* 2008). This suggests that PFOS was not the cause of death. However, this comparison should be viewed cautiously as acute oral dose toxicity assessments are unable to be undertaken for bottlenose dolphins, meaning there may be significant interspecies differences between rat and dolphin toxicities, which may affect the comparison.

Dolphins from the Port River were significantly higher in PFOS than animals from the Adelaide metropolitan coast, and both were significantly higher than the West Coast of SA (Appendix 2: Table 5). This is likely to reflect the proximity to PFAS sources including the Inner Port fuel berths and the stormwater wetlands, as indicated by the water survey, as well as the amount of flushing that occurs throughout the Port River and Barker Inlet system compared to the relatively well flushed waters of the Adelaide metropolitan coast.

Bottlenose dolpins across southern Australia

In order to understand the broader context of dolphin PFAS biomagnification, liver samples from *Tursiops* sp. were sampled from other southern Australian locations with varying degrees of industrialisation and compared to the South Australian data. Figure 4 (and cluster analysis in Appendix 2) shows three main groups:

1 **High** – The Swan River dolphins had significantly higher PFAS concentrations than all other locations, largely due to the prevalence of PFOS and the presence of PFDA, PFNA, PFHxA and PFOA (■).

2 **Middle** – Mandurah (WA), offshore NSW, Port River (SA) and the Adelaide metropolitan coast were all similar in PFAS profiles (▼).

3 **Low** – Animals from Tasmania, Bunbury (WA) and the West Coast (SA) were similar and at much lower levels, likely representing background concentrations in bottlenose dolphins (▲).

