Health impact assessment from consumption of fish from Lake Fiskville

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Executive Summary

As a result of past training practices at Fiskville, the water and sediment of Lake Fiskville has high concentrations of perfluorochemicals (PFCs). From their extensive use in consumer products these chemicals are also ubiquitous in the general and human environment. The biota in Lake Fiskville has assimilated PFCs present in lake water and/or sediment to a much larger extent than expected from background exposure.

In particular redfin fish from the lake have very high concentrations of perfluorooctane sulphonate (PFOS) in their flesh. This is also the PFC which is at the highest concentration in water and sediment and is the PFC of concern within the lake and biota. Concentrations of PFOS in redfin were higher than those in fish considered by overseas agencies as being unfit for consumption. As soon as it became apparent to CFA management that employees were catching and consuming fish or eels from the Lake staff were advised verbally and by newsletter not to fish the lake, and prominent signs were erected at the lake to that effect. Further notices were placed in local newspapers to advise the local community.

Significant uncertainties regarding the extent and frequency that fish or eel were consumed, and lack of PFOS data in eels, precluded assessing health risk from eating fish using a traditional tolerable daily intake (TDI) approach. Because the toxicological effects of PFOS are directly related to serum concentrations, and the sensitive effects in monkeys are changes in blood biomarkers that are routinely evaluated by medical doctors for health status, persons who had eaten fish in the past were invited to voluntarily participate in a health surveillance program. This was also open to persons who may not have eaten fish but were nonetheless concerned they may have been exposed to PFCs while working at Fiskville. This ‘fish consumption’ health surveillance program was an extension of the health status surveillance package already in place for CFA PAD workers. Additional to the existing medical surveillance of medication examination and measurement of routine blood parameters was quantitation of heavy metals in blood and PFC concentrations in serum. Participants were asked if their de-identified results could be made available, via the CFA medical officer, to the consulting toxicologist and thence to the CFA in the form of this report. Participation in the ‘fish consumption’ health surveillance program was not contingent upon agreement to share de-identified information, however all participants agreed their data could be made available.

Serum PFC measurements were undertaken by a commercial laboratory that included appropriate blanks, PFC spikes and duplicate analysis of samples chosen randomly. While internal standard recoveries for some samples were lower than the range regarded as ideal by the laboratory, the data are considered reliable for assessment of potential health risk.
To preserve anonymity, PFC serum concentrations are discussed in a general sense in this report.

Twelve of the 22 participants in the ‘fish consumption’ health surveillance program indicated that they had eaten fish or eel from the Lake in the past. For no person in the surveillance program were there changes in blood clinical chemistry parameters that could be attributed to PFOS. While recognising the very small sample size limits confidence in the data interpretation, regression analysis of a priori individual blood parameters with serum PFOS levels for either the entire cohort or just those that ate fish indicated no associations. Nevertheless there were a number of individuals in both the fish eating and non-fish eating groups that had blood parameter measurements outside the population reference range. The medical officer attributed all these to life style factors (e.g. alcohol consumption), body mass index, existing disease, and/or medication (including non-compliance). Where appropriate the medical officer referred people to their own medical practitioner.

Of the 10 PFCs looked for in human serum (chosen for their presence in Lake water or fish) only two were present at measurable concentrations in the serum of program participants. These were PFOS and perfluorooctanoic acid (PFOA). All PFOA measurements were approximately an order of magnitude less than the expected background concentrations for this compound. This indicates fish consumption has not contributed to human PFOA serum concentrations; not unexpected since redfin did not have measurable concentrations of PFOA in their flesh. PFOA was therefore not considered further in the risk assessment.

Many animal studies have shown toxicological effects of PFOS are directly related to serum concentrations. The potential health impact of serum PFOS concentrations measured in participants of the health surveillance program has been assessed in a number of ways.

- **Comparison with ‘background’ serum concentrations.**
  - A review of many publications reporting PFOS serum concentration in general communities showed the majority of adults would be expected to have a concentration <0.1 mg/L.

- **Comparison with a human serum level considered to be without effects in humans.** Three different methods were used to establish a serum no observed effect level (serum NOEL) of 2 mg/L. These were:
  - Dose response analysis of a number of occupational epidemiology studies,
  - Derivation from monkey and rat serum NOELs using standard uncertainty factors, and
  - Conversion of the TDI set by the European Food Safety Authority into an equivalent steady state serum concentration.
Calculation of margin of exposure (MOE) is a standard risk characterisation method widely used by Australian authorities. However instead of using experimental doses applied to animals and an uncertain estimated human intake in the calculation, the animal serum NOEL from toxicological studies and serum concentrations measured in program participants were used. While an acceptable MOE based on external dose is 100, that based on serum concentrations is 25. MOEs for four different endpoints (low birth weight, blood biomarkers, liver toxicity, and hepatic adenomas) were estimated.

Four persons had serum PFOS concentrations above that identified as the higher end of the normal range expected from background (i.e. resulting from day to day living). All were below the serum NOEL, indicating low risk for adverse health effects. Available information on fishing frequency by some participants in the program suggests serum PFOS concentrations in persons who may not have been included in the cohort were unlikely to be materially different from those measured in the surveillance program.

The Margin of Exposure (MOE) estimations calculated using current measured serum PFOS concentrations and serum NOELs identified in animal toxicity experiments also indicated very low risk for adverse health effects.

When current serum concentrations were extrapolated back to theoretical levels that may have existed 5 or 10 years previously, and assuming no further fish consumption, both comparison with the human serum NOEL and the calculated MOEs indicate adverse health effects were unlikely to have arisen due to these hypothetical serum PFOS concentrations.

Overall, it is concluded existing serum PFOS concentrations, or past theoretical concentrations, are unlikely to give rise to adverse health effects.
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1. Introduction

The ‘Joy’ report (IFI 2012) made a number of recommendations concerning examining potential environmental contamination that may have arisen as a result of historical fire fighting training at the CFA Fiskville training ground. During these investigations it was discovered the sediment and water of Lake Fiskville had become contaminated with perfluorinated chemicals (PFCs). Consultation with a few long term CFA Fiskville employees revealed the Lake had in the past been stocked with Redfin Perch and some employees, over a number of years, had occasionally caught and eaten fish from the Lake.

An initial analysis of a few fish for PFCs showed they, and other organisms in the Lake, had accumulated some of the PFCs found in the water and sediment. In particular perfluorooctane sulphonate (PFOS) was present in very high concentrations in muscle and liver of Redfin. Staff were instructed not to fish the Lake and ‘no fishing’ signs were erected.

The initial analysis of Redfin was on just four fish, which were the largest of those caught in the sampling program undertaken. Based on recollections of a long term CFA employee for fishing frequency, the numbers of fish caught and the concentration of PFOS in muscle of these four fish, a preliminary informal risk assessment was undertaken to determine potential impact to persons who may have eaten fish from the Lake. The assessment utilised human toxicokinetic information from the scientific literature to predict potential PFOS serum concentrations. It canvassed a range of fish consumption patterns constructed around the anecdotal fishing information provided by the long term employee. The modelling of some of the assumed high consumption patterns suggested high PFOS serum concentrations may occur. At this time the Victorian Department of Health were advised of the situation and of the follow up work that was planned to address significant uncertainties in the modelling of the preliminary assessment.

Major uncertainties in the initial assessment were PFOS concentration data being limited to analysis of just four fish, and no real knowledge of how much fish a person ate or when. The former was addressed by analysis of additional Redfin flesh (in total 21) and the latter by extending the existing CFA personnel health surveillance program to include persons who may have eaten fish. Analysis of blood serum PFCs was added to the existing program for these persons.

This brief report is an updated health risk assessment (HRA) for persons who have eaten fish from Lake Fiskville. However, unlike the preliminary risk assessment it does not rely on toxicokinetic modelling of potential PFOS serum concentrations. The modelling is now redundant. The
assumptions and uncertainties inherent in such modelling are replaced by measured serum concentrations.

Cardno Lane Piper (CLP) has produced a series of reports that document the site investigation and chemical concentrations in various media at Fiskville. To enable this report to be read as a standalone document, relevant analytical data have been extracted from the CLP reports to provide contextual information. Nevertheless the reader is encouraged to consult the cited CLP reports for the complete analytical data and how it was gathered and quality assessed.

2. PFC concentration in water and fish

Detailed information on the concentrations of PFCs in Lake Fiskville and organisms in the Lake and the recycled water dams at Fiskville can be found in the Cardno Lane Piper reports entitled “Surface Water and Sediment Contamination Assessment” (CLP 2013c) and “Ecological Assessment” (CLP 2013b). For completeness and ease of reading a summary of the relevant data is provided herein.

2.1 PFCs in Lake Fiskville

Table 2.1 summarises the PFC concentrations in Lake Fiskville. There were measureable concentrations of eight PFCs in the water column and three in sediment. Of these PFOS has the highest concentration. A glossary of PFC nomenclature and abbreviations can be found in Appendix A.

2.2 PFCs in fish

The analysis of PFCs in biological matrices is not straightforward. In particular for PFOS there is potential, but inconsistent interference by unknown substances. In addition, the literature (van Leeuwen et al. 2006, Malinsky 2009) indicates there can be marked variability within and between laboratories. The inclusion of stable isotope internal standards largely, but not completely, overcomes these issues (van Leeuwen et al. 2009). The analytical program for Redfin muscle analysis was cautiously designed by Cardno Lane Piper to include tissue duplicates, laboratory duplicates, split muscle samples for inter-laboratory comparison, and replicates. While there were instances of poor recovery of internal standard and poor replicates, Cardno Lane Piper undertook a careful quality

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1 Personal communication with National Measurement Institute, Sydney and AsureQuality analytical services, New Zealand.
control examination of the data (CLP 2013a)\(^2\) and concluded the analyses were accurate and could be relied upon.

Table 2.2 summarises the PFC concentrations in a range of organisms sampled from Lake Fiskville in December 2012. The information in the table is derived from CLP (2013d, e)\(^3\). In all organisms it is apparent that PFOS bioaccumulates to a much greater extent (by 3 – 4 orders of magnitude) than do other PFCs. This is consistent with the scientific literature (Conder et al. 2008, de Silva et al. 2011, Giesy et al. 2010, Haukås et al. 2007, Houde et al. 2011, Martin et al. 2003a, b, 2004; Morikawa et al. 2006), and that different organisms bioconcentrate PFOS to different degrees. Redfin are at the top of the aquatic food chain in Lake Fiskville and therefore biomagnify PFOS the greatest (McDowell 1996, as cited in CLP 2013b; NSW DPI 2014; Waterwatch Vic undated, Humphries and Walker 2013). While it may appear Mosquito fish and yabby have taken up a range of PFCs dissimilar to those in Redfin muscle this is probably because the former animals were analysed whole (i.e. included internal organs). Redfin liver contained the same PFCs as Mosquito fish and yabby (CLP 2013b); the redfin liver data is not replicated in this report because it is a tissue not eaten by humans.

\(^2\) The information contained in CLP (2013a) is also available in CLP (2014a, b).

\(^3\) The information contained in CLP (2013d) is also available in CLP (2014a, b).
Table 2.1: PFCs in sediment and water of Lake Fiskville.

