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Toxicant default guideline values for aquatic ecosystem protection

Perfluorooctane sulfonate (PFOS) in freshwater

Technical brief

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Summary

The default guideline values (DGVs) and associated information in this technical brief should be used in accordance with the detailed guidance provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality website (ANZG 2018).

Perfluorooctane sulfonate (PFOS) generally refers to a long chain perfluorinated chemical containing eight perfluorinated carbons terminated with a sulfonate or sulfonyl fluoride group. It is a conjugate base anion of perfluorooctane sulfonic acid (i.e. the perfluorooctane sulfonate anion ($C_8F_{17}SO_3^-$; CASRN 45298-90-6)) (NICNAS 2015). Common PFOS salts include the acid (CASRN 1763-23-1), potassium (CASRN 2795-39-3), lithium (CASRN 29457-72-5) and ammonium salts (CASRN 56773-42-3). PFOS is a member of the chemicals referred to as perfluoroalkyl and polyfluoroalkyl substances (i.e. PFAS) (Ankley et al. 2004) and is characterised as a perfluoroalkyl sulfonate within this group. Some PFAS are precursors to the formation of PFOS and other perfluoroalkyl acid breakdown products.

PFOS is classified, globally and in Australia, as a persistent, bioaccumulative and toxic (PBT) substance (UNEP 2006, NICNAS 2015). In 2009, PFOS was added to the Stockholm Convention on Persistent Organic Pollutants (POPs) and in July 2025, it (along with PFOA and PFHxS and related substances) was listed in the Industrial Chemicals Environmental Management Standard (IChEMS) (Register) Instrument 2022, Schedule 7 of the *Industrial Chemicals Environmental Management (Register) Act 2021* (IChEMS Act 2021).

PFOS is moderately soluble in freshwater and has unique surface-active properties, with both lipid-repellent and water-repellent characteristics. Unlike most POPs, PFOS binds to proteins rather than concentrating in the lipid fraction (Oakes et al. 2005). PFOS is resistant to environmental degradation processes such as hydrolysis, photolysis, biodegradation and metabolism (Ginn et al. 2005, Oakes et al. 2005, Hazelton et al. 2012, Ng & Hungerbühler 2014).

The aquatic toxicity data presented in this technical brief are expressed as total PFOS. Aquatic toxicity tests were conducted on either analytical or commercial grade PFOS. It is assumed that the chemicals used in these tests included both linear and branched isomers of PFOS. The toxicity of PFOS to freshwater species ranges over five orders of magnitude, with fish and invertebrates generally more sensitive than plants and algae. Based on the toxicity data considered, the frog *Lithobates pipiens* was the most sensitive species with a reported LOEC of 0.057 µg/L, while the diatom *Navicula pelliculosa* was the least sensitive species, with an EC50 of 263 000 µg/L (although neither of these values were used in the final derivation).

Very high reliability DGVs were derived using chronic EC10, NOEC, and LOEC data for 37 species from 11 taxonomic groups, with a good fit of the species sensitivity distribution (SSD) to the toxicity data. The DGVs for PFOS in freshwater for 99%, 95%, 90% and 80% species protection are 0.02 µg/L, 0.9 µg/L, 4 µg/L, and 20 µg/L, respectively. Because the DGVs do not account for the bioaccumulation of PFOS in aquatic food chains, the 99% species protection DGV for PFOS in freshwater is recommended for application to slightly-to-moderately disturbed ecosystems. The DGVs are expressed as the PFOS anion; therefore, monitoring data must be reported as the anion for comparison with the DGVs.

Although the 99% species protection DGV is recommended to protect species from bioaccumulation of PFOS, biota in the water may have tissue concentrations of PFOS that exceed the PFAS NEMP 3.0 biota guideline values regardless of whether the water quality meets the DGV potentially leading to biomagnification concerns. The 99% DGV alone may not be sufficient to protect organisms that consume these biota (e.g. predators such as birds). To address the risk to higher consumers, a biota screening threshold for PFOS in freshwater of 0.0005 µg/L is recommended – exceedance of this concentration may result in PFOS concentrations in aquatic biota that exceed the PFAS NEMP 3.0 biota guideline values. Exceedance of the biota screening threshold should trigger an assessment of PFOS concentrations in aquatic biota tissues to assess the possible risk to mammalian and avian predators. Assessments of PFOS in freshwater should consider the presence and implications of PFOS precursors.

1 Introduction

Perfluorooctane sulfonate (PFOS) is a member of the chemicals referred to as perfluoroalkyl and polyfluoroalkyl substances (PFAS) (Ankley et al. 2004), and is characterised as a perfluoroalkyl sulfonate within this group. PFOS generally refers to a long chain perfluorinated chemical containing eight perfluorinated carbons terminated with a sulfonate or sulfonyl fluoride group. It is a conjugate base anion of perfluorooctane sulfonic acid (i.e. the perfluorooctane sulfonate anion ($C_8F_{17}SO_3^-$)) (NICNAS 2015). Common PFOS salts include the acid, potassium, lithium and ammonium salts.

PFOS has been commercially produced for many different uses, typically using the electrochemical fluorination process (Martin et al. 2010). This process results in a mixture of linear (70–80%) and branched (20–30%) isomers (Buck et al. 2011). In addition to commercial production, PFOS can be formed as a result of the degradation or metabolism of higher molecular weight PFAS, which are referred to as PFOS precursors (Martin et al. 2010, Chen et al. 2015).

PFOS has been used extensively in metal plating and aviation hydraulic fluids and in the manufacture of clothing and textiles, food wrapper coatings, paper and packaging, coating additives, cleaning products, stain repellents, semi-conductors, surfactants, and firefighting foams (Bots et al. 2010, Buck et al. 2011, OECD 2020; MPCA 2020; ITRC 2023; MPCA 2023). In the 1990s, evidence began to surface that PFOS was present in measurable concentrations in humans and the environment. This raised international concern about the health and environmental hazards posed by PFOS. In 2000, the 3M Company in the United States announced it would voluntarily phase-out production of PFOS (Boudreau et al. 2003a, Brooke et al. 2004, Giesy et al. 2010). The 3M Company phase-out was completed by 2002 (Brooke et al. 2004, Bots et al. 2010). However, since 2002, other companies have begun production of PFAS.

PFOS is classified by international and national regulatory authorities as a persistent, bioaccumulative and toxic (PBT) substance (UNEP 2006, NICNAS 2015). In 2009, PFOS, its salts and perfluorooctane sulfonyl fluoride (PFOSF) were added to the Stockholm Convention on Persistent Organic Pollutants (POPs). At the time of preparing this technical brief, Australia had yet to ratify the Stockholm Convention amendment (i.e. listing PFOS, its salts and perfluorooctane sulfonyl fluoride). On 1 July 2025 PFOS (along with PFOA and PFHxS and related substances) was listed in the Industrial Chemicals Environmental Management Standard (IChEMS) Register, Schedule 7, which is a list of chemicals of the highest risk of causing serious or irreversible harm to the environment (IChEMS 2021).

Australia has never manufactured PFOS, but has imported it for a variety of uses. Following listing on the Rotterdam Convention, Australia enacted import and export controls consistent with the convention (Parliament of Australia 2014, NICNAS 2015). Some state and territory governments have set regulatory controls for the use and disposal of certain PFOS-containing products as well as the direct precursors of PFOS. New Zealand has also never manufactured PFOS, and the import of PFOS firefighting foams ceased in 2006 (NZ EPA 2019). In 2011, New Zealand ceased the import and use of PFOS, except for specific uses such as in laboratory analysis (MfE 2022). The PFAS National Environmental Management Plan 3.0 (HEPA 2025) provides guidance on the management of PFAS contamination in the environment, including preventing the spread of contamination with the aim to protect the environment and human health (HEPA 2025).

Perfluorooctane sulfonate is a strong acid in water. Fluorine atoms (compared to hydrogen) are more strongly bound to the carbon chain, resulting in PFOS being chemically stable and persistent (even in biological tissues) with a long half-life: hydrolysis half-life of ≥41 years; photolysis half-life of >3.7 years; very slow rates of anaerobic and aerobic biodegradation; and slow rates of metabolism (Ginn et al. 2005, Oakes et al. 2005, Hazelton et al. 2012, NICNAS 2015). The strong chemical bonds give PFOS unique surface-active (low surface energy) properties and result in PFOS having lipid-repellent and water-repellent characteristics.

PFOS enters the environment from spills or other releases, and during the use and disposal of products containing PFOS or PFOS precursors. Relevant waste streams include sewage outflows, biosolids and landfill leachate. PFOS enters the environment as the parent compound and also as the degradation product of PFOS precursors following degradation or metabolism therefore, it is likely that PFOS will continue to be detected in the environment in the long-term (UNEP 2006, ITRC 2023). PFOS is moderately soluble in freshwater, with solubilities ranging from 370 mg/L in freshwater to 550 mg/L in pure water (OECD 2002).

Once released into the environment, PFOS disperses via air, surface water, groundwater and food chain transfer (UNEP 2006). Based on the chemical characteristics of PFOS (low Henry's Law coefficient, moderate water solubility), the aquatic environment has the greatest potential for PFOS contamination (OECD 2002, Li 2009). Long-range transport of PFAS is evidenced by concentrations reported in remote locations far from sources of PFAS, such as in the Arctic (UNEP 2006, ITRC 2023). For example, PFOS has been reported in Arctic zooplankton at concentrations of 1.8 ng/g, fish at concentrations ranging from 5.7 to 85.4 ng/g, liver tissues of polar bears and ringed seals at concentrations of 3 770 ng/g and 96 ng/g, respectively (Smithwick et al. 2005 in UNEP 2006), and in Herring Gull eggs (*Larus argentatus*) up to concentrations of 42 200 ng/g (OECD 2002, Swedish EPA 2004, Fair et al. 2019, ATSDR 2021).

In Australia, PFOS has been reported in the liver and breast muscle tissue of ducks at 340 ng/g and 33 ng/g, respectively (Sharp et al. 2021). A study of dolphins from south-east Australia (Foord et al. 2024) reported hepatic PFOS concentrations ranging from an individual concentration of 3.7 ng/g in one Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) to a mean of 9,170 ng/g in Burrunan dolphins (*Tursiops australis*), with the highest concentration of 18,700 ng/g reported in one Burrunan dolphin from Port Phillip Bay. A study by Beale et al. (2024) reported elevated PFOS concentrations in organs including the liver, kidneys and heart of female freshwater turtles (*Emydura macquarii macquarii*) collected from a contaminated site in Queensland. Water quality monitoring at the contaminated site (seven events during 2018 and 2021) reported concentrations ranging from 2.8 to 5 µg/L. Maximum PFOS concentrations reported for female freshwater turtles' liver, kidney and heart tissues were 2 880, 3 000 and 2 500 ng/g, respectively.

PFOS concentrations in Australian surface water are reported in the published literature. Information on PFOS concentrations in surface water in New Zealand is mostly limited to data obtained from the assessment and monitoring of contamination from firefighting foams at airports, air and naval bases, petrochemical facilities and the site of a military plane crash. Some studies on PFOS concentrations in Australian waters are summarised below.

- Thompson et al. (2011) assessed PFAS concentrations in drinking water from 34 sources (33 locations across Australia and one bottled water sample) in 2010. These locations included: one in the Australian Capital Territory; one in South Australia; two in Tasmania; three in Western Australia; three in Victoria; four in the Northern Territory; five in Queensland; and 14 in New South Wales. The highest PFOS concentration was recorded in a residential property in the Adelaide suburb of Glenunga (15.1–15.6 ng/L in tap water without a carbon filter, compared to <0.13 ng/L with a carbon filter attached). Most locations reported low but detectable concentrations in tap and tank water (i.e. 0.76–4.68 ng/L). Nine locations reported concentrations below the instrument detection limit of 0.13 ng/L.

- Gallen et al. (2014) measured PFOS concentrations in an urban catchment flowing into Brisbane River and Moreton Bay during a flood in 2011. Sampling locations included two upstream dams at the origin of Brisbane River in low population density areas, several locations in highly urbanised areas of Brisbane River and Oxley Creek (a tributary of Brisbane River), and Moreton Bay. PFOS concentrations in the upstream dams ranged from below the limit of quantitation (0.03–0.13 ng/L) to 0.2 ng/L. The highest concentration of 34 ng/L was reported in Oxley Creek. Concentrations in Moreton Bay ranged from 0.69 ng/L to 2.6 ng/L.
- Allinson et al. (2019) collected water samples from rivers, creeks and estuaries in the Port Philip Bay catchment in Victoria in 2012. The waterways sampled were located in a variety of different land uses, including forested, agriculture (grazing and horticulture), urban residential, and industrial. Some of the waterways received discharges from sewage treatment plants. PFOS concentrations in rivers and creeks ranged from 6.5 ng/L to 45 ng/L, and concentrations in estuaries ranged from 3.9 ng/L to 7.4 ng/L, with higher concentrations reported in industrial development areas.
- Sardiña et al. (2019) assessed PFOS concentrations in surface water at 25 riverine sites (creeks, wetlands, river impoundments) within *ca.* 40 km of major population centres in Victoria. Sites represented five land uses: background (undeveloped); low intensity agriculture (grazing); high intensity agriculture (cropping, horticulture); urban residential; and urban industrial. PFOS concentrations ranged from below the laboratory limit of reporting (LOR) (<2 ng/L) to 100 ng/L.
- Sharp et al. (2021) assessed PFOS concentrations in surface water from 19 wetlands in duck hunting locations across Victoria. Most sampling locations were in agricultural areas, though two were in urban areas and one was close to an Australian Defence Force air base. PFOS concentrations ranged from below the laboratory LOR (<2 ng/L) to 490 ng/L (location near the air base).
- Baddiley et al. (2020) sampled surface water at 55 locations in Queensland every 2 months for 1 year (2019–2020). Sampling locations were targeted away from known PFAS sources (>1 km) and were adjacent to a variety of land uses (e.g. industrial, residential, conservation, agricultural). The results were as follows.

– Eight sites (15% of total) did not report PFAS.
– 21 sites (38% of total) reported PFOS concentrations at approximately the LOR (0.1 ng/L).
– The highest concentrations and types of PFAS were recorded at sites surrounded by urban and industrial land (with PFOS concentrations up to 37 ng/L).
– In agricultural areas, PFOS concentrations ranged from <LOR to 1.1 ng/L.
– In remote areas, PFOS concentrations ranged from <LOR to 0.1 ng/L.