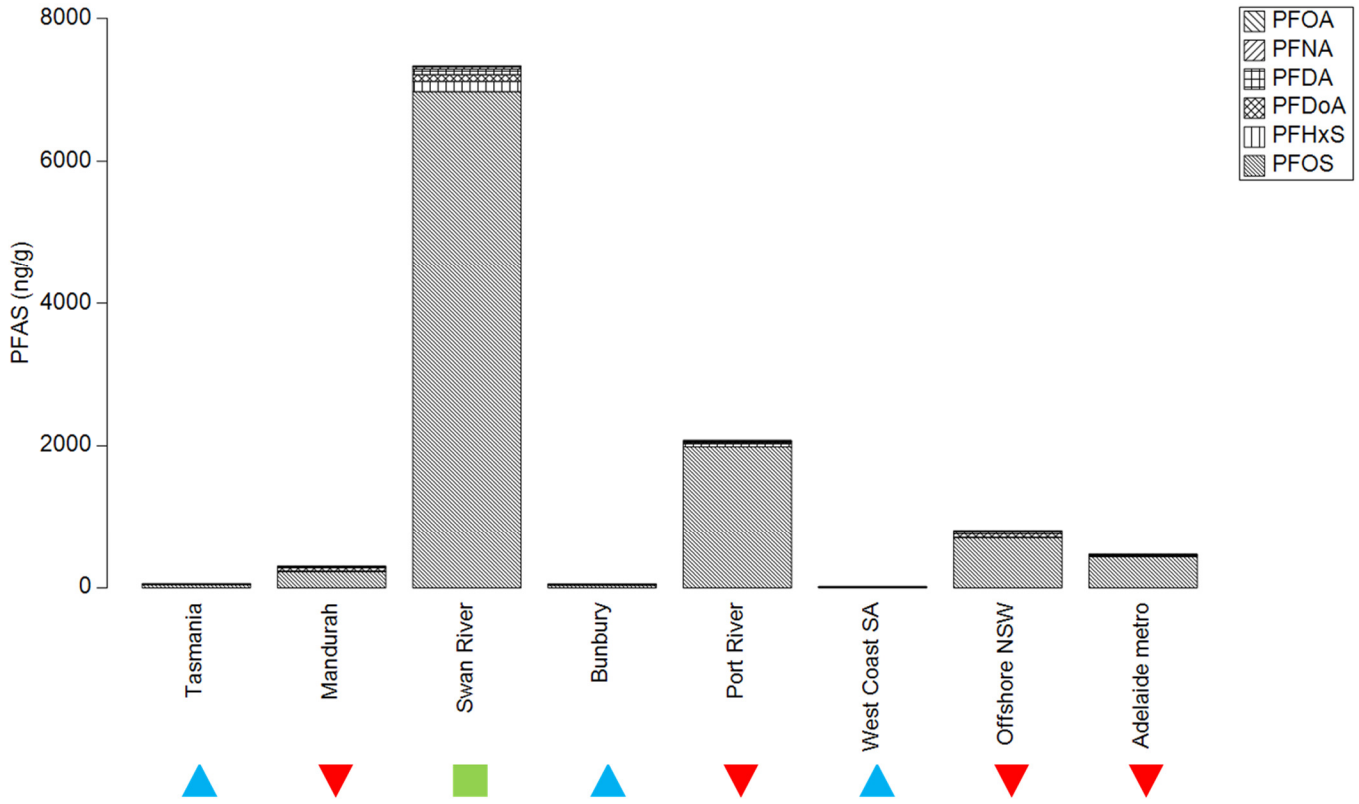

Figure 4 **Regional dolphin liver PFAS composition and concentration (ng/g wet weight). Symbols indicate SIMPROF groupings (Appendix 2: Figure 6) or areas that are similar to each other.**

The three groupings described suggest an interaction with the waterbodies' level of flushing and the proximity to potential PFAS sources. The Swan River, and to a lesser extent the Port River, are both relatively enclosed waterbodies with numerous potential PFAS sources including large fuel shipping ports and stormwater from urban and industrial catchments. Both have populations of resident inshore dolphins. These factors are likely to influence PFAS exposure to resident dolphins rather than volume of PFAS used. Further work on dolphin life histories and information on historical discharges of PFAS from industries in these areas is needed to confirm this link. Comparisons to the New South Wales animals should also be viewed with caution given the high proportion of *Tursiops truncatus* sampled (6 out of 7 samples), which are known to inhabit more offshore areas and are unlikely to be exposed to land-based pollution to the same extent as *Tursiops aduncus* (Lavery *et al* 2008).

Comparing the results of this survey with international literature shows southern Australian dolphins have the highest liver PFOS concentrations found in marine mammals worldwide (Appendix 3: Table 10). This survey should be considered a preliminary investigation and that larger sample sizes are required to have a high degree of confidence in results. PFAS persistence and biomagnification in the marine environment is still considered an emerging issue and studies on ecological presence and biomagnification in dolphin and marine mammals are still limited.

At this point in time there is very little literature detailing the biochemical mode of action of PFOS exposure in dolphins and whether the effects of this chemical can be differentiated from other contaminants known to accumulate in dolphins. Additionally, the ad hoc nature of sampling stranded dolphins after death hinders the ability to have high quality samples for biochemical endpoints. As a result, an evaluation of the actual impact of PFAS on dolphins cannot be determined from the results in this survey.

Despite this, the overall population of *Tursiops* sp. dolphins in Inner Port River has increased over the last 30 years which has been attributed to the widespread improvements in water quality (Bossley *et al* 2016). Therefore, at the population scale, the cumulative effect of all stressors including PFAS, is unlikely to be having a significant effect on the dolphins.

4 Conclusions

This document presents the results of preliminary surveys the EPA has undertaken to assess the presence of PFAS chemicals in the Port River and Barker Inlet. This work demonstrates dolphins residing in heavily industrialised areas, such as the Port River, accumulate large amounts of PFOS in their livers. These levels were compared to dolphins from southern Australia and the results show that the Swan River dolphins and to a lesser extent the Port River dolphins had the highest levels of PFOS seen in marine mammals globally. This is unlikely to reflect extreme usage of PFAS chemicals in these locations. This is more likely to reflect resident dolphins in these heavily industrialised locations increasing exposure which is exacerbated by limited water exchange with the adjacent marine waters. In the Port River, there is no evidence to suggest that the dolphin population is being impacted by these chemicals as the population of dolphins within the inner Port River has increased over time.

A survey of marine waters and local stormwater wetlands was used to identify possible sources of PFAS and lead to further investigation with respect to PFAS management. The findings showed that PFOS was detected in most locations in the Port River and Barker Inlet, and suggested multiple current or historical entry points of PFAS in the Port River, including the Gillman and Barker Inlet stormwater wetlands as well as the waters around the inner Port shipping terminals including Berths M and N which are used for fuel import and Dock One.