<table>
<thead>
<tr>
<th>PFC</th>
<th>Sediment (ng/g)</th>
<th>Water (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFBA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PFPeA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PFBS</td>
<td>ND</td>
<td>1.4 b</td>
</tr>
<tr>
<td>PFHxS</td>
<td>12.6 b</td>
<td>4.4 b</td>
</tr>
<tr>
<td>PFOS</td>
<td>225 a (57 – 785)</td>
<td>13.3 a (8.8 – 17.7)</td>
</tr>
<tr>
<td>PFDS</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PFHxA</td>
<td>ND</td>
<td>4.8 b</td>
</tr>
<tr>
<td>PFHpA</td>
<td>ND</td>
<td>0.7 b</td>
</tr>
<tr>
<td>PFOA</td>
<td>ND c</td>
<td>0.58 a (0.48 – 0.76)</td>
</tr>
<tr>
<td>PFNA</td>
<td>ND</td>
<td>0.04 b</td>
</tr>
<tr>
<td>PFDA</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PFuDA</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PFDoA</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PFTrDA</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PFOSA</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>NEtFOSA</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>NEtFOSAA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NMeFOSA</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>NMeFOSAA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NEtFOSE</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>NMeFOSE</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4:2 FtS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6:2 FtS</td>
<td>12.8 a (&lt;5 – 24)</td>
<td>5 a (3.5 – 7.4)</td>
</tr>
<tr>
<td>8:2 FtS</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

ND = not detected; - = not in analytical suite.

a The data are the average (range in brackets) PFC concentration measured in August 2012 at various locations/depths in the lake. It should be noted that for PFCs other than PFOS, PFOA and 6:2FtS only one sample of water and sediment (LFWE2.0/06082012 or LFSE0.1/02082012) was analysed for the complete suite of PFCs. Information in the table has been compiled from data in CLP (2013c) and ALS analysis certificates (EM1208900, EM1208979, EM1209107) provided by CLP for the water and sediment sample that underwent full PFC analysis.

b Data are the average of the primary sample and its laboratory duplicate.

c For PFOA in sediment 4 of 5 measurements were below the LoR (0.0005 mg/kg), one measurement was marginally above the LoR (0.0007 mg/kg).
Table 2.2: PFC concentrations in organisms sampled from Lake Fiskville

<table>
<thead>
<tr>
<th>PFC conc (ng/g)</th>
<th>Redfin muscle n=21</th>
<th>Mosquito fish whole n=3</th>
<th>Yabby whole n=4</th>
<th>Freshwater shrimp whole n=1</th>
<th>Macrophyte n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFBA</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PFPeA</td>
<td>ND</td>
<td>ND</td>
<td>6 (3.4 – 11)</td>
<td>2.1</td>
<td>3.3 (&lt;2 – 6.2)</td>
</tr>
<tr>
<td>PFOS</td>
<td>9,906 (4,200–23,500)</td>
<td>38,667 (30,000–50,000)</td>
<td>2,540 (560 – 5,000)</td>
<td>260 (440 – 1,440)</td>
<td>1,040 (440 – 1,440)</td>
</tr>
<tr>
<td>PFHxA</td>
<td>ND</td>
<td>ND</td>
<td>2.8 (1.2 – 6.9)</td>
<td>2.1</td>
<td>4.8 (3.2 – 5.7)</td>
</tr>
<tr>
<td>PFHpA</td>
<td>ND</td>
<td>2 (&lt;2 – 2.5)</td>
<td>2.6 (&lt;2 – 4.4)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PFOA</td>
<td>ND</td>
<td>3.3 (2.3 – 4.5)</td>
<td>20.5 (18.2 – 23.2)</td>
<td>ND</td>
<td>1.7 (&lt;2 – 3.2)</td>
</tr>
<tr>
<td>PFNA</td>
<td>ND</td>
<td>4.7 (2.3 – 6.4)</td>
<td>7.6 (4.5 – 10.8)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PFDA</td>
<td>8.1 (4.3 – 13)</td>
<td>9.2 (6.3 – 12.3)</td>
<td>7.4 (2.4 – 15.1)</td>
<td>2.3</td>
<td>ND</td>
</tr>
<tr>
<td>PFuDA</td>
<td>28 (14 – 45.8)</td>
<td>33.6 (25 – 40.2)</td>
<td>26.6 (5.8 – 51.6)</td>
<td>2.5</td>
<td>2 (&lt;2 – 2.6)</td>
</tr>
<tr>
<td>PFDoA</td>
<td>1.9 (&lt;2 – 3.5)</td>
<td>3.4 (2.6 – 3.7)</td>
<td>14.8 (&lt;2 – 40.1)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6:2 FtS</td>
<td>3.6 (1.3 – 5.3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PFBS</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PFHxS e</td>
<td>11.8 (6.5 – 16)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PFDS e</td>
<td>16.5 (11 – 22)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PFTDA e</td>
<td>3.1 (1.6 – 4.8)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PFTeDA e</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PFOSA e</td>
<td>2.2 (1.7 – 2.8)</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>NEtFOSAA e</td>
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<tr>
<td>NMeFOSAA e</td>
<td>ND</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8:2 FtS e</td>
<td>25.4 (20 – 32)</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

n = number of specimens; - = not analysed; ND = Not Detected. Values are mean concentrations with the range provided in parenthesis.

This table is compiled from a Cardno Lane Piper (CLP 2013d) file note4 and spreadsheet (CLP 2013e) as supplied in email from Ashurst 15/08/2013 for sampling undertaken in December 2012 at Lake Fiskville. Measurements reported as less than the detection limit were assumed to be at half the detection limit for calculation of an average. Depending on the PFC, batch run or organism type, limits of detection were 0.5, 1, 2 or 5 ng/g. Note the units (ng/g) are as reported by the analytical laboratory, elsewhere in this report they have been converted to (mg/kg) for ease of comparison with other information.

Redfin data is for 21 specimens, but the calculated mean value includes laboratory duplicate and replicate samples for a maximum total of 34 results for PFOS, PFOA and some other PFC’s. In addition not all fish were analysed for all PFCs, so there may also be less than 21 values for calculating an average, see also Footnote ‘e’.

The information in CLP (2013d) is also available in CLP (2014a, b).
There were two fish with PFOS concentrations of about 23,000 ng/g that were analysed in the initial batch of 4 fish from Lake Fiskville, these were the largest of the redfin that were caught. These two fish were stored frozen and reanalysed by the same laboratory (NMI) some months later and returned concentrations of 15,000 ng/g; PFOS is very stable and freezing and thawing is not expected to result in degradation of PFOS, it may however change the matrix of the fish such that less interfering substances are co-extracted with PFOS. Recent developments for analysing PFCs in fish include a freezing step to enhance protein precipitation after tissue has been homogenised with extraction solvent (Malinsky 2009, Malinsky et al. 2011). In calculating the average all data has been used. The average without the additional analysis of the two fish is 8,260 ng/g. Additional information on the impact of the replicates of these fish is depicted in Figure 2.1.

A macrophyte is an aquatic plant (Potamogeton sp. is a species of pondweed).

These PFCs were only reported by AsureQuality in eight redfin samples used for inter-laboratory comparison. Averages for other PFCs include the results from both NMI (the primary analytical laboratory) and AsureQuality, with the exception of PFBA and PFPeA. The latter PFCs were only reported by NMI.

From the analysis of PFCs in water of Lake Fiskville and associated biota it is patent that PFOS is the PFC of potential concern. In comparison to concentrations of PFOS in Lake Fiskville and biota, the measured levels of other PFCs were not significant. Importantly PFOA was not detected in Redfin muscle.

Information obtained in consultation with CFA Fiskville personnel indicated anglers kept all redfin that were caught at the Lake, regardless of size. An examination of PFOS concentration in Redfin muscle with fish size shows only a weak correlation (Figure 2.1). This is consistent with other investigations which have found PFOS concentrations in fish muscle within or between species were not positively correlated with fish age or size (Becker et al. 2010, De Silva et al. 2011, Exponent 2011, Hoff et al. 2003, Martin et al. 2004, MPCA 2010, Murakami et al. 2011).

The concentration of PFOS in Redfin muscle, across a wide range of fish sizes (approximately 40 – 800g), is about 5,000 – 13,000 ng/g fish. These fish concentrations are approximately ten times higher than levels at which a number of international authorities have made recommendations the fish should not to be eaten (Dutch VWA 2008, German FIRA 2006, Alabama DoPH undated, Minnesota MDH 2008, Ontario MoE 2013). Fish advisories set by various authorities use quite conservative assumptions about lifetime patterns of exposure. Consuming fish with higher concentrations occasionally, or for a short period, does not automatically mean unacceptable health risk for the person, or that adverse health effects will occur. The fish advisories are discussed in more detail in Appendix F.

The availability of serum PFOS concentrations in persons who have acknowledged eating fish from Lake Fiskville negates the need to undertake a ‘traditional’ risk assessment based on PFOS fish concentrations and assumptions about how much fish, or eel, were caught and eaten. If such an assessment were to be done, it is the average PFOS concentration in the consumed flesh that is most

\[5\] This range excludes the two fish that initially analysed at approximately 23,000 ng/g but on re-analysis returned 15,000 ng/g.
appropriate to use for exposure estimations. However it is inappropriate\(^6\) to use a tolerable daily intake (TDI) for risk characterisation when exposure is known to be infrequent and potentially for just a few years (i.e. a small fraction of a lifetime).

2.3 Other substances in fish

In addition to PFCs, the initial four Redfin were also analysed for metals (arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc).

\(^6\) It is inappropriate to use the common risk characterisation method in these circumstances because the TDI is established on the assumption the food commodity is eaten every day for a lifetime (70 yrs). In the situation at Fiskville fish were eaten infrequently for relatively few years; averaging the total intake of PFOS over a life time dilutes the potential risk. For substances with long half-lives it is possible the total intake over a short period may increase body burden (measured as serum concentration) to levels potentially associated with changes in biomarkers of certain common diseases. This may not be recognised if intake was averaged over a life time in order to match the TDI. In addition marked uncertainty with regard to estimating intake of PFOS by persons at Fiskville via their historical fish consumption renders comparison with the TDI spurious.
Concentrations of arsenic, cadmium, chromium, lead and nickel in Redfin muscle were less than or marginally greater than the limit of reporting (0.01 or 0.05 mg/kg wet weight). Concentrations of copper (0.09 – 0.25 mg/kg ww) and zinc (3.4 – 4.3 mg/kg ww) were well within background concentrations in fish (Arellano et al. 1999, Zeynali et al. 2009, Jones et al. 2000) and below maximum residue limits (MRLs) for human consumption (APVMA 2013, EFSA 2012a).

Mercury in Redfin muscle ranged from 0.42 - 0.59 mg/kg wet weight (mean 0.48 mg/kg ww). The mean concentration in the four fish was just below the MRL of 0.5 mg/kg (FSANZ 2013). None of the participants in surveillance program had elevated blood mercury concentrations that were associated with eating fish from Lake Fiskville (Section 3.3).

3. Health surveillance program

3.1 Overview

For some time CFA have had a health surveillance program in place for its personnel. This was extended on a voluntary basis to all persons and their families who had eaten fish from Lake Fiskville, or had concerns about other possible exposure to PFCs at Fiskville. Entry into the program was not restricted to CFA personnel. Fiskville staff were informed verbally and by newsletter of the program, and advertisements were run in the local newspaper. People who thought they knew someone who might have eaten fish from Lake Fiskville were encouraged to inform them of the program, or give CFA hygiene staff their name so they may be contacted. Where possible these persons were contacted by telephone.

In addition to obtaining a blood sample for analysis of PFCs, all persons had additional blood taken for measurement of heavy metals and, as per the existing program, for haematology parameters and clinical chemistry screening that included tests for liver, kidney and thyroid function ⁷. A detailed list of

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⁷ The blood sampling program was coordinated by the Organisational Health & Wellbeing department of the CFA. Blood was obtained by a trained phlebotomist from a pathology laboratory engaged by the medical officer. The pathology laboratory also prepared serum and organised sample shipment to the laboratory measuring PFCs. Blood chemistry parameters and heavy metals were done using standard techniques employed by the pathology laboratory with results reported against the population reference range used by the laboratory.

Serum PFC analysis was undertaken by the National Measurement Institute (NMI). The method of determination was by High Performance Liquid Chromatography tandem Mass Spectrometry (HPLC-MS-MS). Prior to extraction the sample was spiked with a range of isotopically labelled surrogate standards followed by solid phase extraction. An aliquot of extract was injected onto the HPLC and separated PFCs detected and quantitated using mass spectrometry. Results were corrected for recovery of labelled surrogates. Included in batch analysis runs were calf serum matrix blanks that had, or had not, been spiked with known amounts of PFCs.
tests and the suite of PFCs looked for in serum is in Appendix C. Furthermore individuals had their medical history obtained and a general medical examination by the contracted medical officer. At the examination the medical officer made enquiries regarding medications they may be taking and when and how much fish they may have eaten from Lake Fiskville.