PFOS bioaccumulation in aquatic organisms is difficult to predict. In octanol/water partitioning tests, PFOS forms three layers, indicating that a log K_{ow} cannot be reliably determined (Oakes et al. 2005) or used to predict the potential for PFOS to bioaccumulate. In addition, PFOS has a low acid dissociation constant with measured pK_a values reported to range from <0.3 (Vierke et al. 2013 cited in ITRC

2023) to <1 (Cheng et al. 2009 cited in ITRC 2023), and readily dissociates in water (Moermond et al. 2010).

Log Bioconcentration factors (BCFs) for freshwater fish (whole body or tissue-specific) have been reported to range from approximately 0.5 to 3.6 L/kg (Qi et al. 2011, Lu et al. 2015, Burkhard 2021). Log Bioaccumulation factors (BAFs) for PFOS in freshwater fish are reported to range from 3.18 L/kg to 4.9 L/kg (Moermond et al. 2010, Burkhard 2021). PFOS also has high biomagnification potential. Using a weight of evidence approach, Moermond et al. (2010) recommended a biomagnification factor (BMF) of 5 for small fish to larger fish, and a BMF of 5 for larger fish to fish-eating mammals and birds.

Notwithstanding the difficulties of predicting PFOS bioaccumulation, studies on PFOS bioaccumulation in higher trophic levels (e.g. fish, fish-eating organisms) have indicated that accumulation occurs in particular organs (such as liver tissues) (UNEP 2006, Hagenaars et al. 2011, Borg & Håkansson 2012), and that fish-eating organisms, particularly air-breathing organisms, contain greater concentrations of PFOS than their food (Lu et al. 2015). Thus, there is extensive evidence that PFOS bioaccumulates in aquatic organisms and biomagnifies in higher trophic levels (also see Section 2.2).

2 Aquatic toxicology

2.1 Mechanisms of toxicity

The mode of action of PFOS is not fully understood. The predominance of information about possible modes of action for PFOS relates to animals, with little to no information for plants. Following PFOS exposure, molecular-level perturbations can disrupt estrogenic, androgenic, and thyroid endocrine pathways, as well as neuronal, lipid, and carbohydrate metabolic systems. These disruptions may lead to cellular and organ-level dysfunctions, ultimately affecting reproduction, growth, and development at the organism level. For comprehensive overviews, see Lee et al. (2020) and Ankley et al. (2021).

PFOS has been shown to affect fish and amphibian development via reproductive and endocrine effects (vitellogenin induction in male fish, abnormal ovary and testis development, thyroid disruption and embryonic deformities) and via hepatotoxicity such as vacuolation of liver cells (Keiter et al. 2012, Rainieri et al. 2017, Chen et al. 2018b, Sant et al. 2018, Fort et al. 2019, Zhang et al. 2019, Flynn et al. 2022, Britton et al. 2024).

Possible PFOS modes of action include:

- activation of the nuclear peroxisome proliferator activated receptor-alpha (PPAR-α) (Bots et al. 2010, Borg & Håkansson 2012, ECCC 2018)
- alteration of membrane properties such as permeability and fluidity (Jones et al. 2003, Lankadurai et al. 2013)
- binding to proteins such as serum albumin, with weaker binding to proteins involved in fatty acid transport and metabolism (Jones et al. 2003)

- up- and down-regulation of various protein biomarkers in diseased muscle tissues, heart tissues, kidneys and ovary tissues (Beale et al. 2024)
- uncoupling of oxidative phosphorylation (Moermond et al. 2010, ECCC 2018)
- inhibition of intercellular gap junctions (Jones et al. 2003, ECCC 2018)
- endocrine effects (Ankley et al. 2005, Borg & Håkansson 2012, Keiter et al. 2012)
- interaction with transporter proteins (Keiter et al. 2012).

Additional research from a mechanistic perspective is needed to better understand how these different modes of action elicit specific biological responses in fish, aquatic invertebrates, and amphibians. While modes of action are mostly reported in animal studies, the alteration of membrane properties and inhibition of intercellular junctions may also be relevant to plants. The mode of action of PFOS in plants is not well understood (Hanson et al. 2005).

2.2 Toxicity

A literature search of the aquatic toxicity of PFOS on freshwater organisms identified acute and chronic effects for plant and animal species including traditional, ecologically relevant endpoints and non-traditional endpoints, for which ecological relevance is unclear. Traditional endpoints included: survival; growth; development; and reproduction. Non-traditional endpoints included: behavioural effects; endocrine effects, including vitellogenin induction; developmental effects, including malformations; altered gene expression; deoxyribonucleic acid (DNA) damage; histopathological effects; and changes to community structure.

In the literature, study types included: water-borne laboratory tests; field or laboratory mesocosm and microcosm studies; and uptake or bioaccumulation studies via feeding, injection, or water-borne exposure.

Chronic duration studies are preferred over acute studies when deriving DGVs (Warne et al. 2018). Given this, the literature review focussed on chronic effects, with acute data only discussed briefly.

Acute toxicity data

Most acute data represent effects on survival. Survival effects were reported for over 30 species, with LC50 concentrations ranging from 700 µg/L for the nematode *Caenorhabditis elegans* (2 d LC50) (Chen et al. 2018a) to 247 140 µg/L for the snail *Cipangopaludina cathayensis* (4 d LC50) (Yang et al. 2014). Effects of acute exposure on growth, development, behaviour and reproduction were reported for eight species, with toxicity values ranging from 82.8 µg/L for the zebrafish *Danio rerio* (5 d growth LOEC) (Jantzen et al. 2016) to 158 100 µg/L for the mussel *Ligumia recta* (2 d foot movement EC50) (Hazelton et al. 2012).

Chronic toxicity data

Three hundred and seventy seven chronic toxicity data were identified for 45 species from nine phyla (Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cyanobacteria, Mollusca, Platyhelminthes, Rotifera and Tracheophyta). The chronic studies included short-term and long-term partial life cycle, full life cycle and multigenerational exposures for traditional endpoints of survival, growth, reproduction and development, as well as non-traditional endpoints such as behaviour, biochemical responses, and endocrine responses.

The chronic toxicity values ranged from 0.057 µg/L for the amphibian *Lithobates pipiens* (development LOEC 30 d) (Flynn et al. 2021) to 263 000 µg/L for the diatom *Navicula pelliculosa* (5-d EC50) (OECD 2002). Toxicity values for individual taxonomic groups spanned orders of magnitude, as summarised below:

- for insects, values ranged from 0.226 µg/L (14 d growth EC10 for *Neocloeon triangulifer*) (Soucek et al. 2023) to 7 950 µg/L (120 d development LOEC for *Enallagma cyathigerum*) (Bots et al. 2010)
- for fish, values ranged from 0.734 µg/L (F2 generation, 90 d and 180 d post fertilisation growth LOECs for *D. rerio*) (Keiter et al. 2012) to 16 004 µg/L (7 d growth LOEC for *D. rerio*) (Sant et al. 2017). It is noted that an independent technical review of key comments received during the public consultation process (Dawson et al. 2024) concluded that the Keiter et al. (2012) study was unreliable and should not be used in the derivation of the DGVs.
- for crustaceans, values ranged from 1 µg/L (21 d reproduction LOEC for *Daphnia carinata*) (Logeshwaran et al. 2021) to 50 000 µg/L (21 d reproduction LOEC for *D. magna*) (Boudreau et al. 2003a)
- for amphibians, values ranged from 0.057 µg/L (30 d development LOEC for *L. pipiens*) (Flynn et al. 2021) to 10,000 µg/L (21 d weight LOEC for *Xenopus laevis*) (Degitz et al. 2024)
- for molluscs, values ranged from 4.5 µg/L (36 d survival LOEC for *Lampsilis siliquoidea*) (Hazelton et al. 2012) to 125 000 µg/L (14 d survival LOEC for *Physa pomilia*) (Funkhouser 2014).
- for rotifers, values ranged from 250 µg/L (28 d reproduction LOEC) to 2 000 µg/L (5 d survival LOEC) for *Brachionus calyciflorus* (Zhang et al. 2013).
- for flat worms, values ranged from 500 µg/L (10 d development LOEC)(Yuan et al. 2014) to 5 000 µg/L (22 d development LOEC) (Zhao et al. 2018) for *Dugesia japonica*.

For macrophytes and microalgae, the range in toxicity values was smaller, but still differed by orders of magnitude, as follows.

- For macrophytes, values ranged from 100 µg/L (42 d growth EC10 for *Myriophyllum sibiricum*) (Hanson et al. 2005) to 59 100 µg/L (7 d growth IC50 for *Lemna gibba*) (Boudreau et al. 2003a). The EC10 growth effect of 100 µg/L for *M. sibiricum* reported in Hanson et al. (2005) represents growth of the longest root, which is not considered to be an ecologically relevant endpoint.
- For microalgae, values ranged from 5 300 µg/L (4 d growth EC10 for *Raphidocelis subcapitata*) (Boudreau et al. 2003a) to 263 000 µg/L (4 d growth EC50 for *N. pelliculosa*) (OECD 2002).

Studies that report body burden (following water-borne and/or dietary exposure) in association with toxic effects are relevant for setting aquatic ecosystem guideline values for persistent, bioaccumulative and biomagnifying toxicants such as PFOS. The chronic long-term and multigenerational exposures are more likely to report effects from bioaccumulation following water-borne exposure compared to shorter duration studies.

Long-term multigenerational exposures for animal species resulted in some of the lowest effects concentrations and were available for:

- fish, including *D. rerio* (Du et al. 2009, Wang et al. 2011, Keiter et al. 2012, Chen et al. 2013, Du et al. 2018, Christou et al. 2021, Gust et al. 2024), *Oryzias latipes* (Ji et al. 2008), *Pimephales promelas* (Ankley et al. 2005, Suski et al. 2021)
- chironomid *C. riparius* (Stefani et al. 2014, Marziali et al. 2019)
- cladoceran *D. magna* (Jeong et al. 2016)
- rotifer *B. calyciflorus* (Zhang et al. 2013)
- snail *P. pomilia* (Funkhouser 2014).

The multigenerational exposure toxicity values ranged from 0.734 µg/L for *D. rerio* (F2 generation, 90 d and 180 d post fertilisation growth LOEC) (Keiter et al. 2012) to 35 900 µg/L for *P. pomilia* (F1 generation 44 d LC50) (Funkhouser 2014).

The following mesocosm and microcosm studies on PFOS assessed effects on the exposed species, including bioaccumulation and persistence of PFOS in sediment and the water column. Except for Jacobsen et al. (2010), the studies represent non-renewal exposures for the duration of the experiment.

- Sanderson et al. (2002, 2004) reported effects in zooplankton communities of copepods, cladocerans and rotifers at 1 d, 2 d, 4 d, 7 d, 14 d, 21 d, 28 d and 35 d using 30 L indoor microcosms and 12 000 L outdoor mesocosms. Both studies used field collected sediment and water. Apart from the natural zooplankton communities, organism assemblages in the indoor microcosms included snails, algae, macrophytes and macroinvertebrates. The outdoor mesocosms were seeded with macrophytes (*M. sibiricum*) and fish (*P. promelas*). Similarly, Boudreau et al. (2003b) reported effects at 1 d, 2 d, 4 d, 7 d, 14 d, 21 d, 28 d and 35 d for zooplankton communities of copepods, cladocerans and rotifers exposed to PFOS in 12 000 L outdoor mesocosms. In addition to zooplankton, Boudreau et al. (2003b) also assessed the effects of PFOS on the aquatic macrophyte *L. gibba* within the mesocosms for 7 d, 14 d, 21 d, 28 d, 35 d and 42 d, although this appeared to represent a single species study within a mesocosm rather than a mesocosm study. Effects measured included plant number, frond number, frond size, root length, chlorosis and necrosis. The PFOS exposure concentrations in Sanderson et al. (2002, 2004) and Boudreau et al. (2003b) ranged from 0.3 mg/L to 30 mg/L. These studies found that as the PFOS concentrations increased, zooplankton species richness decreased and the abundance of tolerant species increased. The copepod community showed greatest sensitivity. Persistence of PFOS in the water column was also assessed for 285 d and found to remain constant.
- Hanson et al. (2005) assessed the effects of PFOS on the macrophytes *M. sibiricum* and *M. spicatum* in 12 000 L outdoor mesocosms exposed to PFOS concentrations from 0.3 mg/L to 30 mg/L. The water used in the exposures was from a pond supplied with well water circulated for 2 weeks prior to PFOS exposure to provide the microcosms with assemblages of zooplankton and algae. The reported effects included reductions in plant length, biomass, root number, root length, number of nodes, chlorophyll and carotenoid content at 14 d, 28 d and 42 d.
- Jacobsen et al. (2010) reported increased likelihood of parasite infestation in the amphipod *Monoporeia affinis* when exposed to increasing concentrations of PFOS ranging from 0.01 mg/L to 5 mg/L in a semi-static 1 L laboratory microcosm chamber. The exposure used sediment and water containing microfauna and meiofauna from a field collection site, with PFOS added to the water column.

- Fang et al. (2016) assessed the bioaccumulation of PFOS in the carp *Cyprinus carpio* in 70 L aquaria containing 20 kg of sediment; PFOS concentrations were 10 mg/L in water and 1 mg/kg in sediment. Uptake of PFOS in fish was measured during exposure (days 2, 5, 9, 14, 21 and 28) and depuration (days 30, 33, 37, 42, 49 and 56). The study found that carp accumulated PFOS, with linear chains accumulated to a greater extent than branched.
- Foguth et al. (2019) and Flynn et al. (2021) assessed the effects of PFOS on the northern leopard frog *L. pipiens* in 180-L plastic wading pools filled with 75 L of well water and containing sediment spiked with PFOS at concentrations of 0.01, 0.1 and 1 mg/L. The sediment used was from a permanent pond at the Purdue Wildlife Area, the water was well water and the frogs were collected as egg masses from an ephemeral pond also at the Purdue Wildlife Area. Algae and zooplankton were allowed to establish prior to addition of the frog larvae. Flynn et al. (2021) assessed growth and developmental effects both of which were reported after 5 and 30 days and Foguth et al. (2019) assessed the effects of increasing concentrations on neurotransmitter levels. Both studies also assessed bioaccumulation based on both water and sediment concentrations.