The areas identified in the water sampling program were also targeted for common recreationally caught fish and invertebrates. Using advice from SA Health, PFOS results were compared to the interim enHealth guidance values, which demonstrated that all fish and invertebrates were safe to eat, even for high seafood consumers. There was a large difference in PFOS accumulation between species, with Salmon (*Arripis truttaceus*) accumulating more than all other species tested, which is likely due to their prey selection and species-specific biology.

The EPA is currently auditing facilities that may have used PFAS to look at use history, pollution control strategies, potential for site contamination and ongoing management. Significant findings will be reported on the EPA website www.epa.sa.gov.au

PFOS is one of many emerging contaminants that enter the marine environment. Monitoring and evaluation of all chemicals is impossible but the EPA will continue to keep a watching brief on new and emerging chemicals of concern through risk assessments, literature from the scientific community and where appropriate, occasional surveys in high-risk locations.

Future questions

Bottlenose dolphins are long lived top predators that have been shown to accumulate a range of pollutants from natural chemicals such as mercury (Butterfield and Gaylard 2005) and synthetics such as PCBs (EPA 2000). Differentiation of the effects of any one of these chemicals in a mixture accumulated over the dolphin's lifespan is extremely difficult. Further work is needed to understand the toxicology and potential health impacts from these chemical mixtures, but access to very recently stranded animals providing fresh tissue is needed.

The EPA is in discussions with the SA Museum, the Whale and Dolphin Conservation Society and universities to try to understand whether dolphin lifespan or reproductive ability are being impacted in heavily industrialised locations by the range of historical and current contaminants found in our waters.

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6 Glossary

benthic	Living on the sea bed
bioaccumulate	The accumulation of substances in biota at a rate faster than it can excrete, resulting in an increase in concentration over time. Bioaccumulation takes into account uptake of a substance from water and food.
biomagnify	The increase in concentration of a substance at successively higher levels through the food web
demersal	Living near the sea bed
emerging contaminants	Emerging contaminants are new or novel and typically their persistence or environmental impact have not been widely studied. Emerging contaminants can include household chemicals such as pharmaceuticals.
EPA	South Australian Protection Authority
invertebrate	Organisms that do not at any time in their life, have a backbone. They include crabs and mussels
SIMPROF	A statistical method of exploratory data analysis testing whether similarities in profiles of the data are different to those that might occur by chance. Used to show groupings present in hierarchical agglomerative clustering.
pelagic	Living in the water column
PFAS	Per and poly-fluorinated alkyl substances. An umbrella term used to describe any fully fluorinated carbon chain chemical.
PFOA	Perfluoroctanoic acid is a man-made fluoro-surfactant and carboxylic acid. Like PFOS, PFOA is very stable and does not breakdown in the environment. Used in the manufacture of non-stick cookware, waxed paper and fabric stain repellents.
PFOS	Perfluorooctane sulfonate man-made fluoro-surfactant widely used throughout the world since the 1950s. A key ingredient in a range of industrial and household products including stain repellents and firefighting foams. PFOS is very stable and does not breakdown in the environment. As a result is now known as a global pollutant.
PIRSA	Department of Primary Industries and Regions South Australia
TDI	The tolerable daily intake is an estimate of the amount of a substance in food or drinking water which is not added deliberately (eg contaminants) and which can be consumed over a lifetime without presenting an appreciable risk to health

Appendix 1 Laboratory methods

All PFAS samples were analysed at the National Measurement Institute laboratories at North Ryde, NSW.

Analysis was conducted using isotopic dilution, based on reference method USEPA 537. Samples were prepared for analysis by homogenisation using a knife mill or hand-held homogeniser and stored in 50 mL Falcon® polypropylene tubes (Corning) at −20 °C. Samples had known amounts of ^{13}C isotopically labelled analogues of the target analytes added (Wellington Laboratories, Canada) and were extracted with saponification by tumbling with alkaline methanol. The extract was centrifuged, and the supernatant concentrated then purified by solid phase extraction. A ^{13}C isotopically labelled standard was added to the sample to serve as a recovery standard. Qualitative/quantitative analysis for PFASs was performed using an Agilent 1100 HPLC, ABSciex 4000 Qtrap MS/MS high performance liquid chromatograph/triple quadrupole mass spectrometer/computerised data system (LC/MS/MS). Multiple reaction monitoring (MRM) of two characteristic transitions was performed, with identification confirmed when target ions were detected in both the monitored MRMs within established retention time windows.