All persons entering the program were adults and agreed to have the results of their tests made anonymously available for evaluation. However as explained to all participants this was not a condition of entry into the program. Only the medical officer was aware of the identity of the people in the program, he presented the de-identified data to the consulting toxicologist, who with the medical officer interpreted the information.

3.2 Data interpretation

Information from the general medical screening part of the health surveillance program was evaluated as is usually done by medical practitioners. That is, an individual’s blood parameters were interpreted against population reference ranges in conjunction with their medical history and condition, the concomitant medical examination, and the medical expertise of the medical officer.

An important consideration is that clear adverse effects of PFOS have only been documented in animal studies and the effects are directly related to PFOS serum concentrations in the animals. When interpreting serum PFOS concentrations in the Fiskville cohort it also needs to be remembered that the measurement represents an aggregation of several modes of potential exposure. These include background exposure, possible past consumption of fish, and perhaps also historical exposure to firefighting foams that contained PFOS. Included in the cohort were some of the PAD operators.

To interpret the PFCs measured in the serum of program participants, two ‘indicator’ serum concentrations were constructed as comparators (see Appendix B for details). These comparison serum concentrations are:

1. **Background serum levels** usually present in adult populations (Appendix B.1). The PFCs are ubiquitous in the human environment and are found in serum as a result of day-to-day living. The majority of people are expected to have background serum concentrations of:

PFOS is the PFC of concern. Recovery of PFOS from spiked samples ranged from 6 – 124%. Although some recoveries (5 of 22 samples i.e. 23%) were below the ideal range (25 – 125%) of the laboratory, the laboratory considered PFOS to be suitably quantitated due to the inclusion of internal standards in the analysis. Relative Percentage Difference (RPD) of duplicate analysis (n = 2 of 22 analyses) for PFOS was 4 and 10%, this is considered to be acceptable.
2. A serum concentration that is without adverse effects, i.e. a serum no observed effect level (NOEL). Several lines of evidence are presented in Appendix B.2 that indicate a serum PFOS concentration of 2 mg/L (2,000 ng/mL)\(^8\) is a level at which, with current knowledge, it can be confidently stated no effects are likely to be observed in adult individuals. The evidence supporting this serum NOEL comes from:

a) Epidemiology studies in workers making or handling PFOS who individually have serum concentrations up to 13 mg/L (i.e. about 2 – 4 orders of magnitude higher than the mean levels for non-occupationally exposed populations).

b) NOELs observed in monkey and rat experiments where the animals have purposefully been administered high doses of PFOS. The very high serum concentrations produced allows determination of the potential effects of PFOS, the dose response, and the NOEL for the effects. These animal serum NOELs when converted to an equivalent human serum NOEL using the standard default uncertainty (safety) factors for deriving toxicity reference values from animal information give values of 3.3 – 4.4 mg/L. The process is briefly described below and in detail in Appendix B2.2.

In a six month monkey study the most sensitive effects were decreased serum cholesterol, decreased high density lipoprotein (HDL) and slightly decreased circulating total triiodothyronine (T\(_3\)) (Seacat et al. 2002, EFSA 2008). The lower 95% confidence limit on the benchmark dose (as serum concentration) is 35 mg/L (MDH 2008), this was divided by 2.5 to account for differences in toxicodynamics\(^9\) between monkey and human and 3.2 for toxicodynamic differences between humans as per the recommendations of enHealth (2012) and WHO (2004, 2010) to yield a human serum NOEL of 4.4 mg/L.

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\(^8\) Cross sectional epidemiology studies in communities affected by PFOA in drinking water have shown weak positive associations of relatively low PFOA serum concentrations (measured or predicted) with increased serum cholesterol and fatty acids in adults, kidney and testicular cancer and hypothyroidism in children. No such associations have been observed for PFOS. While PFOS and PFOA share a number of common toxicological properties there are also significant differences (primarily in tumourigenicity and reproductive/developmental toxicity, the latter being the most sensitive effect as determined from toxicological studies). Furthermore PFOA is not a substance of concern at Fiskville. It is therefore inadvisable to extrapolate toxicological or health information for PFOA either to PFOS or to the circumstances of PFC exposure at Fiskville.

\(^9\) The lower bound benchmark dose, as a serum concentration, (BMDL) is an outcome of mathematical modelling of the dose response (using either experimental serum concentrations or doses which are subsequently converted to serum concentrations). The BMDL is used in deriving guidelines and standards in a
From a variety of rodent studies the most sensitive effects of reduced pup weight at birth, neonatal weight gain and survival are found in rat two generation reproduction and developmental studies (Lau et al. 2003, Thibodeaux et al. 2003a, b; 3M Company 2003, Luebker et al. 2005a, b; Lau et al. 2007). The serum PFOS BMDLs (lower bound benchmark dose for 5% effect) for decreased neonatal weight gain was 26 – 31 mg/L and for reduced survival 83 – 100 mg/L. Applying the same toxicodynamic uncertainty factors to the lower serum concentrations (i.e. to the most sensitive effect) as for the monkey serum BMDL gives an equivalent serum NOEL for humans of 3.3 – 3.9 mg/L.

c) Conversion of the PFOS exposure guideline (the tolerable daily intake, TDI) established by the European Food Standards Authority (EFSA 2008) to a serum concentration using human toxicokinetic information (Appendix B2.3). The TDI is an intake in units of µg PFOS/kg body weight /day that is considered not to cause adverse effects to people exposed every day over their lifetime. The serum concentration associated with the TDI therefore represents a steady state concentration. Given that the half-life of PFOS in humans is 5.4 years (EFSA 2008), steady state serum concentrations will be achieved after approximately 20 – 27 years of daily exposure at the TDI (i.e. after 4 – 5 half-lives). Using standard one compartment pharmacokinetic equations for a daily dose at the EFSA TDI of 1.5 µg/kg/d yields a steady state serum concentration of 2 mg/L.

**In summary**, the interpretation of PFOS and PFOA measured in serum of persons at Fiskville has been achieved by:

1. Comparison with general population background serum concentrations where the majority of adults are for:
   - PFOS <0.1 mg/L.
   - PFOA <0.05 mg/L.

similar manner as the experimental NOEL but is considered to be a better estimate of the true NOEL than the experimental value (enHealth 2012, EFSA 2009, Gezondheidsraad 2003, US EPA 2012). Because the BMDLs for PFOS are expressed as serum concentrations that elicit the effect, the toxicokinetic processes that influence the serum concentrations associated with any given daily dose of PFOS are inherently incorporated into the assessment process. Thus only potential tissue responsiveness differences (i.e. toxicodynamic differences) need to be accounted for when converting an animal serum NOEL (i.e. the BMDL) to an anticipated human serum NOEL that can be used in risk assessment. This would not be the case if the BMDL’s were expressed as an external dose of mg/kg/d instead of an internal dose of mg/L serum. As applied in this risk assessment the NOEL serum concentrations relate to presumed steady state concentrations.
2. Comparison with a human NOEL of 2 mg/L for PFOS, derived from:
   a) Occupational epidemiology studies.
   b) NOELs in animal toxicology experiments for:
      o Reversible changes in blood cholesterol, lipids and thyroid hormone in monkeys.
      o Decreased neonatal weight gain from rat two generation and developmental studies.
   c) Conversion of the European Food Standards Authority (EFSA 2008) tolerable daily intake to an achieved steady state serum concentration.

In addition to comparison with the above PFOS ‘reference’ serum concentrations, margins of exposure (MOE) were calculated (Section 4.2).

The various human and animal data discussed above and in detail in Appendix B are summarised in Figure 3.1.
Figure 3.1: Summary of serum concentration (dose) - response for critical effects of PFOS in animals and humans. Also shown are adult background serum concentrations and the serum NOEL of 2 mg/L for humans. The latter is derived from occupational epidemiology studies, animal toxicology investigations, and the steady state serum concentration associated with the tolerable daily intake set by the European Food Standards Authority.
3.3 Health surveillance results

3.3.1 General considerations

In order to preserve the privacy of persons who participated in the health surveillance program only broad précises of the data are provided in this report.

Twenty two persons availed themselves of the surveillance program; just over half indicated they had eaten fish or eel from Lake Fiskville in the past. In terms of environmental epidemiology studies this is a very small number of persons potentially exposed to PFOS through eating fish. Accordingly it is difficult to draw general conclusions from the data. Much caution should be used when weighing up the information provided in this section.

Table 3.1 summarises some of the features of the people who joined the program. PFOS serum concentrations are discussed in Section 3.3.2.

There were slightly more males and females in the group that ate fish from Lake Fiskville as in the group that did not eat fish. The age range in each group is about the same.

However, for the people who had their body mass index (BMI) recorded, there were apparent differences in the BMI between the two groups (Figure 3.2). Overall only 2 people in the entire cohort had a BMI considered to be healthy, one fish eater and one non-fish eater. In the group that ate fish from Lake Fiskville, 42% had BMI’s considered to be in the obese range compared to 29% in the non-fish eating group. Importantly, BMI was not correlated with PFOS serum concentrations for either the whole cohort or just the fish eater group who do have higher serum PFOS levels (Table 3.1, Section 3.3.2, Figure 3.3).

Given the small group sizes no importance can be placed on the apparent differences in BMI between the two groups; it is likely to be a random finding. However whether a person is overweight or obese has implications for interpreting their individual clinical chemistry data.

10 In the group that indicated they ate fish there was a septuagenarian person who is not a current employee of CFA. The next oldest male in this group is in his early sixties.

11 The following BMI based health categories are for young and middle-aged adults (Vic Govt 2013):
   o 18.5 to 24.9 - healthy weight range (22-26 may be acceptable for older Australians).
   o 25.0 to 29.9 – overweight.
   o > 30 – obese.

12 The regression analysis equations for the correlation of BMI and PFOS serum concentration are:
   o All persons: \( y = -0.0068x + 30.9 \), \( R^2 = 0.013 \).
   o Fish eaters only: \( y = 0.0138x + 32.8 \), \( R^2 = 0.067 \).
Table 3.1: Summary of cohort characteristics.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Non-fish eaters a</th>
<th>Fish eaters a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total persons</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td># Females (~age range)b</td>
<td>4 (30 - 50 yrs)</td>
<td>5 (35 – 60 yrs)</td>
</tr>
<tr>
<td># Males (~age range)b</td>
<td>4 (45 - 50 yrs)</td>
<td>7 (50 – 70 yrs)</td>
</tr>
<tr>
<td>BMI: % overweight</td>
<td>57</td>
<td>50</td>
</tr>
<tr>
<td>BMI: % obese</td>
<td>29</td>
<td>42</td>
</tr>
</tbody>
</table>

a The ‘fish eater’ descriptor refers to whether or not an individual indicated they had eaten at any time, fish, eels or yabbies that were from Lake Fiskville. Personal data, including the age of the person, was inadvertently not collected for all people in each group. Consequently the numbers of males and females do not add up to the total persons.

b To preserve anonymity the age range has been rounded to the nearest 5 years.
3.3.2 PFC serum measurements

Of the 10 PFCs looked for in human serum (Appendix C) only two were present at measurable concentrations. These were PFOS and PFOA.

**PFOA:**

All PFOA measurements were approximately an order of magnitude less than the expected background concentrations of <0.05 mg/L (Section 3.2 and Appendix B.1) arising from day to day living. The data indicate fish consumption has not contributed to human PFOA serum concentrations. This is not unexpected since redfin (the fish being consumed) did not have measurable concentrations of PFOA in their flesh (Table 2.2).

**PFOS:**

A summary of the serum PFOS concentrations is at Table 3.2. Perhaps not surprisingly the average PFOS serum concentration in the group that self-reported to have eaten fish was higher than in the non-fish eating group, but not statistically significant\(^\text{13}\). Four persons had concentrations higher than the expected background concentration of <0.1 mg/L. The implications of the PFOS measurements are discussed in the risk characterisation section (Section 4).

<table>
<thead>
<tr>
<th></th>
<th>Non-fish eaters(^a)</th>
<th>Fish eaters(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum PFOS (mg/L)(^b)</strong></td>
<td>0.016</td>
<td>0.085</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range(^c)</td>
<td>&lt;0.005 – 0.07</td>
<td>0.002 – 0.4</td>
</tr>
<tr>
<td># persons &gt; background(^d)</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

\(^a\) The ‘fish eater’ descriptor refers to whether or not an individual indicated they had eaten at any time, fish, eels or yabbies taken from Lake Fiskville.