Bioaccumulation data

Uptake and bioaccumulation of PFOS following acute and chronic exposures were reported for the following animals:

- worms, including nematode *Caenorhabditis elegans* (Chen et al. 2018a, Kim et al. 2020) and oligochaete *Limnodrilus hoffmeisteri* (Liu et al. 2016, Meng et al. 2016, Qu et al. 2016)
- midge larva *Chironomus plumosus* (Wen et al. 2016)
- cladoceran *D. magna* (Dai et al. 2013, Xia et al. 2015a, Dai et al. 2018, Jeong and Simpson 2020)
- amphipods *Afrochiltonia subtenuis* (Sinclair et al. 2022)
- snail *Lymnaea stagnalis* (Olson 2017)
- mussels *Dreissena polymorpha* (Fernandez-Sanjuan et al. 2013) and *Lampsilis silquoidea* (Hazelton et al. 2012) and Asian clam *Corbicula fluminea* (Liu et al. 2020, Bi et al. 2022)
- frogs *L. pipiens* (Ankley et al. 2004, Hoover et al. 2017, Abercrombie et al. 2019, Foguth et al. 2019, 2020, Flynn et al. 2021, Hoskins et al. 2022)
- toads *Anaxyrus americanus* (Abercrombie et al. 2019)
- salamander *Ambystoma tigrinum* (Hoover 2018, Abercrombie et al. 2019)
- fish, including carp *C. carpio* (Hoff et al. 2003, Inoue et al. 2012, Chen et al. 2015, Qiang et al. 2016, Zhong et al. 2018, Shan et al. 2022), grass carp *Ctenopharyngodon idealla* (Qiang et al. 2016), rainbow trout *Oncorhynchus mykiss* (Martin et al. 2003, Falk et al. 2015, Vidal et al. 2019, Yi et al. 2020), fathead minnow *P. promelas* (Ankley et al. 2005, Suski et al. 2021), salmon *Salmo salar* (Mortenson et al. 2011, Arukwe et al. 2013), suckermouth catfish *Hypostomus plecostomus* (Qiang et al. 2016), bluegill *Lepomis macrochirus* (Drottar et al. 2001) and zebrafish *D. rerio* (Huang et al. 2010, Wang et al. 2011, Chen et al. 2013, Li et al. 2017, Cheng et al. 2016, Qiang et al. 2016, Tu et al. 2019, Vogs et al. 2019, Gaballah et al. 2020, Jeong and Simpson 2020, Zou et al. 2021, Wang et al. 2023).

However, uptake and bioaccumulation reported in association with effects on ecologically relevant toxicity endpoints following chronic exposures were limited to:

- *D. rerio* (Chen et al. 2013)

- *L. pipiens* (Flynn et al. 2021)
- *L. stagnalis* (Olson 2017)
- *P. promelas* (Ankley et al. 2005, Suski et al. 2021).

Chen et al. (2013) observed increased mortality and PFOS bioaccumulation in embryos produced by adult *D. rerio* exposed for long periods (21–120-d post fertilisation (dpf) and 1–120 dpf) compared to control organisms. Flynn et al. (2021) observed decreased growth and delayed development in *L. pipiens* with increasing PFOS accumulation. PFOS accumulation in tissues of exposed *P. promelas* was highest in blood plasma, followed by the liver and then the gonads of male and female fish, with females accumulating more than males (Ankley et al. 2005). An increase in PFOS concentrations in the tissue of *P. promelas* coincided with the increasing exposure concentrations and also with increased mortality effects. Suski et al. (2021) reported dose-dependent PFOS accumulation in female gonads; however, this could not be determined to be the cause of decreased growth in their offspring, as their offspring were also exposed to PFOS. Olson (2017) reported PFOS bioaccumulation in the snail *L. stagnalis* as exposure to PFOS in water was increased. However, the increased body burden of PFOS did not produce a corresponding effect on snail reproduction (Olson 2017). The evidence that PFOS bioaccumulation in aquatic organisms is linked to ecologically relevant endpoints, such as survival, indicates the importance of measuring body burden in conjunction with effects of PFOS exposures to better understand critical body burdens.

3 Factors affecting toxicity

No studies on factors affecting PFOS toxicity were found during preparation of this technical brief. However, a limited number of studies have measured uptake, accumulation and biochemical effects of PFOS in biota tissue in association with differing water quality parameters. These are discussed below.

Two studies by Kovacevic et al. (2018, 2019) assessed the effects of acute (2 d) exposure on the metabolism of *D. magna* exposed to 30 mg/L of PFOS with and without dissolved organic matter (DOM) (5 mg/L DOM in Kovacevic et al. 2018; 1 mg/L, 2 mg/L, 3 mg/L and 4 mg/L DOM in Kovacevic et al. 2019). The 2018 study reported change to percentages of amino acids in response to the combination of PFOS and DOM compared to PFOS alone. In the 2019 study, no changes to metabolism were noted in response to exposures of 1 mg/L DOM with PFOS. However, at 2 mg/L, 3 mg/L and 4 mg/L DOM, greater metabolic changes in *D. magna* were reported in the PFOS plus DOM exposures compared to PFOS alone. Both studies provide limited information with which to understand if DOM modifies PFOS toxicity at the population level (i.e. effects on development, growth, reproduction, survival).

Dai et al. (2018) measured PFOS bioaccumulation in *D. magna* at different water-borne DOM concentrations, reporting increased uptake of PFOS at 1 mg/L DOM, and decreased uptake of PFOS at 10 mg/L and 20 mg/L DOM. Xia et al. (2015b) measured the effects on PFOS bioaccumulation in *D. magna* at different humic and fulvic acid concentrations. Lower concentrations of fulvic and humic acids (1 mg/L) increased bioaccumulation of PFOS, while higher concentrations of fulvic and humic acids (20 mg/L) decreased bioaccumulation. Similarly, Wen et al. (2016) measured bioaccumulation in the midge larva *C. plumosus* in the presence of fulvic, humic and tannic acids (concentrations of

1 mg/L, 5 mg/L, 10 mg/L, 30 mg/L and 50 mg/L). The study found that PFOS body burden increased as fulvic and tannic acid concentrations increased.

Vidal et al. (2019) assessed the effect of temperature (7°C, 11°C and 19°C) on PFOS bioaccumulation and elimination in the rainbow trout *O. mykiss* following dietary exposure. The uptake of PFOS increased as temperature increased, whereas the effect on elimination rates was less clear and varied for the different organs and temperatures. Xia et al. (2015c) measured the effect of PFOS and water temperature on anti-predator behaviour and fast-start swimming performance in the carp species *Spinibarbus sinensis*. For most endpoints assessed, carp were more sensitive to PFOS exposure at higher temperature (28°C, LOEC of 2 mg/L) compared to lower temperature (18°C, LOEC of 5 mg/L).

Further studies are needed to investigate the relationships between water quality parameters (e.g. organic matter, temperature) and the toxicity of PFOS.

4 Default guideline value derivation

The DGVs were derived in accordance with the method described in Warne et al. (2018) except that the shinyssdtools software (V 0.4.0) (Dalgarno 2018) was used instead of the Burrlioz 2.0 software. Numerous data selection decisions were also informed by the recommendations of an independent technical review of key comments received during the public consultation process (Dawson et al. 2024).

4.1 Toxicity data used in derivation

In accordance with Warne et al. (2018), toxicity data were considered in the DGV derivation if they: had traditional endpoints (Section 2.2); passed quality assessment (quality score >50%); and used a test substance of >80% purity.

Most aquatic toxicology studies were performed using the potassium salt of PFOS, with fewer studies conducted using the acid, lithium, or ammonium salts. Some studies reported effects for the PFOS anion equivalent concentrations. The toxic effect is expected to be from the PFOS anion, and effects from cations such as the potassium and acid are not considered significant.

In the case of PFOS tetraethyl ammonium salt, the reported concentrations were converted to the PFOS anion. Effects using tetraethyl ammonium salt were limited to *E. cyathigerum* (Bots et al. 2010). This is consistent with the approach taken in the Global Hazard Assessment of PFOS (OECD 2002).

In some studies, the form of PFOS used to prepare the test solutions was not stated but the concentrations of PFOS were measured and reported as the PFOS anion. For approximately one-third of the final dataset, the form of PFOS for the toxicity values reported was not stated in the studies; thus, this precluded the ability to convert to the PFOS anion concentration if such a conversion was necessary. Notably, the maximum error that would occur as a result of this would be for studies that reported the results as the concentration of PFOS potassium salt (which has a molecular weight of 539). In such cases, the toxicity value would overestimate the concentration of the PFOS anion (which has a molecular weight of 499) by 8%. For cases where the results were reported as the PFOS acid, the error would be negligible (i.e. ~0.2%). This amount of uncertainty is very low relative to other sources of uncertainty introduced throughout the DGV derivation process (e.g. error in the original toxicity estimates, conversion of chronic LOECs and EC50s, analytical error, model error, etc.) and, thus, would have a negligible effect on the DGVs. Consequently, toxicity values based on unclear forms of PFOS were included in the DGV derivation.

Where only one toxicity value was available for a species, it was included in the dataset for the DGV derivation. For species with more than one toxicity value available, data were selected in accordance with Warne et al. (2018). Because the available chronic toxicity dataset met the minimum species and taxonomic group requirements (at least five species from at least four taxonomic groups), acute toxicity data were not required for the DGV derivation. Some chronic toxicity data selections involved professional judgments, as described below.

Warne et al. (2018) states that toxicity data based on nominal concentrations (i.e. theoretical rather than measured concentrations of a test substance) should not be used to derive a DGV unless a technically defensible justification can be provided. Excluding studies that report only nominal concentrations can substantially reduce the data available for deriving a DGV, which may increase the chance of calculating DGVs that do not provide adequate or appropriate protection. Conversely, including studies with nominal PFOS concentrations increases uncertainty in the toxicity estimates, and including such data could introduce errors into the DGVs derivation. Given PFOS is persistent, loss from toxicity test vessels via processes such as degradation and volatilisation is unlikely to occur and affect test concentrations. However, PFOS sorbs to some materials, notably glass and polytetrafluoroethylene (PTFE or Teflon®), and some loss from the water column may be expected. Renewal and measurement of test concentrations and/or use of plastic materials such as polypropylene or polyethylene are recommended to limit interactions between PFOS and the exposure chamber/vessel (USEPA 2009). Notwithstanding these recommendations, approximately one third of the assessed PFOS chronic aquatic toxicity data (>240 values), including data for 11 of the 37 species selected for the current derivation, are based on nominal concentrations (see accompanying data spreadsheet for details). Review of the nominal and measured toxicity values indicated the nominal values were evenly spread throughout the measured dataset (i.e. did not lie at the extremes of the range of measured values), and the nominal and measured values were similar in concentration within a taxonomic group (where the data were available for comparison). For some species, only nominal concentrations were available and/or the nominal data represented lower concentrations such that their inclusion was more likely to achieve ecosystem protection. Consequently, toxicity values based on nominal concentrations that passed the quality assessment process were considered for the final dataset. A box plot comparing the nominal and measured concentrations is presented in Appendix B.

Zhang et al. (2013) assessed the effect of 5 d PFOS exposure on population growth of *B. calyciflorus*. Although classified as an acute exposure according to the definition provided by Warne et al. (2018), this exposure was considered as chronic for this species given that rotifers undergo a full life cycle within 2 to 5 days (Snell & Moffat 1992, Lavens & Sorgeloos 1996). A LOEC of 250 µg/L (28 d reproduction) (Zhang et al. 2013) for *B. calyciflorus* was selected for the DGV when a NOEC of 1 000 µg/L (5 d reproduction) (Zhang et al. 2013) was available. Although NOECs are preferred, a LOEC was selected because: the concentration was lower than the NOEC; it represents a true effect (i.e. a measurable effect that is statistically significantly different compared to controls); and the exposure was multigenerational (28 d) as opposed to two generations (5 d). A LOEC (90 d growth (length and weight – females) – 100 µg/L) was selected for use in the current derivation for a fish (*Xiphophorus helleri*) (Han & Fang 2010), where NOEC (90 d growth (length and weight – males; condition factor – males and females) of the same concentration as the LOEC from the same study were available. The LOEC was selected in this case because it represents a true effect and, after conversion, the concentration is below the available NOEC which achieves greater protection than if the NOEC were adopted. A LOEC (21-d reproduction – average offspring / total living offspring – 1 µg/L) was selected for use in the current derivation for the cladoceran *D. carinata* (Logeshwaran et al. 2021), where NOECs of 10 µg/L for survival and reproduction (days to first brood) were also available. The LOEC was selected because it represents a true effect and is the lowest available effects concentration for this species. Notably, the results presented in Table 2 of Logeshwaran et al. (2021) for *D. carinata* reproduction (average offspring in each brood) contained errors. A corrected version of Table 2 was published by Logeshwaran et al. (2025) and is reproduced in Appendix C.

For the amphibian *L. pipiens*, two low effect concentrations – a 30 d LOEC for development (0.0569 µg/L gosner stage (Flynn et al. 2021)) and a 30 d LOEC for growth (7.74 µg/L scaled mass index (Flynn et al. 2022)) – were lower than the lowest NOEC, but were not selected for use in the derivation. The gosner stage LOEC (Flynn et al. 2021) was based on spiked sediment rather than spiked water, and the authors noted that the effects may have been due to the combined sediment and water concentrations of PFOS. The growth LOEC (Flynn et al. 2022) was based on data that exhibited no concentration-response relationship. Consequently, the next highest value for *L. pipiens*, a 40-d NOEC (gosner stage development) of 10 µg/L was selected (Hoover et al. 2017).

For the macrophyte *M. sibiricum*, a 42 d growth EC10 of 600 µg/L (Hanson et al. 2005) was selected for the DGV over a 42 d growth NOEC of 300 µg/L (plant length) from the same study. Although the NOEC represents a lower toxicity value, EC10s were available for growth endpoints (plant length, root length, root number, dry mass) and indicated consistency in concentrations ranging from 700 µg/L to 1 500 µg/L. As stated in Section 2, root length was not considered ecologically relevant and, therefore, was not considered for use in the DGV. Given the widespread in the concentration range (control, 0.3 mg/L, 3 mg/L, 10 mg/L and 30 mg/L) and that most effects occurred between the 0.3 mg/L and 3 mg/L concentrations, the EC10s were considered better estimates of the effect threshold, whereas the NOECs were considered to be overly conservative.