The limits of reporting (LORs) were determined for each compound in each sample based on noise and laboratory blank levels, and varied between samples as a result of instrument performance and the level of sample contamination. Quantification of linear and branched isomers of all analytes was based on the use of the ^{13}C labelled surrogates and linear calibration standards, and the total of all isomers reported. Analyte concentrations were corrected for recovery of ^{13}C isotopically labelled surrogates to overcome matrix suppression/enhancement, and results were reported on a wet weight basis. Validation of the method included analysis of a fish standard reference material provided by the National Institute of Standards and Technology (SRM 1946, NIST, USA) which had an assigned reference value for PFOS, and resulted in measurements within 10% of the assigned value. Three samples were analysed in duplicate and the relative percent difference (RPD) of the analyte concentrations were within reasonable levels for both perfluoro-n-hexane sulfonate or PFHxS and perfluoro-n-octane sulfonate or PFOS (no other analytes were present at concentrations greater than the limit of reporting in these samples).

Data analysis

PFAS analysis measures five sulfonate compounds: PFBS, PFHxS, PFOS, 6:2 FTS and 8:2 FTS, and seven carboxylic acid compounds PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA and PFDoA. When a compound was not detected at any site within the given media (water, fish, dolphin), the compound was removed from all statistical analyses. Where compounds are included but samples were found to be below the limit of reporting, half the LOR was used in all analyses. This approach has been shown to reduce biases introduced by using either the LOR or zero (Helsel and Hirsch 2002).

Appendix 2 Statistical analysis

Table 2 **Water: Pearson correlation coefficients for log transformed water PFAS concentrations taken from the Port River and Barker Inlet system on 4 February 2016. PFBuA, PFPeA, PFNA and 8:2 FTS were not detected in any water samples. Strong correlations (> 0.6) are shown in bold (Evans 1996).**

	PFHxA	PFHpA	PFOA	PFBS	PFHxS	PFOS	6:2 FTS
PFHpA	**0.788**	–	–	–	–	–	_
PFOA	**0.721**	**0.963**	**–**	**–**	**–**	**–**	_
PFBS	0.561	**0.850**	**0.916**	–	–	–	_
PFHxS	0.414	**0.738**	**0.818**	**0.947**	**–**	**–**	_
PFOS	0.577	**0.863**	**0.900**	**0.919**	**0.939**	**–**	_
6:2 FTS	**0.704**	**0.864**	**0.874**	**0.804**	**0.729**	**0.853**	_

Table 3 **Fish liver: Pearson correlation coefficients for log transformed fish PFAS concentrations. PFBS, PFHxA, PFHpA, PFNA, 6:2 FTS and 8:2 FTS were not detected in any fish tissue samples. PFHxS, PFOA had insufficient samples above LOR to enable correlation. Strong correlations (> 0.6) are shown in bold.**

	PFOS	PFDA	PFUdA
PFDA	**0.7436**	**–**	–
PFUdA	**0.6426**	**0.9764**	–
PFDoA	0.4798	**0.9012**	**0.9568**

With the exception of PFOS, there were insufficient data detected within fish muscle to undertake correlation.

Table 4 **Dolphin liver: Pearson correlation coefficients for log transformed dolphin liver PFAS from southern Australia. PFHxA, PFHpA, 6:2FTS and 8:2 FTS were not detected in sufficient samples to undertake correlation analysis. Strong correlations (> 0.6) are shown in bold.**

	PFOA	PFNA	PFDA	PFDoA	PFHxS
PFNA	**0.941**	–	–	–	–
PFDA	**0.856**	**0.898**	–	–	–
PFDoA	**0.639**	**0.732**	**0.883**	–	–
PFHxS	**0.924**	**0.885**	**0.771**	0.559	–
PFOS	**0.929**	**0.899**	**0.847**	**0.634**	**0.963**