\(^b\) Of the suite of PFCs looked for in serum, only PFOS and PFOA were measureable. PFOA concentrations were all below background, consequently only PFOS serum concentrations are reported in this table. The PFC analysis method and QA/QC data are described in a footnote to Section 3.3.1. PFC concentrations are reported by the laboratory as ng/mL but for consistency within this report have been converted to mg/L.

\(^c\) To preserve anonymity the values provided have been rounded to one or two significant figures. Due to matrix effects the analytical limit of reporting (LOR) differs slightly between samples. For the entire cohort the LOR’s were 0.002 – 0.01 mg PFOS/L serum.

\(^d\) Background PFOS serum concentrations are expected to be <0.1 mg/L (Section 3.2 & Appendix B.1).

\(^\text{13}\) An unpaired t-test of unequal variances showed mean PFOS concentrations in fish eaters is not statistically different from that in non-fish eaters.
3.3.3 Blood chemistry results

Individual blood chemistry information was assessed by the medical officer who personally discussed them, together with the PFOS results with the person involved.

There were a number of persons, in both the fish eating and non-fish eating groups, that had some clinical chemistry parameters outside of the normal range that signalled increased risk of disease. There were also persons whose blood parameters were abnormal as a result of life style factors, existing disease, or medication. Where necessary the medical officer wrote a referral for the person to follow up with their own general practitioner.

For no individual were blood parameters related to their serum PFOS concentrations.

In addition regression analysis of blood parameters for the whole cohort, or for just the fish eaters, showed no trend association of any parameter with PFOS concentrations (Appendix D).

4. Risk characterisation

4.1 Comparison with referent serum concentrations

The two comparator serum concentrations used for risk characterisation in this report are 0.1 mg/L (a concentration which the majority of people are expected to be below if they are only exposed to background sources) and 2 mg/L (a concentration deemed to be without adverse clinical effects) (Section 3.2 and Appendix B).

As discussed in Section 3.3.2, all except four persons had PFOS concentrations less than 0.1 mg/L. Those who returned levels above the background concentration of 0.1 mg/L were at least 5 times less than the serum NOEL of 2 mg/L (Figure 4.1).

Unfortunately information is poor on how much fish or eel was eaten and how long ago. Consequently, due to the considerable uncertainty, it is not proper to construct exposure scenarios and attempt to predict by toxicokinetic modelling serum PFOS concentrations that may have arisen from eating fish. It is however germane to consider that sometime in the past an individual may have had higher serum PFOS concentrations than has been currently measured. However, there is no indication in the consumption information provided by the four persons who have higher than background PFOS levels that they ate more fish, or more frequently, in the past 5 – 10 years than in recent years. The difference between the current serum concentrations
Figure 4.1: Comparison of measured serum PFOS concentrations with critical levels in animals and humans. See Figure 3.1 for explanation of abbreviations.
and the serum NOEL and the calculated MOE’s (see Section 4.2) are sufficient to cater for this uncertainty. For example, if it is speculated that a person was once a consumer of fish from the Lake but stopped 5 – 6 years ago, then based on the highest current serum concentration measured in the surveillance program cohort that person’s PFOS level 5 – 6 years ago would still be less than half the serum NOEL. Similarly if a person stopped consuming fish from the Lake about 11 years ago their serum PFOS at that time, in order to give rise to the highest current serum concentration, would have been approximately 70% of the serum NOEL.

Based on these considerations there is low likelihood of adverse health effects having arisen, or arising from PFOS concentrations in these persons.

A letter from the consulting medical officer and toxicologist has been written to the CFA Chief Executive Officer expressing this opinion (Appendix E).

4.2 Margin of Exposure calculations

An additional technique commonly used for judging the potential health impact of chemical exposure is to calculate a margin of exposure (MOE) against NOELs derived from well conducted animal experiments (enHealth 2012, EFSA 2012b, WHO 2004, 2010). These studies are described in Appendices B2.2 and B.3. In Australia, public health risks that may arise from use of agricultural chemicals, veterinary chemicals applied to food producing animals, or from non-occupational exposure to industrial chemicals are deemed to be acceptable if the MOE, based on exposure dose, is equal to, or greater than 100 (APVMA 2006, NICNAS 2007). This MOE is informally based on the 10 x 10 fold safety factor15 widely used to account for uncertainty in intra- and inter-species differences in the effects of chemicals. It addresses toxicokinetic and toxicodynamic differences between animals and between humans. However since ‘exposures’ in the surveillance program are measured as serum concentration rather than the external applied dose, toxicokinetic variability between animals and humans is inherently assimilated into the MOE calculation when using serum concentrations. Hence the usual acceptable MOE of 100 needs to be adjusted to account for the inherent inclusion of toxicokinetic species differences in calculating the MOE. This is particularly the

14 The half-life of PFOS in humans is approximately 5.4 years (EFSA 2008). This is the time for the serum concentration to decrease by half. If the highest current PFOS serum concentration is 5 times less than the serum NOEL for humans (i.e. about 0.35 mg/L), then 5.4 years ago in the absence of further exposure the concentration would be around 0.7 mg/L (i.e. less than half the human serum NOEL). Eleven years ago the serum concentrations in this hypothetical person may have been 1.4 mg/L (70% of the serum NOEL of 2 mg/L).

15 The 10 x 10 safety factors (also called uncertainty factors) are firstly for interspecies differences (between animal and human) in toxicodynamics (tissue responsiveness) and toxicokinetics (chemical metabolism) respectively, these are 2.5 x 4 respectively, and secondly for interindividual differences between humans in toxicodynamics (3.2) and toxicokinetics (3.2) (enHealth 2012).
case for compounds such as PFOS which aren’t metabolised and whose distribution in the body is confined to extracellular water (i.e. primarily serum) and effects are directly related to serum concentrations. Thus an acceptable MOE based on serum measurement in humans and serum NOEL in animals would be 25 \((100 ÷ 4)\)\(^{16}\).

In this HRA, MOE’s for a number of toxicological end points identified in animal studies have been calculated. Developmental effects in rodents are the most sensitive ones observed in animal studies (Appendix B) and patently this endpoint is only germane for females of reproductive age. These persons are also therefore the most sensitive sub-population. Thus:

For males and females \(\geq 45\) years MOEs are calculated with:
- Serum NOELs (35 mg/L) from monkey experiments for the same blood parameters as evaluated in the health surveillance program (Seacat et al. 2002).
- Serum NOELs for sensitive effects in chronic toxicity studies.
  - 60 mg/L for production of liver adenomas in a two year bioassay (Butenhoff et al. 2012b, Thomford 2002, 3M Company 2003).
  - 45 mg/L for liver toxicity in a two year bioassay (Butenhoff et al. 2012b, Thomford 2002, 3M Company 2003).

For females \(\leq 45\) years (i.e. considered to be of reproductive age [DFG 2005, 2013]) MOEs are calculated
- As above, plus
  - 26 mg/L in maternal serum for decreased weight gain in offspring in two generation and/or developmental rodent studies (Luebker et al. 2005a, 2005b) (Appendix B).

In order that potential reproductive risk (low birth weight) is addressed to the extent possible, females of reproductive age (\(\leq 45\) years old) have been assessed as a separate group. There are currently only 3 persons in this category, but based on the current ages of females in the cohort, five and ten years ago there were potentially 6 and 8 females in the cohort who were \(\leq 45\) years old. Assuming these persons were eating fish from the Lake up to that time but stopped 5 or 10 years ago their serum PFOS concentrations would have to have been higher to account for the current measured concentrations. Using an approximation of the current maximum serum PFOS concentration that is

\[^{16}\text{In this calculation the divisor of 4 is the toxicokinetic uncertainty factor used in risk assessments and public health guideline setting that addresses toxicokinetic differences between animals and humans (i.e. the interspecies uncertainty factor, }AK_{UF}\text{) (enHealth 2012, WHO 2005). That is the toxicokinetic differences between humans (3.16), toxicodynamic differences between animals and humans (2.5) and toxicodynamic differences between humans have been retained (3.16) in the MOE for a total of 25.}\]
higher than actual for females, serum concentrations for hypothetical females eating fish 5 or 10 years ago have been estimated (Table 4.1).

All MOEs, except one, are larger than the acceptable MOE of 25 (Table 4.1). This indicates low potential for health effects, either now or in the past. The MOE that is lower than the acceptable value is for a theoretical female of reproductive capacity who, ten years ago, may have had serum concentrations markedly higher than the current maximum female concentration. Given that this MOE of 22 is only marginally less than acceptable, and the approximations that have been made in the MOE calculations, this MOE of 22 is not an indication of unacceptable risk at that time.

**Table 4.1: Margin of Exposures (MOEs) for current and assumed past serum PFOS concentrations**

<table>
<thead>
<tr>
<th>Person category</th>
<th>Approx max human serum conc (mg/L)</th>
<th>MOEs (Calculated against serum NOELs from animal studies)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Animal serum NOEL (Critical effect)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26 mg/L (Developmental - rodent)</td>
</tr>
<tr>
<td>Male or Female (&gt;45 yrs)</td>
<td>Current ~ 0.35 c</td>
<td>N/A f</td>
</tr>
<tr>
<td></td>
<td>5 yr ago ~ 0.7 d f</td>
<td>N/A f</td>
</tr>
<tr>
<td></td>
<td>10 yr ago ~ 1.4 d f</td>
<td>N/A f</td>
</tr>
<tr>
<td>Female b (≤ 45 yrs)</td>
<td>Current n = 3</td>
<td>All have &lt;&lt;0.1 mg/L.</td>
</tr>
<tr>
<td></td>
<td>5 yr ago n = 6</td>
<td>Current max for this group is ~ 0.3 mg/L so 5 yr ago ~ 0.6 mg/L d</td>
</tr>
<tr>
<td></td>
<td>10 yr ago n = 8</td>
<td>10 yr ago ~ 1.2 mg/L d</td>
</tr>
</tbody>
</table>

**Notes:**

a MOEs are calculated against a number of serum No Observed Effect Levels (NOEL) for a range of effects observed in animal toxicity studies (Appendix B.3). The acceptable MOE is ≥25 (see text).

b Females in the cohort who are currently of reproductive age (≤ 45 yrs old), or were so 5 or 10 years ago, are assessed against animal serum NOELs for reproductive effects (low birth weight of offspring) in addition to the other toxicological endpoints for males and non-reproductive capacity females. Using a high approximation of the uppermost current serum PFOS concentration in females, serum concentrations 5 or 10 years ago have been estimated using a serum half-life for PFOS of 5.4 years (EFSA 2008).

To preserve anonymity the serum concentrations in this table are not actual measured values. They are higher than those actually measured.
These concentrations are estimated from the appropriate approximate maximum concentration assuming a serum PFOS half-life of 5.4 years. Because the exposure patterns are not known they do not relate to a particular individual but rather are hypothetical concentrations, but nonetheless grounded in current PFOS serum measurements.

This MOE is marginally lower than the critical MOE for low risk of 25. Given the approximations in the MOE calculations this does not represent an unacceptable risk of low birth weight ten years ago.

Because the reproductive effect of concern is low birth weight, mediated by maternal serum PFOS concentrations it is not applicable (N/A) to calculate MOEs for males or females of non-reproductive capacity using an animal serum NOEL for this endpoint.

5. Conclusions

Serum PFC measurements were undertaken by a commercial laboratory that included appropriate blanks, PFC spikes and duplicate analysis of samples chosen randomly. While internal standard recoveries for some samples were lower than the range regarded as ideal by the laboratory, the data are still considered reliable for assessment of potential risk.

Twelve of the 22 participants in the ‘fish consumption’ health surveillance program indicated that they had eaten fish or eel from the Lake in the past. For no person in the surveillance program were there changes in blood clinical chemistry parameters that could be attributed to PFOS. While recognising the very small sample size limits confidence in the data interpretation, regression analysis of a priori individual blood parameters with serum PFOS levels for either the entire cohort or just those that ate fish indicated no associations. Nevertheless there were a number of individuals in both the fish eating and non-fish eating groups that had blood parameter measurements outside the population reference range. All these were attributed to life style factors (e.g. alcohol consumption), body mass index, existing disease, and/or medication (including non-compliance). Where appropriate the medical officer referred people to their own medical practitioner for follow up.