Data for two macrophyte species (*M. sibiricum and M. spicatum*) were from a mesocosm study (Hanson et al. 2005) that was conducted outdoors in 12 000 L test chambers with no exogenous food source. Despite having a diverse taxonomic diversity, the indoor microcosm study of Sanderson et al. (2002) was not considered a mesocosm study because it was conducted in the laboratory using a relatively small volume (30 L) per test chamber volume, and an exogenous algal food source was added throughout the experiment.

Data for ten species were from studies with at least a 10-fold increase between test concentrations. These studies were used because they provided the only available data for these species or were the lowest toxicity values for these species. These species include: three macrophytes, *M. spicatum* and *M. sibiricum* (Hanson et al. 2005) and *Lemna gibba* (Boudreau et al. 2003a); three crustaceans, *Cyclops diaptomus*, *Cyclops cantcampus staphylinus* (Sanderson et al. 2002) and *D. carinata*

(Logeshwaran et al. 2021); an insect, *E. cyathigerum* (Bots et al. 2010); one fish, *O. latipes* (Ji et al. 2008); and two frogs, *L. pipiens* (Hoover et al. 2017) and *Xenopus laevis* (Degistz et al. 2024).

The hierarchy of statistical estimates of toxicity in Warne et al. (2018) preferences EC10s over NOECs, and NOECs over LOECs, but allows for the use of professional judgement in making such decisions. There were twelve EC/IC/LC10s (from eight taxonomic groups – amphibians, blue-green alga, crustaceans, diatom, fish, green alga, insects and macrophytes) available, which was sufficient to derive a DGV. However, many other statistical estimates of toxicity were also available, and which could potentially be included in the final dataset. NOECs were available for an additional 17 species (from seven taxonomic groups – amphibians, crustaceans, fish, green alga, insects, macrophytes and molluscs), six of which were lower than the lowest EC/IC10. Thus, to ensure adequate species protection, and to increase sample size and decrease uncertainty in the DGVs, the NOECs were included in the final dataset. One NOEC represented a '≥' value (≥11 µg/L for the eel *Anguilla anguilla*). No other data were available for this species. According to Warne et al. (2018), toxicity values expressed as greater than (>) should not be used if they are too far outside the existing data range. Review of this '≥' NOEC indicated that it was within the existing data range for the taxonomic group, and inclusion in the DGV derivation did not have a large influence on the final DGVs. This value was considered acceptable for inclusion in the derivation. For two of the 18 species for which NOECs were reported (two crustaceans, *D. magna* and *Moina macrocopa* (Ji et al. 2008), sufficient data were available to enable EC10s to be calculated and used for the final dataset over the published NOEC data.

In addition to the EC/IC10s and NOECs, LOECs were available for an additional seven species (from five taxonomic groups – amphibians, crustaceans, fish, platyhelminths and rotifers), of which two were lower than the lowest NOEC and all seven were within the lower (i.e. more sensitive) half of the dataset. For three of the six species, *Cyclops diaptomis* (Sanderson et al. 2002), *D. japonica* (Yuan et al. 2014) and *Lithobates catesbeiana* (Flynn et al. 2019), the LOECs were the only acceptable data available. For *X. helleri* (Han and Fang 2010) and *D. carinata* (Logeshwaran et al. 2021), as discussed above, the LOEC was selected over a NOEC of the same concentration as it achieves greater protection. For *O. latipes* (Ji et al. 2008) and for *B. calyciflorus* (Zhang et al. 2013), LOECs for F1 generation were selected over available NOECs as the duration exposures were longer, and the endpoints selected (reproduction) were considered more sensitive than survival. Thus, the exclusion of the LOECs was considered likely to result in DGVs that may be under-protective and, as such, they were included in the final dataset (after being converted to 'negligible effect' (i.e. EC10/NOEC-equivalent) concentrations by dividing by the default factor of 2.5). An EC50 value was available for the green alga *Desmodesmus communis* (Yang et al. 2014). As with the two crustaceans (*D. magna* and *M. macrocopa*) mentioned above, sufficient information was available to calculate an EC10, which was used in the final dataset.

For two species, *P. promelas* (Suski et al. 2021) and *Tetradesmus obliquus* (Zhang et al. 2012), NOECs were selected over LC10 and IC10 values, respectively. For *P. promelas* the NOEC for growth was selected over the LC10 as the NOEC was for the F1 generation in a multi-generation study, growth is considered a more sensitive endpoint compared to lethality and the effect concentration for the NOEC was significantly lower (44 µg/L compared to 3,500 µg/L). For *T. obliquus* the NOEC for reproduction was selected over the IC10 for growth as the NOEC was below the available IC10 which achieves greater protection than if the IC10 was adopted.

Although the lowest toxicity value for *D. rerio* was a LOEC of 0.734 µg/L (F2 generation, 90 d and 180 d post fertilisation growth) from Keiter et al. (2012), Dawson et al. (2024) concluded that the study was unreliable and the data from it should not be used in the derivation of the DGVs (see Dawson et al. 2024 for details). Instead, a F1 generation (weight and length) NOEC of 16 µg/L (Gust et al. 2024) was selected for use. While there was a lower effect concentration of 3.4 µg/L for the parental exposure (180 d length) NOEC available from the same study, the effect size for the corresponding LOEC of 24 µg/L (i.e. the concentration immediately above 3.4 µg/L) was very small (i.e. ~3% reduction in length relative to the control), which was considered to be not ecologically relevant. The PFOS Independent Review Committee (Dawson et al. 2024) suggested that consideration be given to the use of one of two geometric mean NOECs of 28 µg/L and 31 µg/L calculated by Pandelides et al. (2023) in a review of PFOS toxicity to *D. rerio*. However, neither of these values were selected as they were calculated using data from different exposure durations and for different endpoints, which does not follow the Warne et al. (2018) methodology for calculating geometric means.

As noted above, new EC/IC10s were estimated for *D. magna* (Ji et al. 2008), *M. macrocopa* (Ji et al. 2008) and *D. communis* (Yang et al. 2014) based on data reported in the primary source references, using the ecotoxicity testing statistical software package CETIS™ v2.1.5.5.

Based on the above decisions, the dataset included chronic toxicity values for 37 species from 11 taxonomic groups, comprising 15 EC/IC10s, 15 NOECs and seven LOECs. A modality assessment was performed on the dataset according to the method in Warne et al. (2018) and is provided in Appendix B. The dataset did not exhibit bimodality or multimodality; thus, the chronic toxicity data for 37 species from 11 taxonomic groups were used to derive the DGVs (Table 1). These species included: one diatom, one cyanobacterium, four species of green microalgae, three macrophytes, one rotifer, one flatworm, nine crustaceans, four insects, two molluscs, six fish and five amphibians. The toxicity values for these species span over five orders of magnitude. The dataset (37 values) consisted of toxicity values from a mix of single species, single generation studies and single species multigenerational studies: 32 values were from single species, one generation (or less) studies and five values were from single species, multigenerational (≥2 generation) studies (*D. rerio*, *O. latipes*, *P. promelas*, *P. pomilia* and *B. calyciflorus*). Additionally, of the 37 values, two (*C. diaptomus* and *Cyclops canthocamptus staphylinus*) were from a microcosm and two (*M. spicatum*and *M. sibiricum*) were from a mesocosm study.

A summary of the toxicity data (one value per species) used to calculate the DGVs for PFOS in freshwater is provided in Table 1; additional details are in Appendix A. Details of the data quality assessment and the data that passed the quality assessment are provided as supporting information.

Table 1 Summary of single chronic toxicity values, all species used to derive default guideline values for PFOS in freshwater

Taxonomic group	Species	Life stage	Duration (days)	Toxicity measure [a]	Toxicity value (µg/L)	Estimated chronic value (µg/L) [e]
Amphibian	*Bufo gargarizans*	Tadpole	30	LC10	2 000	2 000 [b]

Taxonomic group	Species	Life stage	Duration (days)	Toxicity measure [a]	Toxicity value (µg/L)	Estimated chronic value (µg/L) [e]
	Lithobates catesbeiana	Tadpole	72	LOEC (growth)	144	57.6 [c, f]
	Lithobates pipiens	Tadpole	40	NOEC (development)	10	10 [b]
	Xenopus laevis	Tadpole	36	NOEC (growth)	1 250	1 250 [b]
	Xenopus tropicalis	Embryo	150	NOEC (growth)	590	590 [b]
Blue–green alga	*Anabaena flos-aquae*	–	4	EC10 (growth, biomass)	82 000	82 000 [b]
Crustacean	*Ceriodaphnia dubia*	Neonate	6	EC10 (reproduction)	6 900	6 900 [b]
	Cyclops diaptomus	–	28	LOEC (survival)	1 000	400 [c]
	Cyclops canthocamptus staphylinus	–	35	NOEC (survival)	1 000	1 000 [b]
	Daphnia carinata	Neonate	21	LOEC (reproduction)	1	0.4 [c]
	Daphnia magna	Neonate	21	EC10 (intrinsic rate of population growth)	933	933 [b, f]
	Daphnia pulicaria	Neonate	21	NOEC (survival)	6 000	6 000 [b, f]
	Hyalella azteca	Juvenile	42	EC10 (reproduction)	700	700 [b]
	Moina macrocopa	Neonate	7	EC10 (reproduction)	90.6	90.6 [b, f]
	Procambarus fallax f. virginalis	Juvenile	28	NOEC (survival)	200	200 [b]
Diatom	*Navicula pelliculosa*	–	4	EC10 (growth, cell density)	<62 300	62 300 [b]
Fish	*Anguilla anguilla*	Adult	28	NOEC (growth)	≥11	11 [b]
	Danio rerio	Embryo, F1 generation	180	NOEC (growth)	16	16 [b]
	Oryzias latipes	Embryo, F1 generation	24	LOEC (reproduction)	10	4 [c, f]
	Pimephales promelas	Larvae, F1 generation	21	NOEC (growth)	44	44 [b]
	Pseudorasbora parva	Adult	30	LC10	2 120	2 120 [b]
	Xiphophorus helleri	Fry	90	LOEC (growth)	100	40 [c, f]
Green microalga	*Chlorella vulgaris*	–	4	IC10 (growth, biomass)	8 200	8 200 [b, f]

Taxonomic group	Species	Life stage	Duration (days)	Toxicity measure [a]	Toxicity value (µg/L)	Estimated chronic value (µg/L) [e]
	Desmodesmus communis	Exponential growth phase	4	EC10 (growth)	49 790	49 790 [b]
	Raphidocelis subcapitata	–	4	IC10 (growth, biomass)	5 300	5 300 [b, f]
	Tetradesmus obliquus	Exponential growth phase	4	NOEC (growth, biomass)	25 000	25 000 [b, g]
Insect	*Aedes aegypti*	Larva, 1st instar	40	NOEC (survival)	50	50 [b]
	Chironomus dilutus	Larva	16	LC10	1.36	1.36 [b]
	Enallagma cyathigerum	Larva	120	NOEC (development)	7.95	7.95 [b, d, f]
	Neocloeon triangulifer	Larva	14	EC10 (growth)	0.23	0.23 [b]
Macrophyte	*Lemna gibba*	–	42	NOEC (growth)	300	300 [b]
	Myriophyllum sibiricum	–	42	EC10 (growth)	600	600 [b]
	Myriophyllum spicatum	–	28	EC10 (growth)	3 300	3 300 [b]
Mollusc	*Lymnaea stagnalis*	Adult	21	NOEC (survival)	3 000	3 000 [b]
	Physa pomilia	Egg, F1 generation	44	NOEC (reproduction)	10 000	10 000 [b]
Flatworm	*Dugesia japonica*	Fragment	10	LOEC (reproduction)	500	200 [c, f]
Rotifer	*Brachionus calyciflorus*	Neonate	28	LOEC (population)	250	100 [c]

a The measure of toxicity being estimated/determined: EC/IC/LCx: x% effect/inhibition concentration; NOEC: no observed effect concentration; LOEC: lowest observed effect concentration.
b Actual chronic negligible effect value (i.e. NOEC or EC/IC/LC10).
c Default conversion from chronic LOEC to chronic negligible effect value: chronic LOEC ÷ 2.5 = chronic NOEC.
d Exposure concentrations converted from PFOS tetraethyl ammonium salt to PFOS anion.
e Estimated chronic values are reported to no more than three significant figures.
f Nominal concentration.
– : not stated/no data.

4.2 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 37 chronic PFOS freshwater toxicity data reported in Table 1 is shown in Figure 1. The SSD was plotted using shinyssdtools (V 0.4.0) software. The model was judged to provide a good fit to the data. Further details on the SSD fitting process are provided in Appendix D.