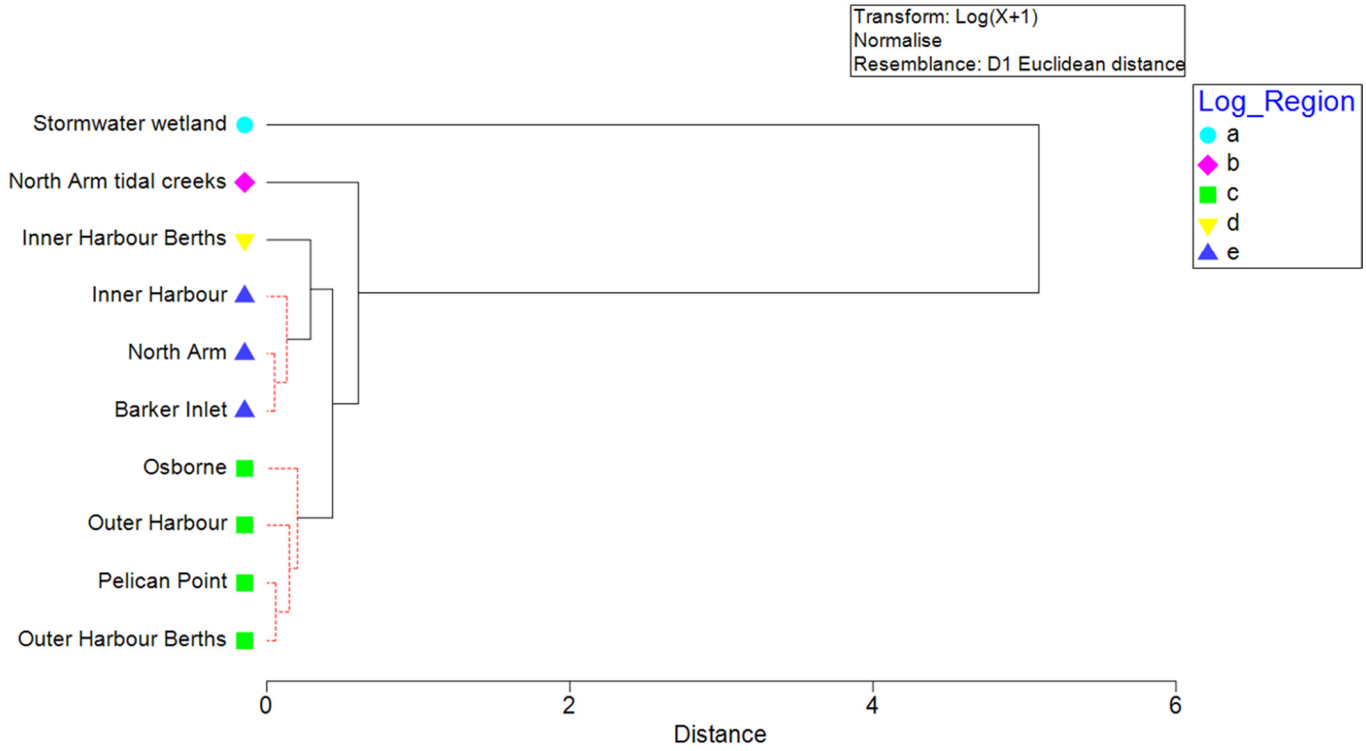

Figure 5 **Cluster analysis of water PFAS from the Port River and Barker Inlet on 4 February 2016. Symbols represent groupings determined by SIMPROF analysis indicating significant similarity in PFAS profile.**