Of the 10 PFCs looked for in human serum (chosen for their presence in Lake water or fish) only two were present at measurable concentrations in the serum of program participants. These were PFOS and PFOA. All PFOA measurements were approximately an order of magnitude less than the expected background concentrations for this compound. This indicates fish consumption has not contributed to human PFOA serum concentrations; not unexpected since redfin did not have measurable concentrations of PFOA in their flesh. PFOA was therefore not considered further in the risk assessment.

The potential health impact of measured serum PFOS concentrations has been assessed using two comparator serum concentrations. The first being a background concentration where it is expected the majority of the population will be below. The second is a serum concentration at which no effects in humans are expected, termed the serum NOEL.
Four persons had serum PFOS concentrations above that identified as the higher end of the normal range expected from background (i.e. resulting from day to day living). All were below the serum NOEL indicating low risk for adverse health effects. Available information on fishing frequency suggests exposure patterns were unlikely to have been materially different in the past and so serum PFOS concentrations were also unlikely to be markedly different from those measured in the surveillance program.

The Margin of Exposure (MOE) estimations calculated using current measured serum PFOS concentrations and serum NOELs for sensitive toxicological endpoints identified from animal toxicity experiments also indicated very low risk for adverse health effects.

When current serum concentrations were extrapolated back to theoretical levels that may have existed 5 or 10 years previously, and assuming no further fish consumption, both comparison with the human serum NOEL and the calculated MOEs indicate adverse health effects were unlikely to have arisen due to the hypothetical serum PFOS concentrations.

Overall, it is concluded existing serum PFOS concentrations or past theoretical concentrations are unlikely to give rise to adverse health effects.

6. Uncertainty analysis

As with all human health risk assessments (HHRAs) there are uncertainties in this assessment that potentially affect the conclusions. They have been addressed either by conservative assumptions or inclusion of hypothetical exposure scenarios.

Exposure:

The major uncertainty in HHRAs usually resides with exposure estimations. In this HHRA much of the exposure ambiguity associated with determination of external dose is negated by use of serum PFOS concentrations as a measure of internal dose. The residual exposure uncertainty lies with the analytical measurement of PFCs in human serum. Since appropriate spiked matrix samples, blanks and duplicates were included in the analytical regime which all returned consistent, expected results uncertainty in the determination of current PFOS serum concentrations is considered to be minimal.

Current measurement of PFOS serum concentrations provides information allowing assessment of health impacts at the time of measurement and, because of the long serum half in humans, also in the recent past. However there is uncertainty regarding past PFOS serum concentrations. This has been
addressed by assuming no PFOS contaminated fish consumption for the past 5 or 10 years and extrapolating the maximum measured current PFOS serum concentration back to those times. While this theoretical past serum concentration may be under or over estimated it is our opinion it is more likely an overestimate of past serum levels.

Toxicological reference values and risk characterisation:
HHRAs often use toxicological reference values (e.g. TDI or RfD) established by competent authorities for judging the impact of the calculated external exposures. Since the exposure metric is serum concentration rather than dose such guidance values are inappropriate. The risk characterisation has been carefully undertaken using:

- Two comparator serum concentrations developed for this assessment, and
- with MOE calculations.

The latter not being reliant on assumptions made in the development of the comparator serum concentrations.

- The first PFOS serum comparator is a maximum PFOS serum concentration that might arise due to PFOS exposure in the general human environment (i.e. background exposures). More than 40 peer reviewed papers reporting blood/serum PFOS concentrations from around the world were included in this assessment. Care was taken not to include occupational exposures, populations near PFC manufacturing/handling facilities, or communities affected by PFC ground water contamination. We have a high degree of confidence that the majority (~95%) of people should have background PFOS serum concentrations <0.1 mg/L.

- The second PFOS serum comparator was the establishment of a serum concentration that would be expected to be without adverse health effects. To reduce the uncertainty in setting the human serum NOEL, three independent methods were employed (described in Appendix B). These were:
  - a NOEL from occupational epidemiology studies,
  - application of standard techniques for setting toxicological reference values using sensitive effects observed in monkeys and rats, and
  - using human toxicokinetic data to convert the TDI set by the European Food Safety Authority to an equivalent steady state serum concentration.

We have a high degree of confidence in the robustness of the human NOEL (2 mg/L) used in this assessment.
Cohort sample size:
The number of people entering the PFOS health surveillance program was small (22 individuals), with just over half of these reporting they had eaten fish from Lake Fiskville. Consequently there is uncertainty in making group deductions about the relationship between serum PFOS concentrations and any particular health parameter measured in the program. Nevertheless correlations have been constructed that show no association between the health parameters and serum PFOS for the group. Due to the small sample size these need to be interpreted with caution.

Possible risk to an individual was done according to standard medical practice using the expertise of the medical officer and the consultant toxicologist. While this advice is subject to the usual uncertainties associated with medical diagnosis it has been professionally provided and we are confident it has been appropriate for the circumstance of the individual(s).
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(This list includes references for the appendices in this report)


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German FIRA (2006). High levels of perfluorinated organic surfactants in fish are likely to be harmful to human health (in German). German Federal Institute for Risk Assessment. Statement No. 21/2006.


### Appendix A: Glossary

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate Aminotransferase</td>
</tr>
<tr>
<td>BMDL</td>
<td>Lower bound Benchmark Dose</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CFA</td>
<td>Country Fire Authority</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>GGT</td>
<td>Gamma Glutamyl Transferase</td>
</tr>
<tr>
<td>HDL</td>
<td>High Density Lipoprotein</td>
</tr>
<tr>
<td>HPLC-MS-MS</td>
<td>High Performance Liquid Chromatography Tandem Mass Spectrometry</td>
</tr>
<tr>
<td>HRA</td>
<td>Health Risk Assessment</td>
</tr>
<tr>
<td>LDL</td>
<td>Low Density Lipoprotein</td>
</tr>
<tr>
<td>MOE</td>
<td>Margin Of Exposure</td>
</tr>
<tr>
<td>NMI</td>
<td>National Measurement Institute</td>
</tr>
<tr>
<td>NOEL</td>
<td>No Observed Effect Level</td>
</tr>
<tr>
<td>PFC</td>
<td>Perfluorinated Compound</td>
</tr>
<tr>
<td>RPD</td>
<td>Relative Percentage Difference</td>
</tr>
<tr>
<td>SS</td>
<td>Steady State</td>
</tr>
<tr>
<td>T3</td>
<td>Triiodothyronine</td>
</tr>
<tr>
<td>T4</td>
<td>Thyroxine</td>
</tr>
<tr>
<td>TDI</td>
<td>Tolerable Daily Intake</td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid Stimulating Hormone</td>
</tr>
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</table>
## PFC Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>PFC</th>
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<tbody>
<tr>
<td>PFBA</td>
<td>Perfluorobutanoic acid</td>
</tr>
<tr>
<td>PFPeA</td>
<td>Perfluoro-n-pentanoic acid</td>
</tr>
<tr>
<td>PFBS</td>
<td>Perfluorobutane sulphonic acid</td>
</tr>
<tr>
<td>PFHXS</td>
<td>Perfluorohexanesulphonic acid</td>
</tr>
<tr>
<td>PFOS</td>
<td>Perfluoroctane sulphonic acid</td>
</tr>
<tr>
<td>PFDS</td>
<td>Perfluorodecane sulphonic acid</td>
</tr>
<tr>
<td>PFHxA</td>
<td>Perfluorohexanoic acid</td>
</tr>
<tr>
<td>PFHpA</td>
<td>Perfluorheptanoic acid</td>
</tr>
<tr>
<td>PFOA</td>
<td>Perfluoroctanoic acid</td>
</tr>
<tr>
<td>PFNA</td>
<td>Perfluorononanoic acid</td>
</tr>
<tr>
<td>PFDA</td>
<td>Perfluorodecanoic acid</td>
</tr>
<tr>
<td>PFUDA</td>
<td>Perfluoroundecanoic acid</td>
</tr>
<tr>
<td>PFDoA</td>
<td>Perfluorododecanoic acid</td>
</tr>
<tr>
<td>PFTrDA</td>
<td>Perfluorotridecanoic acid</td>
</tr>
<tr>
<td>PFTeDA</td>
<td>Perfluorotetradecanoic acid</td>
</tr>
<tr>
<td>PFOSA</td>
<td>Perfluorooctane sulphonamide</td>
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<tr>
<td>NETFOSA</td>
<td>N-ethyl-perfluorooctane sulphonamide</td>
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<tr>
<td>NETFOSAA</td>
<td>N-ethyl-perfluorooctanes sulphonamidoacetic acid</td>
</tr>
<tr>
<td>NMeFOSA</td>
<td>N-methyl-perfluorooctane sulphonamide</td>
</tr>
<tr>
<td>NMeFOSAA</td>
<td>N-methyl-perfluorooctane sulphonamidoacetic acid</td>
</tr>
<tr>
<td>NMeFOSE</td>
<td>N-methyl-perfluorooctane sulphonamidoethanol</td>
</tr>
<tr>
<td>NMeFOSE</td>
<td>N-methyl-perfluorooctane sulphonamidoethanol</td>
</tr>
<tr>
<td>4:2 FtS</td>
<td>1H,1H,2H,2H-perfluorohexane sulphonic acid</td>
</tr>
<tr>
<td>6:2 FtS</td>
<td>1H,1H,2H,2H-perfluorooctane sulphonic acid</td>
</tr>
<tr>
<td>8:2 FtS</td>
<td>1H,1H,2H,2H-perfluorodecane sulphonic acid</td>
</tr>
</tbody>
</table>
Appendix B: Determination of serum PFC concentrations for risk characterisation.

B.1 Background human PFC serum concentrations

A wide range of PFCs are found in consumer and industrial products. They are used to treat leathers and paper so they repel water and grease (e.g. grease proof paper, pizza boxes, popcorn, hamburger and chip containers, fruit boxes, etc), water proof shoes and textiles, make breathable water repelling fabrics (e.g. Cortex), apply stain resistance to carpets and furniture (e.g. Scotchguard). They are in a range of cosmetics and personal care products, and in some surface coatings. PFOS has been used in certain firefighting foams. In the environment or in the body many of the PFCs in these products breakdown or are metabolised to PFOS or PFOA. These are both very stable and are persistent in the environment and long lived in the body.

To identify background serum concentrations of PFOS and PFOA a literature search was undertaken for data in populations around the world that were not occupationally exposed, did not live near PFC manufacturing sources, and were not influenced by local contamination of groundwater or soil.

Figures B.1 and B.2 summarise background PFOS and PFOA concentrations in human serum from a large number of studies, the information is consolidated in Table B.1. Individual data for the studies was not available to statistically construct a ‘normal’ background reference range. However inspection of Figure B.1 compellingly indicates the majority of the general population would be expected to have a PFOS serum concentration less than 0.1 mg/L. This agrees with 3M Company (2003) and Olsen et al (2003b) who statistically calculated 95% of the general population have PFOS serum concentration less than 0.1 mg/L.

Similarly, Figure B.2 indicates the majority of persons would be expected to have less than 0.05 mg/L PFOA in their serum as a result of normal day-to-day living.