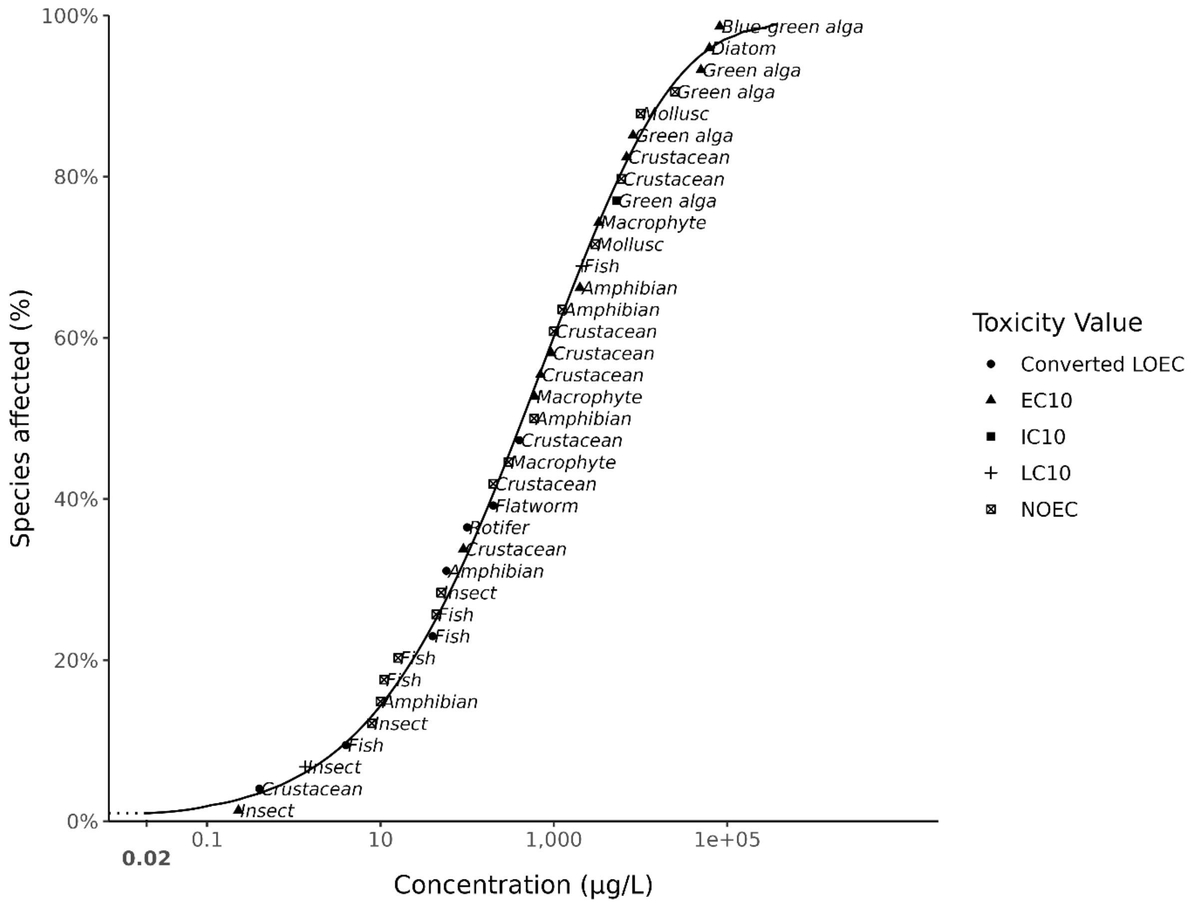


Figure 1 Species sensitivity distribution, PFOS in freshwater

4.3 Default guideline values and advice on protection of higher order biota

It is important that the DGVs (Table 2) and associated information in this technical brief are used in accordance with the detailed guidance provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality website (ANZG 2018).

4.3.1 Freshwater default guideline values

The PFOS freshwater DGVs for 99%, 95%, 90% and 80% species protection are shown in Table 2. The DGVs are expressed as the PFOS anion; therefore, monitoring data must be reported as the anion for comparison with the DGVs.

ANZG (2018) recommends a conservative approach when applying DGVs for bioaccumulative toxicants such as PFOS (e.g. 99% species protection DGV for slightly-to-moderately disturbed ecosystems rather than 95% species protection DGV) unless the DGVs have been derived based on a significant proportion of (a) long-term mesocosm/field effects data or (b) multigenerational laboratory data for a range of taxa (e.g. >30% of the dataset and for >3 taxa). The PFOS freshwater toxicity dataset included long-term mesocosm data or multigenerational laboratory toxicity data for only seven (or 19%) of the 37 species represented in the final dataset. Therefore, it is recommended that the conservative approach to protecting aquatic ecosystems is adopted, with the 99% species protection DGV being recommended for application to slightly-to moderately-disturbed freshwater ecosystems. Moreover, the ANZG (2018) principle of continual improvement dictates that, where the concentration of a contaminant is below the appropriate guideline value, the over-riding objective should be to continue to improve, or at least maintain, water quality (i.e. not to allow increases in concentration up to the guideline value).

The DGVs were compared with the freshwater chronic toxicity data that were compiled from the literature review and passed the quality assessment (i.e. 377 chronic values for 37 species). The theoretical protection offered by the DGVs for 99%, 95%, 90% and 80% species protection is considered to be sufficient for the protection of non-air breathing aquatic species from direct toxicity, but may not be protective of longer-term effects through bioaccumulation (see section 4.3.2 below).

Future aquatic toxicity data may lead to DGVs that meet the minimum requirements, as detailed in ANZG (2018), for relaxing the default approach of increased percent species protection, or a more mechanistically based approach may involve, for example, the future development of tissue residue guidelines.

Table 2 Default guideline values, PFOS anion in freshwater, very high reliability

Level of species protection (%)	DGV for PFOS anion in freshwater (µg/L) [a, b]
99	0.02
95	0.9
90	4
80	20

a Default guideline values were derived using the shinyssdtools (V 0.4.0) software, and have been rounded according to the details provided in Appendix E.
b The DGVs may not adequately protect higher order biota from effects due to bioaccumulation of PFOS. Refer to Section 4.3.2 for further details and guidance.

Environmental monitoring data for comparison with a DGV must be rounded according to the Australian Standard AS 2706-2003 (Standards Australia 2003) to the number of significant figures in the DGV (see ANZG (2018) for further guidance).

4.3.2 Protection of air-breathing aquatic species and air-breathing predatory species

It is important to note that the toxicant DGVs for aquatic ecosystem protection are not intended to specify species protection concentrations for air-breathing animals that live in aquatic ecosystems, or prey on aquatic organisms. Consequently, the DGVs may not account for effects which result from the biomagnification of toxicants such as PFOS in air breathing animals. For example, data collected as part of the Queensland Ambient PFAS Monitoring Program (Baddiley et al. 2020) indicated that biota in some environments are accumulating PFOS to levels that that would constitute a risk to mammalian and avian aquatic and terrestrial predators (on the basis of biota guideline values in the PFAS NEMP 3.0 (HEPA 2025) – see below) in waters where the concentrations (median of 0.0017 µg/L) were below the 99% species protection DGV of 0.02 µg/L. Moreover, a recent field study in South East Queensland (Vardy et al. 2025) found that PFOS concentrations as low as 0.0005 µg/L are associated with PFOS bioaccumulation in freshwater aquatic fauna (fish and macroinvertebrates) to concentrations that exceed the PFAS NEMP 3.0 (HEPA 2025) biota guideline values for mammals if only fauna (e.g. macroinvertebrates and fish) were considered as food sources (i.e. not also plants).

In relation to assessments for bioaccumulation, the PFAS NEMP 3.0 (HEPA 2025) recommends sampling and analysis of aquatic biota, and comparison of these data with the PFAS NEMP 3.0 biota guideline values discussed below. Based on the recent data from South East Queensland, Vardy et al. (2025) recommended an interim field derived water screening value of 0.0005 µg/L, above which biota tissue concentration data should be collected for risk assessments as part of a multiple lines of evidence approach. Based on the findings of Vardy et al. (2025), a biota screening threshold is provided in Table 3 which, if exceeded, should trigger an assessment of PFOS concentrations in aquatic biota tissues in order to assess the possible risk to mammalian and avian predators (see Table 4, below). The biota screening threshold is also supported by data from other studies available at the time of publication of the current technical brief, as discussed in Appendix F, and may be updated if significant additional data are published. The recommended use of the biota screening threshold represents a practical risk-based approach that is consistent with the advice in the PFAS NEMP 3.0 (HEPA 2025) that "*bioaccumulation in aquatic species cannot currently be accurately predicted based on water concentrations. This is evident from site assessment data, where bioaccumulation in fish tissues has been measured, despite water concentrations being at or below the laboratory detection limits. Therefore, to consider risks as a result of bioaccumulation, direct measurement of aquatic biota is the preferred approach where exposure pathways and sensitive receptors (ecological and/or human) exist*.". However, given the above advice in the PFAS NEMP 3.0, persons investigating potential impacts of PFAS on wildlife diet may choose to proceed directly to monitor concentrations in wildlife diet rather than utilise the biota screening threshold as a trigger for this.

Table 3 Biota screening threshold for PFOS in freshwater for triggering an assessment of PFOS concentrations in aquatic biota

Biota screening threshold (µg/L) [a]	Supporting references
0.0005	Vardy et al. (2025); also see Appendix F

a The threshold value relates to a concentration of PFOS in freshwater that, if exceeded, may result in PFOS concentrations in aquatic biota that exceed the PFAS NEMP 3.0 biota guideline values (HEPA 2025).

The PFAS NEMP 3.0 (HEPA 2025) includes advice on biota guideline values (also known as wildlife diet values) for the sum of PFOS and PFHxS, which are reproduced in Table 4, below. These were derived by using the approach in ECCC (2018), adjusted to consider the Australian context by using wildlife consumption data for a representative Australian mammalian species. Further information is available in the PFAS NEMP 3.0 (HEPA 2025), especially Supporting Document 2, which includes the details on the derivation of ecological guidelines for wildlife, and Supporting Document 5 on the applicability of the Canadian ecological guidelines (ECCC 2018) to Australia.

Table 4 Biota guideline values (from HEPA 2025)

Exposure scenario	Sum of PFOS and PFHxS [a]	Description
Ecological direct exposure for wildlife diet [b]	3.1 µg/kg	Mammalian diet – consumption of biota as wet weight food
	8.2 µg/kg [c]	Avian diet – consumption of biota as wet weight food
Ecological exposure protective of birds [d]	0.2 µg/g	Whole bird egg as wet weight

Note:

a Where the criteria refer to the sum of PFOS and PFHxS, this means concentrations of PFOS only, PFHxS only, and the sum of the two. The ECCC (2018) guidelines refer to the criterion for PFOS only; in the PFAS NEMP the guideline values for ecological direct exposure for wildlife diet refer to the levels of PFOS and PFHxS in food consumed by mammals or birds. This has been adapted to allow for uncertainties and potential similar properties and toxicities of PFHxS with PFOS.

b The guidelines for the sum of PFOS and PFHxS, and the guideline for PFOA, are based on the ECCC (2018) approach using representative local species. For the relevant tolerable daily intakes and approach adopted, see Supporting Document 2. For reference, TDIs (µg/kg bw/day) used in deriving the guidelines are: Mammalian – PFOA 1, PFOS + PFHxS 1.1; Avian – PFOA 2.3, PFOS + PFHxS 7.7. As the PFOA mammalian toxicity derivation is based on adverse effects that occur during development and lactation, food intake rates are based on lactating females. c. The avian diet value may not be protective of migratory wading birds that have a high food intake due to the need to gain weight rapidly. d. Adapted from ECCC (2018) using an additional uncertainty factor. The adjusted uncertainty factor is 100 while the uncertainty factor used in ECCC (2018) was 10. This guideline value is to be used on sampled bird eggs to assess risk to sensitive avian ecological receptors. The guideline value for ecological exposure protective of birds refers to the levels of PFOS and PFHxS in bird eggs. Measurements are shown as micrograms per gram (µg/g) as reported in research on these issues.

The NEMP PFAS 3.0 (HEPA 2025) and Supporting Document 2 for the bird tissue egg value, provide guidance on sampling wildlife tissues. Further work that would assist in this area is underway, which will be published separately when it is available.

4.3.3 PFOS Precursors and PFAS mixtures

Environmental assessments should also consider the presence of PFOS precursors in water (HEPA 2025), as biotransformation of precursors to PFOS is an additional contribution (potentially isomer-specific) to PFOS body burden as observed by *in vivo* and *in vitro* experiments (Chen et al. 2015). Moreover, there is an inherent uncertainty in the level of protection of the PFOS DGVs when other PFAS are present. For situations where multiple PFAS are present, refer to the ANZG (2018) guidance for assessing chemical mixtures.

Environmental regulators or local catchment managers may be able to provide additional jurisdiction-specific information and guidance.

4.4 Reliability classification

The PFOS freshwater DGVs have a very high reliability classification (Warne et al. 2018) based on the outcomes for the following three criteria:

- sample size—37 (preferred)
- type of toxicity data—chronic negligible effect and estimated negligible effect values
- SSD model fit—good.

It is important to note that the reliability classification applies only to the DGVs and not the biota screening threshold, for which there is no reliability classification.

Glossary

Term	Definition
acute toxicity	A lethal or adverse sub-lethal effect that occurs as the result of a short exposure period to a chemical relative to the organism's life span.
bioaccumulation	The process by which chemical substances are accumulated by aquatic organisms by all routes of exposures (dietary and the ambient environment).
bioaccumulation factor (BAF)	The ratio of the concentration of a contaminant in an organism to its concentration in the ambient environment at a steady state, where the organism can take in the contaminant through ingestion with its food as well as through direct contact. It can be expressed on a wet weight, dry weight or lipid weight basis.
bioconcentration	The process by which chemical substances are accumulated by aquatic organisms via absorption through the respiratory and dermal surfaces (dietary exposure is excluded).
bioconcentration factor (BCF)	The ratio of the concentration of a contaminant in an organism to its concentration in the ambient water (or sediment) at a steady state. It can be expressed on a wet weight, dry weight or lipid weight basis.
biomagnification	The process by which tissue concentrations of chemicals increase as the chemical passes up through two or more trophic levels in a food chain.
biomagnification factor (BMF)	The ratio of contaminant concentration in an organism to that in its diet at steady state.
chronic toxicity	A lethal or sublethal adverse effect that occurs after exposure to a chemical for a period of time that is a substantial portion of the organism's life span or an adverse effect on a sensitive early life stage.
default guideline value (DGV)	A guideline value recommended for generic application in the absence of a more specific guideline value (e.g. a site-specific guideline value) in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Formerly known as 'trigger values'.
DOM	Dissolved organic matter.
EC50 (median effective concentration)	The concentration of a substance in water or sediment that is estimated to produce a 50% change in the response being measured or a certain effect in 50% of the test organisms relative to the control response, under specified conditions.
ECx	The concentration of a substance in water or sediment that is estimated to produce an x% change in the response being measured or a certain effect in x% of the test organisms, under specified conditions.
endpoint	The specific response of an organism that is measured in a toxicity test (e.g. mortality, growth, a particular biomarker).
fulvic acid	One of two classes of natural acidic organic polymer that can be extracted from humus in soil, sediment, or aquatic environments. Fulvic acids are soluble in water at all pH values.
Fx	Filial generation, where x represents the number of the generations since the parent generation (e.g. F1 represents offspring of the parent generation, F2 represents offspring of the F1 generation).
humic acid	One of two classes of natural acidic organic polymer that can be extracted from humus in soil, sediment, or aquatic environments. Humic acids are insoluble at very low pH (<2) but soluble at higher pH values.
humic substances	Organic substances only partially broken down that occur in water mainly in a colloidal state. They can be divided into three main categories: humic acids, fulvic acids and humin.
ICx	The concentration of a substance in water or sediment that is estimated to produce an x% inhibition of the response being measured in test organisms relative to the control response, under specified conditions.