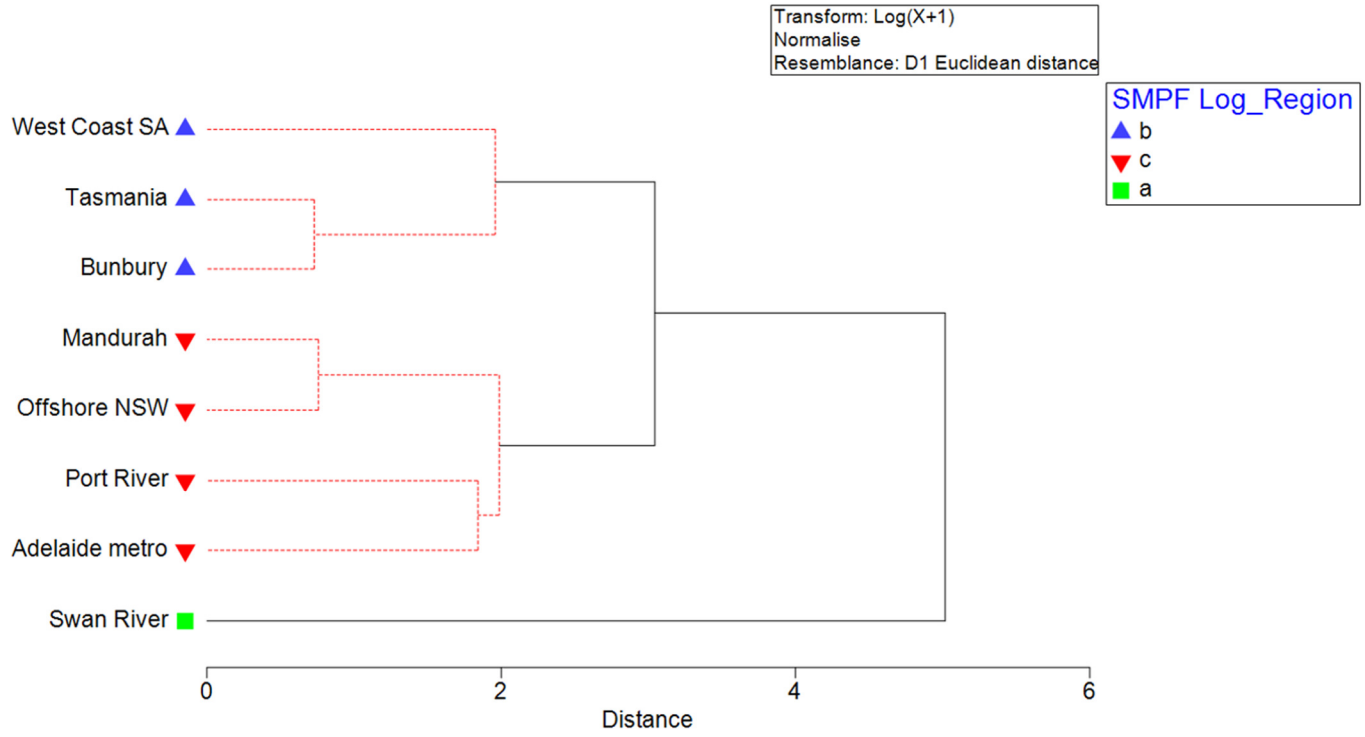

Figure 6 **Cluster analysis of dolphin liver PFAS from southern Australia. Symbols represent groupings determined by SIMPROF analysis indicating significant similarity in PFAS profile.**

Table 5 Regional dolphin liver PFOS mean concentration (ng/g), range and statistical significance (one-way pairwise PERMANOVA)

Region	symbol	n	Mean PFOS (ng/g)	Range	Sig (p< 0.05)
Adelaide metropolitan, SA	*a*	5	436.0	290–690	*c d h*
Port River & Barker Inlet, SA	*b*	9	1,986	510–5,000	*a c d e g h*
West Coast SA	*c*	6	7.250	< 5–13	–
Bunbury, WA	*d*	8	36.92	< 5–97	–
Mandurah, WA	*e*	2	227.0	34–420	*c*
Swan River, WA	*f*	4	6,975	2,800–14,000	*a b c d e g h*
Offshore NSW	*g*	7	705.1	58–1,800	*c d h*
Tasmania	*h*	3	46.0	11–71	–

Appendix 3 Comparison of available published PFOS data

Table 6 Comparison of water PFOS concentrations (ng/L; samples, mean and range) for the groupings in the Port River and elsewhere in the world

Location	n	Mean	Range	Source
Port River (tidal creeks), SA	6	5.95	3.7–10	This survey
Port River (Outer Harbor), SA	17	1.48	<0.5–3.5	This survey
Port River (Inner Port terminal berths), SA	10	5.93	4.2–8.2	This survey
Port River (North Arm, Inner Port River & Barker Inlet), SA	12	3.57	1.5–4.6	This survey
Parramatta River, Sydney Harbour, NSW	20	14	7.5–21	Thompson *et al* 2011
Charleston Harbour, Florida	18	12	*	Houde *et al* 2006
Sarasota Bay, Florida	10	0.9	*	Houde et a 2006
Tokyo Bay, Japan	8	*	0.34–57.7	Yamashita *et al* 2005
Coastal waters off Hong Kong, China	12	*	0.07–2.6	Yamashita *et al* 2005
Guanabara Bay, Brazil	12	0.56	*	Quinete *et al* 2009