Table B.1: Summary of background PFC serum concentrations

<table>
<thead>
<tr>
<th></th>
<th>Population means</th>
<th>Range for individuals</th>
<th>Majority of individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOS</td>
<td>0.005 - 0.05</td>
<td>0 - 0.3</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>PFOA</td>
<td>0.0002 – 0.06</td>
<td>0 – 0.09</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

a Information in the table is a summary of that visually presented in Figures B.1 and B.2.

b It is expected from Figures B.1 and B.2 that the majority of individuals would have serum concentrations less than these values. These concentrations are therefore used as the upper end of ‘background’ serum concentrations for PFOS and PFOA.
Figure B.1: Serum PFOS concentrations in the general community

- **Mean**
- **95th percentile**
- Bars represent range (minimum and maximum)
Figure B.2: Serum PFOA concentrations in the general community

- Mean
- 95th percentile
- Bars represent range (minimum and maximum)
B.2 Human no effect serum concentration (PFOS)

B2.1 Occupational epidemiology studies
Workers who handle or make PFCs have much higher serum PFOS concentrations than the general population; they also tend to have higher PFOA levels. In such workers PFOS concentrations may be as high as 12 - 13 mg/L, but the majority are <6 mg/L (Olsen et al. 1999a, 2003a, 2003f) (Figure AB.1). These levels of exposure are primarily confined to three manufacturing plants in the US and Belgium. Over more than a decade several occupational epidemiology studies have been undertaken on this cohort. The studies have primarily focussed on the *a posteriori* toxicological knowledge gained from monkey and rodent studies; the most sensitive effects being decreased cholesterol and circulating thyroid hormones which are totally reversible when serum concentrations decrease (Section B2.2). At higher serum concentrations (BMDL\textsubscript{10} 60 mg/L) in 2 year rat experiments liver adenomas are observed (PFOS is not genotoxic) and in developmental and multi-generation studies PFOS causes decreased pup weight and neonatal survival (BMDL\textsubscript{5} pup weight 31 mg/L, BMDL\textsubscript{5} perinatal mortality 83 mg/L) (Butenhoff et al. 2012b, Thomford 2002, 3M Company 2003). Potential effects investigated in the epidemiology studies included thyroid and lipid metabolism disorders, mortality, cancer incidence, liver, cardiovascular and gastrointestinal diseases, and pregnancy outcomes (Alexander et al. 2003; Alexander and Olsen 2007; Olsen 1999a, 2003a, 2004c; Grice et al. 2007).

There were no changes in haematological, lipid, hepatic, thyroid, or urinary parameters consistent with the known toxicological effects of PFOS in cross-sectional or longitudinal analyses of workers who had PFOS serum levels < 2 mg/L. At concentrations higher than 6 mg/L slight positive associations with altered cholesterol, triglyceride and high density lipoprotein have been reported but these are inconsistent with the known biochemistry of PFOS and the effects observed in animals, including monkeys. Consequently these associations should be interpreted with care, they may be random findings, or due to a different variable other than PFOS.

Although an initial study reported an association between PFOS and urinary bladder cancer (Alexander et al. 2003) this was based on just three cases, when the study was expanded with more accurate exposure measures and confounders controlled, no association between PFOS and bladder cancer was apparent\textsuperscript{17} (Alexander and Olsen 2007). No changes in other endpoints investigated have been reported.

\textsuperscript{17} A chemical or biological basis for induction of bladder cancer by PFOS is obscure. It does not appear to have the properties of known bladder carcinogens and has not shown any bladder effects in toxicology studies. It is neither genotoxic nor insoluble in urine at room temperature.
The overall size of the occupational cohort is greater than 3,500 however it was smaller subgroups that were investigated in the epidemiology studies, with 100 – 300 persons in any particular exposure strata. As with many cross sectional epidemiology investigations, the individual studies are open to criticism. Some of these are study design, lack of control for certain confounders, participation being

**Figure AB.1: Serum PFOS concentration in workers at two manufacturing plants (Decatur, Alabama and Antwerp, Belgium).**

Production of perfluorinated sulphonated compounds began in Decatur in 1961 and Antwerp in 1976. PFOS measurements started in the early 1990’s when specific analytical techniques became available. The total number of persons who have been exposed and studied in these factories is greater than 3,500. Adverse effects in workers have not been reported at serum concentrations < 2mg/L, this is taken to be a No Observed Effect Level (NOEL) for adults.

The data in the figure has been compiled from the following publications which have studied various sectors of the worker population. The PFOS serum concentrations are the geometric or arithmetic means of the study population, with either the 95% confidence limit (CL) of the mean or the range of serum concentrations when reported.

voluntary rather than random recruitment, uncertainty in assignment to an exposure group based on job description and years of service with serum PFOS bands allocated by measurement of workers with similar job task profiles. Nevertheless the cohort represents the most highly exposed humans in the world. Overall PFOS serum levels in individual workers are up to 4 orders magnitude greater than the population means of the general public, the lowest occupational sub-cohort is approximately 1 - 2 orders greater. Thus, if humans are susceptible to the adverse effects observed to be induced by high serum PFOS in animals, they would be expected to be detected in this occupational cohort. As per the philosophy of administering high doses of chemical to small groups of animals to identify hazards, the high serum concentrations in workers counters the less than ideal number of subjects in the occupational epidemiology studies.

**Conclusion:**
From the occupational epidemiology information it is concluded that a serum PFOS concentration of 2 mg/L represents a level at which no effects have been observed in adults. The actual no effect level may be higher than this but there are insufficient numbers of persons with concentrations around this level for implications to be drawn.
B2.2 Animal serum PFOS no observed effect level (NOEL)

The procedures employed in this section of Appendix B for deriving human serum NOELs are part of standard risk assessment methodologies for setting toxicity reference guideline values used by WHO, the EC and recommended in Australia (WHO 2004, 2010; enHealth 2012).

In general, observations from toxicological studies with PFOS include reductions in body-weight and weight gain, increases in liver weight (characterised by increased centrilobular hepatocellular hypertrophy), mild-to-moderate peroxisome proliferation in rats, increased incidence of hepatocellular adenoma\(^{18}\) in rats, and hypo-cholesterolemia. Effects appear to be related to a threshold body burden and often are associated with a steep dose–response.

The mechanisms of PFOS induced toxicity are not fully understood but may include effects on fatty acid transport and metabolism, membrane function, and/or mitochondrial bioenergetics. Cumulative toxicity, occurring at high serum concentrations, is expressed as metabolic wasting in adult experimental animals, decreased neonatal survival and weight gain in offspring. Sensitive effects are observed in monkey studies which provide serum concentrations for changes in blood biomarkers for potential effects on lipid metabolism and energy production. Developmental and 2-generation reproduction studies in rats deliver benchmark doses (BMD and BMDL) for conversion to serum concentrations for the sensitive effects of neonatal survival and weight gain.

**Monkey:**
The pivotal study for PFOS is a 28 week oral (0, 0.03, 0.15 & 0.75 mg/kg/d via capsule) study in cynomolgus monkeys (Seacat et al. 2002). A range of blood parameters and serum PFOS concentrations were monitored throughout the study and during a one year recovery period. At serum concentrations not causing overt toxicity (approximately 60 – 100 mg/L) the primary findings are changes in biochemical parameters associated with lipid metabolism. The animals show increased liver weight and decreases in body weight, together with decreased cholesterol and high density lipoprotein (HDL), decreased triglycerides and thyroid hormone (T3) (without marked compensatory increase in TSH). These changes have been shown to be readily and completely reversible within 30 weeks of treatment cessation as serum concentrations decrease.

For each of the doses and sampling times serum PFOS was measured. Dose response modelling gave a BMDL serum concentration of 35 mg/L for no or minimal impact on sensitive effects in the liver

\(^{18}\) Hepatocellular hypertrophy and liver adenomas induced in rats by PFOS are mediated through the non-genotoxic mechanisms of PPAR\(\alpha\) and CAR activation and are considered irrelevant modes of action for human risk assessment (Klaunig et al. 2003, Elcombe et al. 2012a, 2013).
(decreased cholesterol) (MDH 2008). The fact that this serum concentration is the low 95th confidence limit estimate means it is conservative and is taken as the no observed effect level (NOEL).

Supporting use of the serum concentration in monkeys as a surrogate for the human internal dose to the target tissue is the liver:serum ratios being similar in monkeys and humans. These ratios are 1.4 and 1.3 respectively (Olsen et al. 2003c, Seacat et al. 2002). There is however undefined uncertainty with regard to the responsiveness of monkey and human liver to the same internal dose (serum concentration) of PFOS. This is despite the majority of hepatic effects being mediated via the PPARα receptor, and humans and monkeys being approximately equally sensitive to its activation by peroxisome proliferators (Cariello et al. 2005, FDA 2005, Kane et al. 2006). To account for human liver possibly being more sensitive than that of monkeys (i.e. for interspecies toxicodynamic differences), the standard default uncertainty factor of 2.5x has been applied to the NOEL of 35 mg/L. In addition the usual default for response variability (toxicodynamic) between humans (3.2x) has been added.

The total uncertainty factor applied to extrapolate the monkey NOEL serum concentration is therefore 8x and the equivalent human serum NOEL derived from the monkey BMDL10 of 35 mg/L is 4.4 mg/L.

Rat:
In rat toxicity studies the most sensitive effect is decreased pup weight gain observed in two generation reproduction experiments (Lau et al. 2012; 3M Company 2003; Luebker 2005a, 2005b; Thomford 2002).

BMDL₅ on pup weight gain is 26 mg/L – 31 mg/L (3M Company 2003) and pup survival 83 mg/L (Lau et al. 2007). The 26 mg/L is derived from data from the two-generation reproduction/developmental study (pup weight gain through lactation) (Luebker et al. 2005a) and the serum PFOS concentration measurements made in a separate toxicokinetic study during pregnancy at the same dose levels (Luebker et al. 2005b). The 31 mg/L is for in reduced pup weight gain during lactation using the mean of gestation day 21 and pre-gestational serum levels in dams (Luebker et al. 2005b).

Lau et al. (2007) is a review of the toxicology of perflouroalkyl acids, primarily PFOS and PFOA. In this review ‘no effect’ doses [i.e. the BMD and BMDL as reported by Luebker et al. (2005b) and Lau et al. (2003)] were translated into equivalent no effect serum concentrations using linear relationships between dose (mg/kg) and serum concentration (mg/L). Thus Lau et al. (2007) converted the BMD₅ and BMDL₅ of:

- 1.06 and 0.89 mg/kg/d from Luebker et al. (2005b) into serum concentrations of 67 and 59 mg/L for postnatal survival at lactation day (LD) 5, and
1.07 and 0.58 mg/kg/d from Lau et al. (2003) for postnatal survival to day 8 were translated into serum concentrations\(^{19}\) of 25 and 16 mg/L.

Unfortunately Lau et al. (2007) did not fully consider the serum data reported in these studies and the animal serum BMDs derived by this author are not the most appropriate for defining serum concentrations for deriving human equivalent serum NOELs for PFOS. It is also noted that Lau et al. (2007) only considered neonatal survival and not the more sensitive endpoint of decreased birth weight and weight gain. The studies and derivation of suitable human serum NOELS are described below.

The Luebker et al. (2005b) study:

Luebker et al. (2005b) dosed rats at 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg PFOS/kg/d for 42d prior to mating and through to gestation day 20, or LD 4 depending on the study phase. The BMDL\(_5\) based on decreased gestation length, birth weight, pup weight at LD 5, pup weight gain through LD 5, and pup survival through LD 5 were relatively tight at 0.31, 0.39, 0.27, 0.28, and 0.89 mg/kg/day, respectively. There is a steep dose–response relationship that begins to appear between 0.8 and 1.2 mg/kg before becoming statistically significant at 1.6 mg/kg. According to Luebker et al. (2005b) this observation, together with other reports in the literature (Lau et al. 2003; Luebker et al. 2005a), suggests a critical body burden in dams is required to influence viability in neonates. In the Luebker et al. (2005b) study maternal serum concentrations on gestation days 1, 7 and 15 were relatively constant indicating the animals were at steady state after 42 days of dosing prior to mating. However there was a 40 – 60% decrease in maternal serum concentrations at gestation day 21. The decline may have been the result of increased volume expansion and other physiological changes during the last trimester, including changes in serum protein content. Patently, post gestational serum concentrations do not reflect the potential extent of foetal exposure during pregnancy.