Term	Definition
K_{ow} or P_{ow}	The octanol:water partition coefficient. The ratio of a chemical's solubilities in n-octanol and water at equilibrium. The logarithm of P_{OW} is used as an indication of a chemical's propensity for bioconcentration by aquatic organisms.
LC50 (median lethal concentration)	The concentration of a substance in water or sediment that is estimated to be lethal to 50% of a group of test organisms, relative to the control response, under specified conditions.
LOEC (lowest observed effect concentration)	The lowest concentration of a material used in a toxicity test that has a statistically significant adverse effect on the exposed population of test organisms as compared with the controls.
macrophyte	A member of the macroscopic plant life of an area, especially of a body of water; large aquatic plant.
NOEC (no observed effect concentration)	The highest concentration of a material used in a toxicity test that has no statistically significant adverse effect on the exposed population of test organisms as compared with the controls.
PBT	Persistent, bioaccumulative and toxic.
PFAS	Perfluoroalkyl and polyfluoroalkyl substances, containing the perfluoroalkyl moiety.
PFOS	Perfluorooctane sulfonate.
pK_a	The acid dissociation constant. A quantitative measure of the strength of an acid in solution, and the equilibrium constant for the acid-base dissociation reaction.
POPs	Persistent Organic Pollutants. As defined under The Stockholm Convention on Persistent Organic Pollutants, POPs are organic compounds that possess toxic properties, resist degradation, bioaccumulate and are transported, through air, water and migratory species, across international boundaries and deposited far from their place of release, where they accumulate in terrestrial and aquatic ecosystems.
PPAR-α	Peroxisome proliferator activated receptor-alpha. PPARs are nuclear hormone receptors involved in lipid and lipoprotein metabolism (Lankadurai et al. 2013, Zhang et al. 2019).
species (biological)	A group of organisms that resemble each other to a greater degree than members of other groups and that form a reproductively isolated group that will not produce viable offspring if bred with members of another group.
species sensitivity distribution (SSD)	A method that plots the cumulative frequency of species' sensitivities to a toxicant and fits a statistical distribution to the data. From the distribution, the concentration that should theoretically protect a selected percentage of species can be determined.
toxicity	The inherent potential or capacity of a material to cause adverse effects in a living organism.
toxicity test	The means by which the toxicity of a chemical or other test material is determined. A toxicity test is used to measure the degree of response produced by exposure to a specific level of stimulus (or concentration of chemical) for a specified test period.

Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values

Table A 1 Summary, chronic toxicity data that passed the screening and quality assessment processes, PFOS in freshwater

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure [a] (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L)	Reference
Amphibian	*Bufo gargarizans*	Tadpole	30	LC10	Dechlorinated tap water	22±2	7.0±0.5	2 000 [b]	Yang et al. 2014
	Lithobates catesbeiana	Tadpole, Gosner stage 25	72	LOEC (growth)	Filtered well water	21	–	144 [c]	Flynn et al. 2019
	Lithobates pipiens	Tadpole	40	NOEC (development)	UV irradiated well water	20±2	–	10 [b]	Hoover et al. 2017
	Xenopus laevis	Tadpole	36	NOEC (growth)	Lake Superior Water	19-21	–	1 250 [b]	Degitz et al. 2024
	Xenopus tropicalis	Embryo, NF stage 10	150	NOEC (growth)	Dechlorinated tap water	25.5–26.5	7.5±0.3	590 [b]	Fort et al. 2019
Blue-green alga	*Anabaena flos-aquae*	–	4	EC10 (growth, biomass)	Algae culture medium and reverse osmosis-purified well water	22.8–23.8	7.4	82 000 [b]	OECD 2002
Crustacean	*Ceriodaphnia dubia*	Neonate	6	EC10 (reproduction)	Moderately hard reconstituted water	24-26	6.77-8.02	6 900	Krupa et al. 2022

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure [a] (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L)	Reference
	Cyclops diaptomus	–	28	LOEC (survival)	Natural pond water	10–18 (increased 2°C per week to 18°C)	8.28–8.37	1 000 [c, e]	Sanderson et al. 2002
	Cyclops canthocamptus staphylinus	–	35	NOEC (survival)	Natural pond water	10–18 (increased 2°C per week to 18°C)	8.28–8.37	1 000 [b, e]	Sanderson et al. 2002
	Daphnia carinata	Neonate	21	LOEC (reproduction)	Cladoceran growth medium	19-23	-	1 [c]	Logeshwaran et al. 2021, 2025
	Daphnia magna	Neonate, <24 h old	21	EC10 (intrinsic rate of population growth)	Moderately hard reconstituted water	25	–	933 [c]	Ji et al. 2008
	Daphnia pulicaria	Neonate, >24 h old	21	NOEC (survival)	Moderately hard clean well water	21±1	–	6 000 [b, g]	Sanderson et al. 2004
	Hyalella azteca	Juvenile	42	EC10 (reproduction)	Carbon-filtered tap water	22-24	7.12-8.10	700 [b]	Krupa et al. 2022
	Moina macrocopa	Neonate, <24 h old	7	EC10 (reproduction)	Moderately hard reconstituted water (USEPA 2002a)	25±1	–	90.6 [g]	Ji et al. 2008
	Procambarus fallax f. virginalis	Juvenile	28	NOEC (survival)	Moderately hard water	20±1	–	200 [b]	Funkhouser 2014

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure [a] (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L)	Reference
Diatom	*Navicula pelliculosa*	–	4	EC10 (growth, cell density)	Algae culture medium and reverse osmosis-purified well water	23.1–24.6	7.5–7.7	<62 300 [b]	OECD 2002
Fish	*Anguilla anguilla*	Adult	28	NOEC (growth)	Tap water	20±2	–	≥11 [b]	Roland et al. 2014
	Danio rerio	Embryo, F1 generation	180	NOEC (growth)	Deionised water and tap water	25-36	7.2	16	Gust et al. 2024
	Oryzias latipes	Embryo, F1 generation	24	LOEC (reproduction)	Dechlorinated tap water	25±1	–	10 [c, g]	Ji et al. 2008
	Pimephales promelas	Adult, F1 generation	21	NOEC (growth)	Dechlorinated tap water	25	7.9	44	Suski et al. 2021
	Pseudorasbora parva	Adult	30	EC10 (survival)	Dechlorinated tap water	22±2	7.0±0.5	2 120 [b]	Yang et al. 2014
	Xiphophorus helleri	Fry	90	LOEC (growth)	Dechlorinated tap water	27±1	–	100 [c, g]	Han & Fang 2010
Green alga	*Chlorella vulgaris*	–	4	IC10 (growth, biomass)	Bristol's algal growing media in laboratory-grade distilled water	23±1	–	8 200 [b, g]	Boudreau et al. 2003a
	Desmodesmus communis	Exponential growth phase	4	EC10 (growth)	M4 medium in dechlorinated tap water	22±2	7.0±0.5	47 790 [b]	Yang et al. 2014

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure [a] (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L)	Reference
	Raphidocelis subcapitata	–	4	IC10 (growth, biomass)	Bristol's algal growing media in laboratory-grade distilled water	23±1	–	5 300 [b, g]	Boudreau et al. 2003a
	Tetradesmus obliquus	Exponential growth phase	4	NOEC (growth, biomass)	HB-4 culture medium	24	–	25 000 [b, g]	Zhang et al. 2012
Insect	*Aedes aegypti*	Larva, 1st instar	40	NOEC (survival)	Moderately hard water deionised laboratory water	25	–	50 [b]	Olson 2017
	Chironomus dilutus	Larva	16	LC10	Moderately hard reconstituted water	23	–	1.36	McCarthy et al. 2021
	Enallagma cyathigerum	Larva	120	NOEC (development)	Dechlorinated tap water	21	>7.5	7.95 [d, g]	Bots et al. 2010
	Neocloeon triangulifer	Larva	14	EC10 (growth)	Hard water	22-24	8.3	0.23	Soucek et al. 2023
Macrophyte	*Lemna gibba*	–	42	NOEC (growth)	Irrigation pond water	15.9–20.5	8.3–8.8	300 [b]	Boudreau et al. 2003b
	Myriophyllum sibiricum	–	42	EC10 (growth)	Irrigation pond water	–	–	600 [b, f]	Hanson et al. 2005
	Myriophyllum spicatum	–	28	EC10 (growth)	Irrigation pond water	–	–	3 300 [b, f]	Hanson et al. 2005
Mollusc	*Lymnaea stagnalis*	Adult	21	NOEC (survival)	Aerated synthetic fresh water	20±1	–	3 000 [b]	Olson 2017

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure [a] (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L)	Reference
	Physa pomilia	Egg, F1 generation	44	NOEC (reproduction)	Moderately hard water	22±1	–	10 000 [b]	Funkhouser 2014
Flatworm	*Dugesia japonica*	Fragment	10	LOEC (reproduction)	Aerated tap water	22	–	500 [c, g]	Yuan et al. 2014
Rotifer	*Brachionus calyciflorus*	Neonate, <2 h old	28	LOEC (population)	USEPA (2002b) culture medium for algae	20	–	250 [c]	Zhang et al. 2013

a The measure of toxicity being estimated/determined: EC/IC/LCx: x% effect or inhibition concentration; NOEC: no observed effect concentration; LOEC: lowest observed effect concentration.
b Value included in the dataset to derive the default guideline values.
c Value included in the dataset to derive the default guideline values, after application of a default chronic LOEC to negligible effect value conversion factor of 2.5.
d Exposure concentrations converted from PFOS tetraethyl ammonium salt to PFOS anion.
e Values taken from a microcosm study.
f Values taken from a mesocosm study.
g Nominal concentration.
– : not stated / no data.
Note: *Lithobates catesbeiana* (formerly *Rana catesbeiana*), *Lithobates pipiens* (formerly *Rana pipiens*), *Xenopus tropicalis* (formerly *Silurana tropicalis*), *Desmodesmus communis* (formerly *Scenedesmus quadricauda*), *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata* and *Selenestrum capricornutum*), *Tetradesmus obliquus* (formerly *Scenedesmus obliquus* and *Acutodesmus obliquus*)

Appendix B: Discussion of modality and concentrations for PFOS dataset

Modality assessment

A modality assessment was undertaken for perfluorooctane sulfonate (PFOS) according to the four questions stipulated in Warne et al. (2018). These questions and their answers are listed below.

Is there a specific mode of action that could result in taxa-specific sensitivity?
The mode of action of PFOS is not fully understood. The information on possible modes of action for PFOS predominantly relates to animals, with little to no information for plants. Modes of action that have been proposed for PFOS include:

- activation of PPAR-α (Bots et al. 2010, Borg & Håkansson 2012, ECCC 2018)
- alteration of membrane properties such as permeability and fluidity (Jones et al. 2003, Lankadurai et al. 2013)
- binding to proteins such as serum albumin, with weaker binding to proteins involved in fatty acid transport and metabolism (Jones et al. 2003)
- uncoupling of oxidative phosphorylation (Moermond et al. 2010, ECCC 2018)
- inhibition of intercellular gap junctions (Jones et al. 2003, ECCC 2018)
- endocrine effects (Ankley et al. 2005, Borg & Håkansson 2012, Keiter et al. 2012)
- interaction with transporter proteins (Keiter et al. 2012).

Although these modes of action are mostly reported in animal studies, the alteration of membrane properties and inhibition of intercellular junctions may be relevant to plants. The mode of action of PFOS in plants is not well understood (Hanson et al. 2005).

Based on mode of action alone, there is no clear reason to suspect large differences in taxa-specific sensitivity.

Does the dataset suggest bimodality?
Visual representation of the data, calculation of the bimodality coefficient (BC), and consideration of the range in the effect concentrations are recommended lines of evidence in evaluating whether bimodality or multimodality of the dataset is apparent. This is discussed as follows.

- The raw effect concentration data (Figure B 1) appear to follow a log-normal distribution, and the log-transformed data (Figure B 1) appear to follow a normal distribution. The distributions are typical of concentration-based data (Warne et al. 2018).
- Data that span large ranges (>4 orders of magnitude) indicate potential for underlying bimodality or multimodality (Warne et al. 2018); the PFOS data span >4 orders of magnitude.
- When the BC is greater than 0.555, it indicates that the data do not follow a normal distribution and may be bimodal; the BC of the log-transformed data is 0.396, which does not support bimodality.

- In addition, the lognormal-lognormal distribution fitted by shinyssdtools can be used to assess if a dataset is bimodal (as one line of evidence). This model received a very low weight (Appendix D) despite the very high sample size and, therefore, provides evidence that the dataset is unimodal.

Based on these lines of evidence, the dataset appears to be more likely to be unimodal than bimodal.