* denotes metric not provided in reference document

Table 7 Comparison of PFOS concentrations (ng/g) in fish muscle from this survey and published literature

Organism	Location	n	Mean	Range	Source
Salmon[1]	Port River & West Lakes, SA	4	5.93	4.2–10	This survey
Bream[2]	Port River, West Lakes & Patawalonga, SA	9	0.36	0.15–0.96	This survey
Whiting[3]	Port River, SA	3	1.10	0.39–1.7	This survey
Mullet[4]	Parramatta River, NSW	10	2.2	0.8–4.9	Thompson *et al* 2011
Fish[5]	Fullerton Cove, NSW	14	8	0.3–19	NSW Government 2015b
	Tilligerry Creek, NSW	23	3	0.3–18	NSW Government 2015b
Mullet[6]	Guanabara Bay, Brazil	8	3.49	1.95–5.44	Quinete *et al* 2009

1 *Arripis truttaceus*

2 *Acanthopagrus butcheri*

3 *Sillaginodes punctata*

4 *Mugil cephalus*

5 *Platycephalus fuscus, Sillago ciliata, Mugil cephalus, Acanthopagrus australis*

6 *Mugil liza*

Table 8 Comparison of PFOS concentrations (ng/g w.w.) in fish liver from this survey and published literature

Organism	Location	n	Mean	Range	Source
Salmon[1]	Port River & West Lakes, SA	4	65.25	44–120	This survey
Bream[2]	Port River, SA	3	2.43	0.81–5.60	This survey
Mullet[4]	Parramatta River, NSW	10	70	44–107	Thompson *et al* 2011
Mullet[5]	Guanabara Bay, Brazil	15	4.30	2.17–9.44	Quinete *et al* 2009

Table 9 Comparison of PFOS concentrations (ng/g w.w.) in bivalve flesh from this survey and published literature

Organism	Location	n	Mean	Range	Source
Mussels[7]	Port River, SA	2	0.68	0.5–0.86	This survey
Oyster[8]	Parramatta River, NSW	10	1.2	0.6–2.3	Thompson *et al* 2011
Oyster[9]	Tilligerry Creek, NSW	7	1.0	< 0.3–2.0	NSW Government 2015a
Mussels[10]	Guanabara Bay, Brazil	17	2.58	< 0.95–4.65	Quinete *et al* 2009

Table 10 Comparison of PFOS concentrations (ng/g w.w.) in dolphins from this survey and published literature

Species		n	PFOS (ng/g)	Range	Source
Dolphin[11]	Adelaide metropolitan, SA	5	436	290– 690	This survey
	Port River, SA	9	1,986	510–5,000	This survey
	West Coast, SA	6	7.250	< 5–13	This survey
	Bunbury, WA	8	36.92	< 5–97	This survey
	Mandurah, WA	2	227	34–420	This survey
	Swan River, WA	4	6,975	2,800–14,000	This survey
Dolphin[12]	Offshore NSW	7	705.1	58–1,800	This survey
	Tasmania	3	46	11–71	This survey
Dolphin[13]	NW Atlantic	20	489	48–1,520	Kannan *et al* 2001

7 *Mytilus* sp.

8 *Saccostrea glomerata*

9 *Saccostrea glomerata* & *Crassostrea gigas*

10 *Perna perna*

11 *Tursiops aduncus* liver

12 *Tursiops* sp. liver

13 *Tursiops truncatus*

Species		n	PFOS (ng/g)	Range	Source
Dolphin[14]	Paraiba do Sul River, Brazil	10	90.5	25.9–149	Quinete *et al* 2009
	Guanabara Bay, Brazil	23	268	13–902	Dorneles *et al* 2008
Dolphin[15]	Indian River Lagoon, Florida	81	597.8	69.2–3,620	Fair *et al* 2012
	Charleston, South Carolina	76	1,246	316.7–6,260	Fair *et al* 2012
	Sarasota Bay, Florida	12	340	*	Houde *et al* 2006a

* denotes metric not provided in reference document

[14] *Sotalia guianensis* liver

[15] *Tursiops truncatus* plasma