Lau et al. (2007) converted the BMD\(_5\) and BMDL\(_5\) of 1.06 and 0.89 mg/kg/d as determined by Luebker et al. (2005b) for pup survival at LD 5 into equivalent serum concentrations using the linear association between dose and maternal serum concentration at gestation day 21. As noted above there is a substantial decrease in serum concentrations between the steady state concentrations up to gestation day 15 and concentrations measured on gestation day 21. It would appear that the dose-

\(^{19}\) Although both Luebker et al. (2005b) and Lau et al. (2003) have modelled similar BMD’s from their data (1.06 and 1.07 mg/kg/d respectively), Lau et al. (2007) derived corresponding serum concentrations that are very different from each other, i.e. 59 and 16 mg/L respectively.
serum concentration relationship at steady state is a better indication of foetal exposure. This relationship yields a regression equation of \( y = 85.656x + 6.8086 \) \((r^2 = 0.9949)\) \(^{20}\); and at a BMDL of:

- 0.89 mg/kg/d for pup survival at LD 5, the maternal steady state serum concentration is 83.1 mg/L.
- 0.28 mg/kg/d for pup weight gain through to LD 5, the maternal steady state serum concentration is 30.9 mg/L. Thus pup weight gain is the more sensitive indicator.

The Lau et al. (2003) study:

Lau et al. (2003) treated rats with 1, 2, 3, 5 and 10 mg PFOS/kg/d on gestation days 2 to 21. In this study there was decreased pup survival and in survivors decreased weight gain. While serum and liver concentrations of pups after birth were measured, serum PFOS in the dams was not. There was a decrease in pup survival at and above 2 mg/kg. The BMD\(_5\) and BMDL\(_5\) were 1.07 and 0.58 mg/kg/d for postnatal survival to day 8. Since Lau et al. (2003) did not report maternal serum PFOS concentrations, Lau et al. (2007) used the maternal serum concentrations at gestation day 21 from Thibodeaux et al. (2003a, b) to convert the Lau et al. (2003) BMDs to equivalent serum maternal concentrations. Rats in Lau et al. (2003) and Thibodeaux et al. (2003a, b) were given the same PFOS dose regime.

Thibodeaux et al. (2003a, b) is a developmental investigation in which skeletal variations occurred in the presence of decreased maternal weight gain. The graphical data in Thibodeaux et al. (2003a) indicates the maternal PFOS serum concentrations are steeply rising for most doses at gestation days 7 and 14 when serum was drawn. This indicates serum PFOS concentrations were not at steady state. Indeed the serum concentrations were markedly less than reported in Luebker et al. (2005b) despite the fact the doses were approximately 5 times higher. Nevertheless, as observed in Luebker et al. (2005b) maternal serum concentrations were somewhat lower at gestation day 21 than at day 15, particularly for the top three doses. Although Lau et al. (2003) and Thibodeaux et al. (2003a, b) are reporting different aspects of the same study, because the dose regime was short and there were marked changes in maternal serum PFOS concentrations between days 14 and 21 it is very difficult to determine the serum concentrations that may be associated with the effects observed in Lau et al. (2003).

\(^{20}\) This correlation is stronger than the \(R^2\) of 0.862 reported by Lau et al. (2005b) using the gestation day 21 serum data of Luebker et al. (2005b). The data for the correlation is provided in table below:

<table>
<thead>
<tr>
<th>Premating dose (mg/kg/d)</th>
<th>15-d serum concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>8.81</td>
</tr>
<tr>
<td>0.4</td>
<td>41.4</td>
</tr>
<tr>
<td>1.6</td>
<td>156</td>
</tr>
<tr>
<td>3.2</td>
<td>275</td>
</tr>
</tbody>
</table>
Conclusions:

For pup survival and weight gain the data of Luebker et al. (2005b) is preferred over Lau et al. (2003) because of the longer dose time (42d vs 19d), lower doses employed, serum concentrations are reported as values that can be used in independent analysis, and the serum and effects data are consistent with the two generation reproduction study (Luebker et al. 2005a). Thus the favoured serum NOELs (as BMDLs) for rat neonatal survival and decreased neonatal weight gain are 83 and 31 mg/L respectively.

The appropriate BMDLs for deriving a human serum PFOS no observed effect level from 2-generation and developmental studies are:

- 26 – 31 mg/L for reduced pup weight gain.
- 83 – 100 mg/L for reduced neonatal survival.

Applying the same uncertainty factors (i.e. 2.5x for interspecies toxicodynamic differences and 3.2x for toxicodynamic variability between humans) to the most sensitive reduced pup weight gain BMDLs of 26 – 31 mg/L as for the monkey serum BMDL gives an equivalent NOEL for humans of 3.25 – 3.9 mg/L.

In summary:

- The equivalent human serum NOEL from the monkey investigation of Seacat et al. (2002) is 4.4 mg/L.
- The human serum LOEL from rat reproduction and developmental studies in which the most sensitive effect was decreased weight gain of neonates is 3.25 – 3.9 mg/L.
B2.3 Conversion of TDI to serum concentration

Four TDIs for PFOS have been established by international authorities:

- The UK Committee on Toxicity (COT 2006): 0.3 µg/kg/d.
- The European Food Standards Authority (EFSA 2008): 0.15 µg/kg/d.
- The Minnesota Department of Health (MDH 2008): 0.08 µg/kg/d.
- US Environmental Protection Authority (US EPA 2009): 0.08 µg/kg/d.

They have all based their deliberations on the 26 week oral monkey study by Seacat et al. (2002) described in Appendix B2.2 but have arrived at different TDI values as a result of different methodologies, different uncertainty factors and/or different science policy.

Generally Australian authorities have a preference for World Health Organisation and European deliberations because these tend to match science policy and risk assessment methods used in Australia more closely than those in North America. Thus Food Standards Australia New Zealand (FSANZ 2011) refer to the ESFA TDI when they reported the results of a survey of chemical migration, including PFCs, from food contact packaging materials into Australian food. In this assessment the TDI of 0.15 µg/kg/d from EFSA (2008) has been adopted. Furthermore it is noted that the average of all the above TDIs is 0.15 µg/kg/d.

The TDI is an estimate of the amount of a contaminant or natural toxicant, expressed on a body weight basis that can be ingested daily over a lifetime without appreciable risk. Thus the long term serum concentrations associated with this dose are steady state concentrations.

The standard pharmacokinetic equation (Birkett 1999) used in medicine to calculate steady state blood concentrations is:

$$C_{SS} = (DR \times t_{1/2}) ÷ (0.693 \times Vd)$$

Where:

- $C_{SS}$ = Steady state serum concentration.
- $DR$ = Dose Rate. In this case 0.15 µg/kg/d (0.00015 mg/kg/d)
- $t_{1/2}$ = Serum half-life (1971 days, EFSA 2008, Olsen et al. 2007, DFG 2010).
- $Vd$ = Apparent volume of distribution is extracellular water (0.2 L/kg bw, Olsen et al. 2007, DFG 2010, Chang et al. 2012).

Substituting values into Equation B1

$$C_{SS} = (0.00015 \times 1971) ÷ (0.693 \times 0.2) = 2.13 \text{ mg/L}$$

Thus the steady state serum concentration of PFOS associated with a TDI of 0.15 µg/kg/d is 2 mg/L (rounded).
B.3 Studies supporting margin of exposure calculations

The serum NOELs used in the calculation of MOEs in Section 4.2 are:

- 26 mg/L in maternal serum for decreased weight gain in offspring in two generation and/or developmental rodent studies (Luebker 2005a, 2005b).
- 35 mg/L from monkey experiments for the same blood parameters as evaluated in the health surveillance program (Seacat et al. 2002).
- 45 mg/L for liver toxicity in a two year bioassay (Thomford 2002, 3M Company 2003, Butenhoff et al. 2012b).
- 60 mg/L for production of liver adenomas in a two year bioassay (Thomford 2002, 3M Company 2003, Butenhoff et al. 2012b).

The Luebker (2005a, 2005b) and Seacat et al. (2002) studies, with the identification of the serum NOELs, are described in Appendix B2.2.

The two year bioassay supporting serum NOELs for chronic liver toxicity and induction of liver adenomas is described below. The study was sponsored by 3M, conducted at Covance Laboratories Ltd under good laboratory (GLP) standards, with the report authored by Thomford (2002). The laboratory report is not publically available but was submitted to EFSA as part of a data package for the PFOS/PFOA review that was being undertaken. EFSA (2008) describes the essential features of the study. The terminal pathology obtained in the study was reported at a toxicology science conference (Seacat et al. 2002b). The 3M Company (2003), in consultation with independent toxicologists, used data from the study to model serum concentration and effects, with the objective of determining serum PFOS concentrations equivalent in status to the lower confidence limit of a benchmark dose for 5% response for liver toxicity (i.e. a serum BMDL₅) or 10% incidence of liver adenomas (i.e. a serum BMDL₁₀). Sometime after this work was completed, Butenhoff et al. (2012b), with Thomford as co-author, published the study in a peer reviewed journal. The description of the study below is primarily derived from Butenhoff et al. (2012b).

The two-year dietary toxicity and cancer bioassay was conducted with potassium PFOS in male and female Sprague Dawley rats. Dietary concentrations were 0, 0.5, 2, 5, and 20 µg/g (ppm). Included in the study was a recovery group that was fed 20 ppm for the first 52 weeks, after which they were fed control diet through to study termination. Scheduled interim sacrifices occurred on Weeks 4, 14, and 53, with terminal sacrifice between Weeks 103 and 106. The PFOS dietary treatment appeared to be well-tolerated, however there were sporadic decreases in body weight during the treatment period that were not clearly dose related. Interestingly male rats had a statistically significant decreased mortality with significantly increased survival to term at the two highest treatment levels. Decreased
serum total cholesterol, especially in males, and increased serum urea nitrogen were consistent clinical chemistry observations that were clearly related to treatment. The reduced serum total cholesterol, seen at earlier time points, was no longer apparent after 104 weeks of treatment. This may have been due to lower liver PFOS concentrations compared to earlier time points.

The principal non-neoplastic effect included liver hypertrophy, with proliferation of endoplasmic reticulum. The effect was dose related from 5 ppm upward. This was also evident in the 20 ppm recovery group, probably as a result of sufficient PFOS being retained in the liver to stimulate PPAR and CAR receptors. In males there were also increased serum enzymes indicative of liver toxicity. Statistically significant increases in benign hepatocellular adenoma\textsuperscript{21} were observed in surviving males and females of the 20 ppm treatment group. There were no treatment-related findings for thyroid tissue and no evidence of kidney or bladder effects.

Butenhoff et al. (2012b) determined dietary doses corresponding to the estimated BMDL\textsubscript{10} for liver adenomas was 7.9 ppm for male rats and 8.0 ppm for female rats. Aging of animals, characterised by progressive nephritis, resulted in high variability in PFOS serum and liver concentrations of PFOS beyond week 53, PFOS concentrations were somewhat less at week 105. At week 53 serum concentration data was only obtained for the controls and high dose (20 ppm) group. BMDL\textsubscript{10} values expressed as serum PFOS concentration after 14 weeks of dosing were 62 µg/mL and 92 µg/mL respectively for male and female.

Butenhoff et al. (2012b) did not determine serum BMDL for liver toxicity, however 3M Company (2003) report a serum BMDL\textsubscript{5} of 44 mg/L in male rats for non-neoplastic liver effects, and BMDL\textsubscript{10} of 62 mg/L for liver tumours. These values have been used in calculation of MOEs for these endpoints.