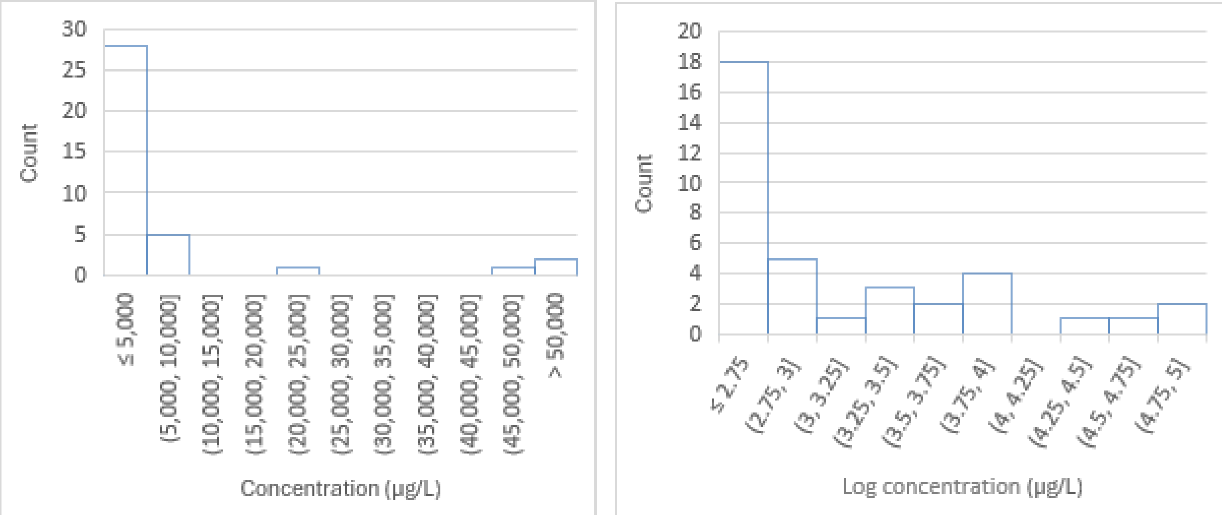
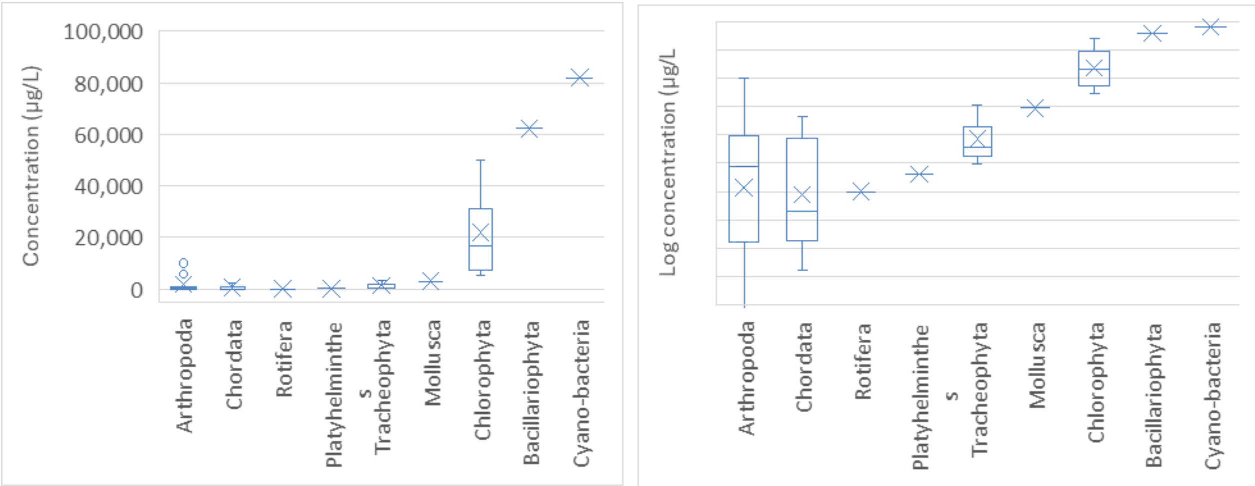


Figure B 1 Histogram, raw (left) and log transformed (right) PFOS data

Do data show taxa-specific sensitivity (i.e. through distinct groupings of different taxa types)?
The potential for taxa-specific sensitivity in the data was examined using box plots of the PFOS data with the grouping variables of phyla, major types of organisms, and feeding strategy (autotrophs and heterotrophs). In addition to these, nominal and measured concentrations were compared.

Figure B 2 indicates the following phyla had similar sensitivities to PFOS: Arthropoda, n=14; Chordata, n=11; Mollusca, n=1; Platyhelminthes, n=1; Rotifera, n=1; Tracheophyta, n=3. In contrast, some phyla show reduced sensitivity to PFOS, namely: Bacillariophyta, n=1; Cyanobacteria, n=1; Chlorophyta, n=4.

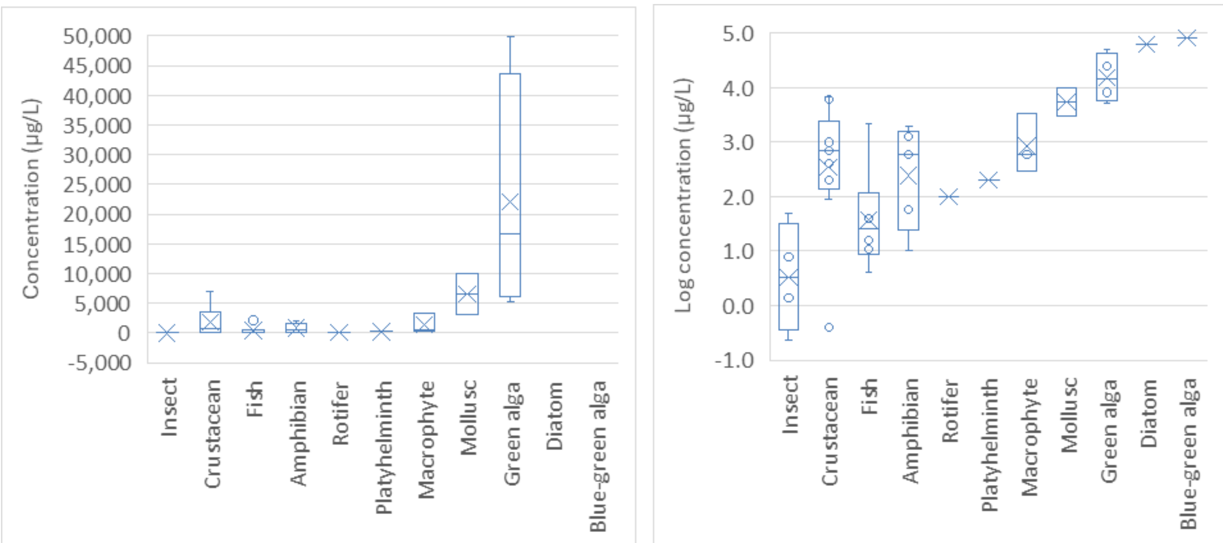
The less sensitive phyla are simple (planktonic) plants. The sample sizes for the planktonic plants (i.e. Ochrophyta and Cyanobacteria) are too small to draw conclusions regarding differences in sensitivity. Furthermore, without a confirmed mode of action, the reason for the apparent differences in sensitivity between the phyla is unknown.



Note: asterisk represents an outlying value >1.5x the interquartile range; open circle represents an outlying value >3x the interquartile range.

Figure B 2 Box plots, raw (left) and log transformed (right) data for PFOS toxicity, grouped by phyla

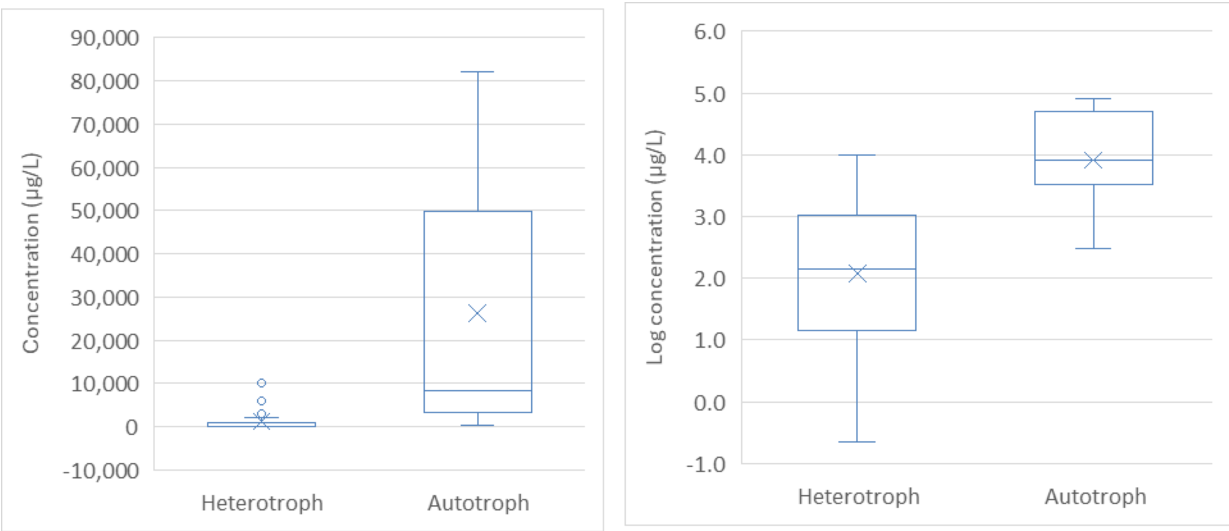
Figure B 3 presents box plots of ‘major types of organisms’ as defined in Warne et al. (2018). These plots indicate that invertebrates (insects, crustaceans, rotifers, platyhelminths and molluscs) (n=17) and vertebrates (fish and amphibians) (n=11) are generally more sensitive to PFOS than algae (green and blue-green alga) (n=5), diatoms (n=1) and plants (macrophytes) (n=3). However, the sample size for cyanobacteria is too small to draw conclusions regarding differences in sensitivity and, without a confirmed mode of action, the reason for the apparent differences in sensitivity between the types of organisms is unknown.



Note: asterisk represents an outlying value >1.5x the interquartile range; open circle represents an outlying value >3x the interquartile range.

Figure B 3 Box plots, raw (left) and log transformed (right) data for PFOS toxicity, grouped by ‘major types of organisms’

The box plots comparing feeding strategy (Figure B 4) indicate that heterotrophs are more sensitive to PFOS than autotrophs. The sample size for heterotrophs (n=28) is larger than for autotrophs (n=9), and the inclusion of the less sensitive planktonic plants (Chlorophyta, Bacillariophyta, and Cyanobacteria) in the autotroph group increases the separation between autotrophs and heterotrophs. However, without a confirmed mode of action, the reason for the apparent differences in sensitivity between the groups is unknown.



Note: asterisk represents an outlying value >1.5x the interquartile range; open circle represents an outlying value >3x the interquartile range.

Figure B 4 Box plots, raw (left) and log transformed (right) data for PFOS toxicity, grouped by 'feeding strategy'

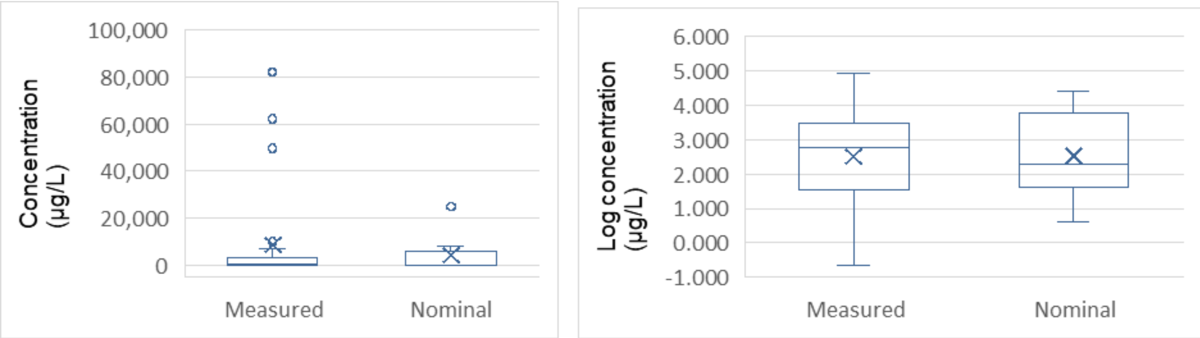
Is it likely that indications of bimodality or multimodality or distinct clustering of taxa groups are not due to artefacts of data selection, small sample size, test procedures, or other reasons unrelated to a specific mode of action?

Review of the data did not indicate discernible trends associated with artefacts of data selection, test procedures, or other reasons unrelated to a specific mode of action.

Although autotrophs may, in general, be less sensitive to PFOS than heterotrophs, this does not appear to result in a bimodal distribution of species sensitivity. The weight of evidence supports use of the 37 species identified in preparation of the SSD.

Nominal and measured concentrations

Box plots of nominal (n=11) and measured (n=26) concentrations are presented in Figure B 5 to inform the decision-making process for inclusion or exclusion of data based on nominal concentrations. Nominal concentrations span a similar interquartile range to measured concentrations and are within the maximum and minimum values of the measured concentrations. Although the mean and median for nominal concentrations are lower compared to those of the measured concentrations (Figure B 5), the differences are small. The fact that the nominal concentration data were neither consistently lower or higher than the measured concentration data led to the conclusion to not exclude toxicity values based on nominal concentrations from the final dataset.



Note: open circle represents an outlying value >3x the interquartile range, the "X" represents the mean and the horizontal line within the box represents the median.

Figure B 5 Box plots, raw (left) and log transformed (right) data for PFOS toxicity, grouped by 'nominal' and 'measured' concentrations

Appendix C: Corrected *Daphnia carinata* data reported in Logeshwaran et al. (2025)

Treatments	Mortality (%)		Days to first brood (d)		Average offspring in each brood (ind)		Total living offspring (ind)	
Control	0.0[a]		7.28 ± 0.84[a]		24.59 ± 1.40[a]		175.24 ± 25.87[a]	
Conc. (mg L^{-1})	**PFOA**	**PFOS**	**PFOA**	**PFOS**	**PFOA**	**PFOS**	**PFOA**	**PFOS**
0.001	0.0[a]	0.0[a]	7.42 ± 0.70[a]	8.05 ± 0.56[a]	24.14 ± 1.36[a]	13.08 ± 1.42[b]	167.47 ± 24.31[a]	81.58 ± 7.50[b]
0.01	0.0[a]	2.5 ± 0.61[ab]	8.03 ± 0.99[a]	10.71 ± 0.68[b]	23.51 ± 1.58[ab]	12.17 ± 1.22[c]	139.41 ± 8.82[ab]	48.63 ± 5.65[c]
0.1	4.0 ± 1.21[b]	9.47 ± 0.64[b]	9.07 ± 0.31[ab]	11.43 ± 0.19[b]	22.93 ± 1.35[b]	11.05 ± 2.17[d]	100.66 ± 2.88[bc]	8.01 ± 2.89[d]
1.0	9.33 ± 1.52[c]	13.87 ± 1.86[c]	10.74 ± 0.70[bc]	nd	12.08 ± 1.58 [cd]	nd	73.01 ± 3.48[cd]	nd
10.0	12.26 ± 1.00[d]	23.33 ± 2.51[d]	12.04 ± 0.30[c]	nd	11.47 ± 1.19[d]	nd	40.70 ± 1.90[d]	nd

Appendix D: Details of the shinyssdtools SSD fitting process

The SSD software package shinyssdtools (V 0.3.1) uses a model averaging approach to generating a SSD (as described by Fox et al. 2021). Figure C 1 shows the full set of default distributions fitted to the PFOS freshwater toxicity dataset, while Table C 1 provides the relevant goodness of fit and weighting estimates for each of the distributions. The final, model-averaged SSD is shown in Figure 1 of the main report.