\textsuperscript{21}Hepatocellular hypertrophy and liver adenomas induced in rats by PFOS are mediated through the non-genotoxic mechanisms of PPAR\(\alpha\) and CAR activation and are considered irrelevant modes of action for human risk assessment (Klaunig et al. 2003, Elcombe et al. 2012a, 2013).
Appendix C: Program surveillance tests

1. **Full blood examination:**
   a. Haemoglobin
   b. Packed cell volume (PCV)
   c. Red cell count (RCC)
   d. Mean cell volume (MCV)
   e. Mean cell haemoglobin (MCH)
   f. Red cell distribution width (RDW)
   g. White cell count (WCC)
   h. Platelets

2. **Blood lipids:**
   a. Total cholesterol
   b. Triglyceride
   c. HDL cholesterol
   d. LDL cholesterol

3. **General biochemistry (serum):**
   a. Sodium
   b. Potassium
   c. Chloride
   d. Bicarbonate
   e. Urea
   f. Estimated glomerular filtration rate (GFR)
   g. Creatinine
   h. Total bilirubin
   i. Alanine aminotransferase (ALT)
   j. Aspartate aminotransferase (AST)
   k. Alkaline phosphatase (ALP)
   l. Gamma glutamyl transferase (GGT)
   m. Total protein
   n. Albumin
   o. Globulin
   p. Urate

4. **Thyroid function (serum):**
   a. Free thyroxine (FT4)
   b. Thyroid stimulating hormone (TSH)
c. Free triiodothyronine (FT3)

5. Other (serum):
   a. Glucose
   b. Creatine kinase (CK)
   c. Prostate specific antigen (PSA)

6. Metals (blood):
   a. Mercury
   b. Cadmium
   c. Lead
   d. Copper
   e. Arsenic

7. Physical examination:
   a. Height
   b. Weight

8. PFCs in serum (see Table C.1 for suite of PFCs)

Table C.1: Suite of PFCs that were analysed in serum

<table>
<thead>
<tr>
<th>PFC</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfluoro-n-pentanoic acid</td>
<td>PFPeA</td>
</tr>
<tr>
<td>Perfluorohexanoic acid</td>
<td>PFHxA</td>
</tr>
<tr>
<td>Perfluoroheptanoic acid</td>
<td>PFHpA</td>
</tr>
<tr>
<td>Perfluoroctanoic acid</td>
<td>PFOA</td>
</tr>
<tr>
<td>Perfluorononanoic acid</td>
<td>PFNA</td>
</tr>
<tr>
<td>Perfluorodecanoic acid</td>
<td>PFDA</td>
</tr>
<tr>
<td>Perfluoroundecanoic acid</td>
<td>PFUdA</td>
</tr>
<tr>
<td>Perfluorododecanoic acid</td>
<td>PFDoA</td>
</tr>
<tr>
<td>Perfluorooctanesulphonic acid</td>
<td>PFOS</td>
</tr>
<tr>
<td>1H,1H,2H,2H-perfluorooctanesulphonic acid</td>
<td>6:2 FtS</td>
</tr>
</tbody>
</table>
Appendix D: Regression analysis of blood parameters with PFOS levels.

**Lipids**

**Cholesterol (♦) & Triglycerides (■)**

**All persons**

- $y = 0.0019x + 4.8105, R^2 = 0.043$
- $y = -0.0025x + 1.8288, R^2 = 0.053$

**Fish eaters only**

- $y = 0.0025x + 4.614, R^2 = 0.084$
- $y = -0.0017x + 1.6258, R^2 = 0.051$

**LDL (×) & HDL (▲)**

**All persons**

- $x = 0.0008x + 2.7482, R^2 = 0.007$
- $y = 0.0023x + 1.2313, R^2 = 0.174$

**Fish eaters only**

- $x = 0.0008x + 2.672, R^2 = 0.009$
- $y = 0.0025x + 1.1969, R^2 = 0.284$
Thyroid function

Free T4 (◦) & Free T3 (■)

All persons

Ref. range FT4 (pmol/L)

Fish eaters only

Ref. range FT3 (pmol/L)

y = 0.0043x + 15.243, R^2 = 0.037
y = -0.0045x + 5.6173, R^2 = 0.473

TSH (▲)

All persons

Ref. range TSH (mIU/L)

Fish eaters only

Ref. range TSH (mIU/L)

y = 0.0004x + 1.4776, R^2 = 0.00007

y = 0.0062x + 14.685, R^2 = 0.107
y = -0.0047x + 5.6627, R^2 = 0.509

y = 0.0015x + 1.1117, R^2 = 0.056
**Liver function**

**ALT (♦) & AST (☐)**

**All persons**

- PFOS (ng/mL)
  - \( y = -0.0174x + 37.681, R^2 = 0.006 \)
  - \( y = 0.0244x + 26.787, R^2 = 0.023 \)

**Fish eaters only**

- PFOS (ng/mL)
  - \( y = -0.0411x + 43.809, R^2 = 0.036 \)
  - \( y = 0.013x + 29.816, R^2 = 0.007 \)

**ALP (▲) & GGT (✗)**

**All persons**

- PFOS (ng/mL)
  - \( ▲ y = -0.0475x + 85.455, R^2 = 0.053 \)
  - \( ✗ y = -0.0668x + 50.766, R^2 = 0.026 \)

**Fish eaters only**

- PFOS (ng/mL)
  - \( ▲ y = -0.0838x + 96.337, R^2 = 0.289 \)
  - \( ✗ y = -0.0848x + 56.334, R^2 = 0.053 \)
**Glucose (♦️)**

- **All persons**
  - Equation: \( y = -0.0015x + 5.878, R^2 = 0.003 \)
  - Reference Glucose (mmol/L)

- **Fish eaters only**
  - Equation: \( y = -0.005x + 6.8493, R^2 = 0.033 \)
  - Reference Glucose (mmol/L)

**Urate (🔺)**

- **All persons**
  - Equation: \( y = -0.0001x + 0.3652, R^2 = 0.013 \)
  - Reference Urate (mmol/L)

- **Fish eaters only**
  - Equation: \( y = -0.00004x + 0.3402, R^2 = 0.003 \)
  - Reference Urate (mmol/L)
Appendix E: Letter to CFA CEO

Mr Mick Bourke,
Country Fire Authority
8 Lakeside Drive,
Burwood East,
Vic, 3151

ToxConsult document: ToxCL281013-R
26th October 2013

Re: PFOS blood tests

Dear Mr Bourke,

To date twenty four persons have volunteered to have blood samples taken for measurement of perfluorinated chemicals (PFCs) in their serum. Approximately 50% have indicated that in the past they have eaten fish from Lake Fiskville. Included in the overall group are people who are not involved with training operations at Fiskville, and some who are not employees of CFA. All persons have had additional blood taken for measurement of heavy metals, haematology parameters, and clinical chemistry screening that included tests for liver, kidney and thyroid function. Furthermore all CFA personnel in the group have had a general medical examination given by the CFA medical officer. All persons have agreed to have the results of their tests made anonymously available for evaluation.

Only two of the eight PFCs looked for in serum were measurable. These were PFOA and PFOS. The PFOA concentrations for all individuals were well within what is expected for the general population. The majority of the PFOS measurements were also comfortably within the values for the general population. A few individuals had PFOS concentrations at, or slightly above, the upper edge of the background range. These results are higher than what is expected for the majority (95%) of the general population. Nevertheless they were still markedly less than serum concentrations in factory workers making PFOS, and for whom there are no PFOS associated changes in blood parameters or demonstrable illness.

None of the individuals examined had changes in their blood parameters characteristic of PFOS, or which correlated with their PFOS serum concentration. Some persons had blood parameters
outside the reference ranges but these were associated with existing health conditions, medication or admitted lifestyle factors.

The CFA medical doctor has discussed the results of their medical examination and testing with each person. Where necessary he has encouraged them to follow up their health condition with their GP and has supplied a facilitating letter.

**In conclusion**, we do not expect there to be any health implications arising from the concentrations of PFOS measured in the serum of the persons investigated.

Yours faithfully,

Roger Drew, PhD, DABT,
Toxicologist & Health Risk Assessor,
ToxConsult Pty Ltd.

Adjunct Associate Professor,
Department of Epidemiology & Preventative Medicine,
Monash University

Dr Michael Sargeant,
CFA Medical Officer,
Public Health Management Pty Ltd.
Appendix F: International fish advisories

A number of authorities have provided advice regarding consumption of fish containing PFOS (Dutch VWA 2008, German FIRA 2006, Alabama DoPH undated, Minnesota MDH 2008, Ontario MoE 2013). These fish advisories are not regulatory standards. The technical derivation of many could not be found (Dutch VWA 2008, Alabama DoPH undated, Minnesota MDH 2008, Ontario MoE 2013). However when the basis of the fish advisories was available it is apparent they are very conservative, primarily because large amounts of fish are assumed to be eaten every day of a person’s life (this is patently not the case for fish consumed from Lake Fiskville). In addition, despite the fact that fish are by far the greatest contributors to PFOS intake by humans, only a small fraction of the TDI is assigned by some agencies to fish. The resulting fish advisories are precautionary, occasional consumption of fish with higher PFOS concentrations does not necessarily indicate an unacceptable health risk or that adverse health effects are likely.

Information on the derivation of guidance concentrations for PFOS in fish from some countries is below.

Netherlands:
A maximum permissible concentration (MPC) for PFOS in fish has been calculated by RIVM (2010) based on the European Food Safety Authority TDI of $1.5 \times 10^{-4}$ mg/kg bw/d (EFSA 2008), assuming a body weight of 70 kg, a daily intake of 115 g fish, and a maximum contribution to the TDI from fish of 10%. The math are $(0.1 \times 1.5 \times 10^{-4} \times 70) / 0.115 = 9.1 \times 10^{-3}$ mg/kg = 9.1 μg/kg (9.1 ng/g) fish wet weight.

If more realistic assumptions are made (e.g. 90% of the TDI for fish and 30 g fish eaten on average per day) the resulting MPC is 315 ng/g fish.

RIVM (2010) indicates that after a fire fighting foam incident at Schipol airport in 2008 in which foam containing PFOS was washed into a nearby canal, the Dutch Food and Consumer Product Authority (“Voedsel en Warenautoriteit”, VWA) concluded that PFOS concentrations in fish from the canal were high (400-1,500 μg/kg as compared to 30 μg/kg in fish caught upstream from the incident location) and consumption was advised against. The advice was for the particular incident and was not underpinned by quantitative considerations of risk to health.
Germany:
In order to evaluate the significance of high PFOS concentrations measured in fish from an aquaculture pond in North Rhine Westphalia, Germany, the German Federal Institute for Risk Assessment (German FIRA 2006) used a TDI of 0.1 µg/kg/day to derive a theoretical tolerable intake of 6 µg PFOS per day for a 60 kg individual.

At an assumed fish consumption rate of 300 g/day, it was determined 100% of the TDI would be exhausted at a PFOS fish concentration of 0.02 µg/g fish (6 µg PFOS/day ÷ 300 g fish/day). However FIRA reasoned it was unlikely for a person to continually eat this amount of fish each day for their lifetime. It was therefore concluded that PFOS concentrations under 0.02 µg/g (i.e. 20 ng/g) in fish are tolerable.

Alabama:
The Alabama Department of Public Health (Alabama DoPH, undated) combined the RfD for PFOS derived by the US EPA (2009) of 0.08 µg/kg/day with standard information for national body weight and food consumption patterns to determine the following advisories for PFOS in fish:

- No restriction: 0 - 40 µg/kg
- 1 meal/week: >40 – 200 µg/kg
- 1 meal/month: >200 – 800 µg/kg
- Do Not Eat: >800 µg/kg

Details on how the calculations were performed and the values used were not provided.

- However assuming 100% of the RfD was assigned to fish and 70 kg body weight, the amount of fish assumed by Alabama DoPH to be consumed per day can be calculated from the maximum value of the “no restriction” range:
  - A TDI of 0.08 µg/kg/day equates to 5.6 µg/d PFOS for a 70 kg individual. Therefore 5.6 µg/d PFOS ÷ 40 µg PFOS/kg fish = 0.14 kg/d fish (i.e. 140 g/d).
**Minnesota:**
The Minnesota Department of Health (MDH 2008) have the same PFOS fish advisories as Alabama. The scientific derivation of the Minnesota fish advisories could not be found.

**Ontario:**
The Ontario Ministry for the Environment (Ontario MoE 2013) provides consumption guidelines for various contaminants in sporting fish. Included is PFOS. Details for the derivation of the guidelines are not provided. However, it is stated that consumption guidelines are based on tolerable daily intakes provided by the Food Directorate of Health Canada. There are five areas in Ontario where consumption of fish is restricted due to concentrations of PFOS they contain. The restrictions are attributed to PFOS released from historic use of firefighting foams.

In Ontario consumption restrictions for PFOS begin at 80 ng/g fish, with complete restriction on consumption advised for levels above 160 ng/g for the sensitive population and 640 ng/g for the general population. The ‘sensitive population’ is defined by Ontario MoE (2013) to include women of child-bearing age and children less than 15 years. Other agencies do not sub-categorise the population, presumably because the TDI is set to include the sensitive sub-populations.

Details for the derivation of the Ontario PFOS fish guidelines are not provided in Ontario MoE 2013.