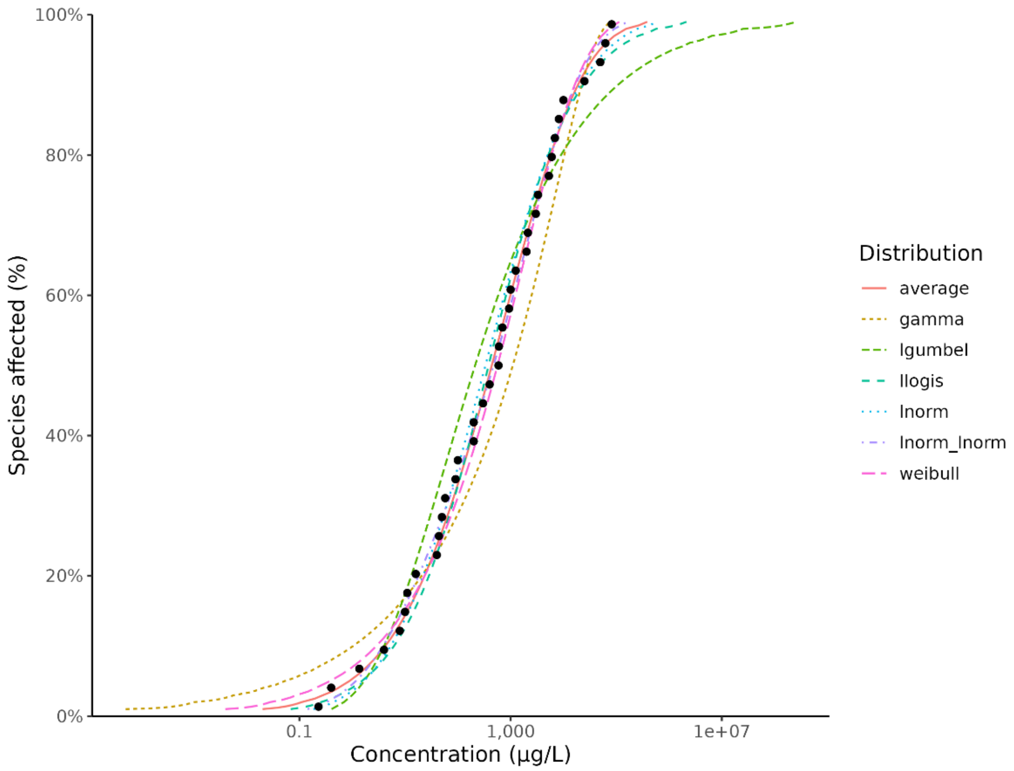


Figure C 1 SSDs for set of six default distributions used to model the PFOS freshwater toxicity dataset

Table C 1 Goodness of fit and weighting estimates for six default distributions used in model averaging for the PFOS freshwater toxicity dataset

Distribution	Akaike's Information Criterion (AIC)	Corrected Akaike's Information Criterion (AICc)	Delta	Weight
Gamma	630	631	5.260	0.030
Weibull	625	625	0.000	0.419
Log normal	625	626	0.307	0.359
Log logistic	627	627	1.880	0.163
Log gumbel	633	634	8.350	0.006
Log normal - Log normal	629	631	5.910	0.022

Appendix E: Assessment of significant figures for default guideline values

The number of significant figures to which DGVs are reported is determined by the degree of uncertainty in the values, based on their standard errors, according to the following rule (ANZG 2018):

The significant figure to which a DGV is reported must correspond to the place of the first significant figure in the standard error of the DGV. Where the standard error is greater than the DGV, the DGV must be reported to one significant figure.

For the PFOS in freshwater DGVs, the raw PC values and their standard errors are shown in Table D 1. Standard errors were determined using shinyssdtools V 0.3.1 with the default set of distributions (see Appendix C). For all PC values, the standard errors were greater than the raw PC values, dictating that all the DGVs are reported to one significant figure.

Table D 1 Assessment of appropriate number of significant figures, PFOS in freshwater

Level of species protection	Raw PC value (µg/L)	Standard error	Interpretation of significant figures rule	Final DGV (µg/L)
99	0.020	0.369	SE > PCx value; therefore, report DGV to one significant figure	0.02
95	0.883	2.304	SE > PCx value; therefore, report DGV to one significant figure	0.9
90	4.226	6.827	SE > PCx value; therefore, report DGV to one significant figure	4
80	23.368	26.682	SE > PCx value; therefore, report DGV to one significant figure	20

Appendix F: Supporting evidence for the screening threshold for PFOS in freshwater

The interim field derived water screening value proposed by Vardy et al. (2025) to protect air-breathing wildlife from PFOS in their diet was developed from biota data collected across eight different sites in Southeast Queensland nine months apart. A number of studies in other jurisdictions of a similar nature have been undertaken, with the associated data sets also supporting the findings of Vardy et al (2025), as described below.

Burkhard (2021) proposed a quality assessment framework for evaluating relevant studies that reported bioconcentration and bioaccumulation factors for PFAS. For the purposes of this summary, freshwater datasets containing whole-organism data alongside corresponding water concentrations that were classified as high quality according to Burkhard's criteria were selected for analysis. To ensure alignment with the methodology of Vardy et al. (2025), only studies involving the same species collected from multiple sites were included. Furthermore, only data pertaining to fish and macroinvertebrates were considered, consistent with the scope of the Vardy et al. (2015) study. Three high-quality studies identified by Burkhard (2021) met these criteria (1-3). One study published after Burkhard (2021) was also included in (Brase et al 2022). This study was assessed as being high quality using the Burkhard criteria. The four studies are briefly summarised, below.

1. Awad et al. (2011) conducted sampling of fish and water across ten sites in waterways northwest of Lake Ontario, Canada, over a three-year period. Yearling common shiners (*Notropis cornutus)* were collected, and composite samples comprising five to ten whole fish of similar size were analysed.
2. De Silva et al. (2011) collected water and fish samples from the Great Lakes, Canada, analyzing individual specimens of lake trout (*Salvelinus namaycush*) and walleye (*Sander vitreus*).
3. Lescord et al. (2015) sampled juvenile char (*Salvelinus alpinus*) (individual fish), benthic macroinvertebrates (chironomids - pooled), and water from six high Arctic lakes in Canada.
4. Brase et al. (2022) examined PFOS in eight freshwater benthic macroinvertebrate (BMI) taxa across four sites in the Hudson River watershed near Albany, New York.

Data for fish and for macroinvertebrate samples were compiled with corresponding water concentrations and compared to the screening threshold, the ANZG 99% species protection DGV and the NEMP biota guideline values (mammalian and avian) (Figures 1 and 2). Data from the different BMI taxa in the Brase et al. (2022) study were averaged at each site. Concentrations of PFOS in water were plotted against the average concentration of PFOS reported in fish at each site for each study. Of the fish data compiled from the collated studies (n=51 sites), 12% of biota results were at or below the screening threshold and below the mammalian biota guideline value, 53% fell between the screening threshold and the 99% DGV, with 96% of this data subset exceeding the mammalian biota guideline value, and 92% exceeding the avian biota guideline value. This demonstrates that the 99% species protection DGV will not protect against bioaccumulation in aquatic organisms to a level that exceeds biota guideline values. The additional fish data from the four studies were consistent with the data from Vardy et al. (2025) (Figure 1). For macroinvertebrates, a smaller number of extra data (n=4) were available, and were plotted with the Vardy et al. (2025) data (n=16) and found to be consistent (Figure 2). For macroinvertebrates, 10% of the data were below the screening threshold and below the mammalian biota guideline value, while 10% were above the screening threshold and below the mammalian biota guideline value. Eighty percent of the macroinvertebrates collected at different sites had PFOS concentrations above the mammalian biota guideline value, at sites where the water concentrations exceeded the screening threshold but were below the ANZG 99% species protection DGV.

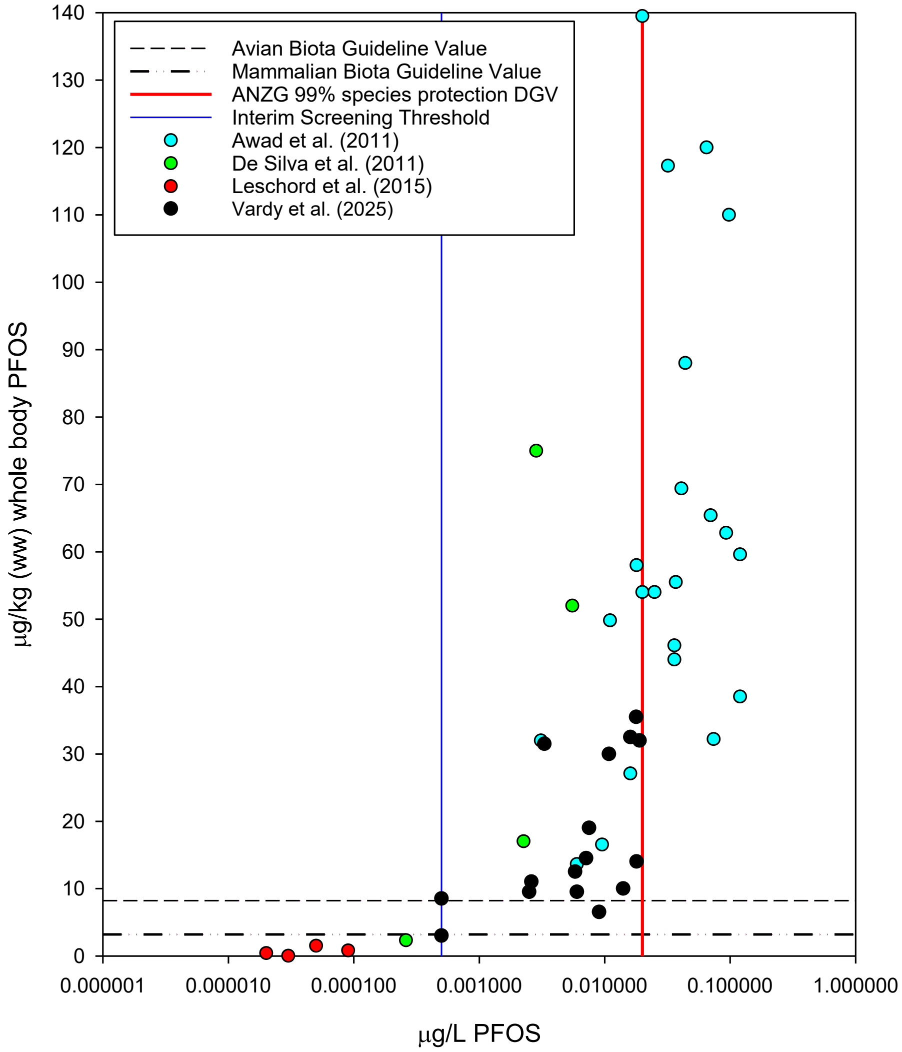


Figure F 1 Concentration of PFOS in water against the concentration of PFOS in fish samples from studies undertaken by Awad et al. (2011), De Silva et al. (2011), Lescord et al. (2015) and Vardy et al. (2025). Grey shaded area indicates samples collected that were above the screening threshold but lower than the mammalian biota guideline value (3.1 ug/kg PFOS w.w.).

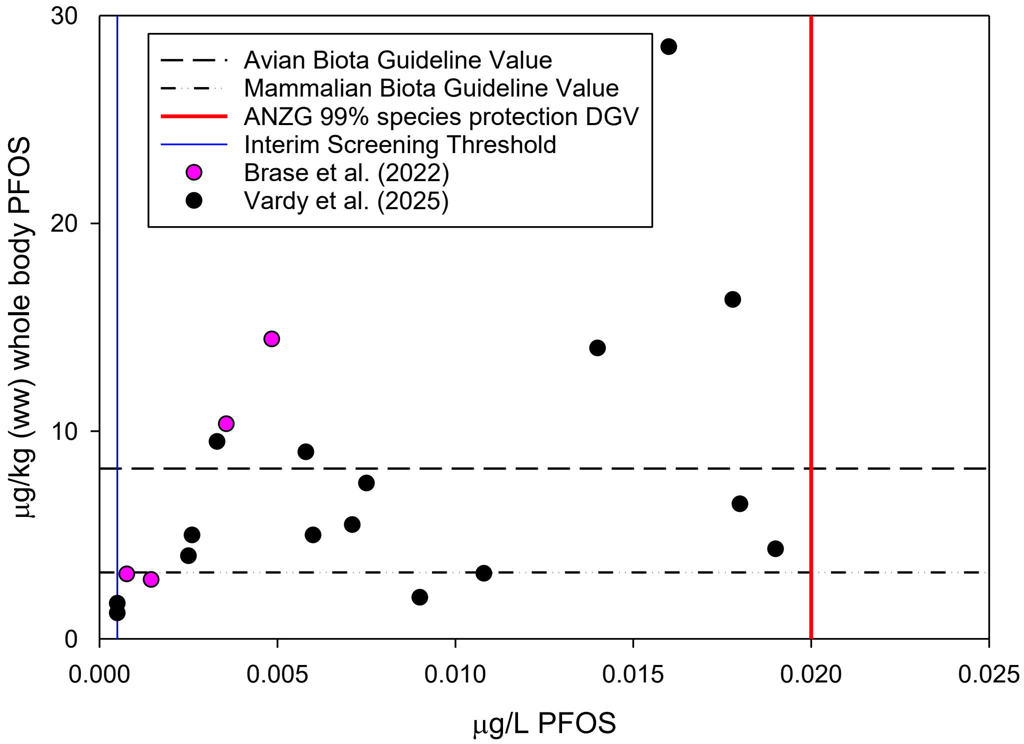


Figure F 2 Concentration of PFOS in water against the concentration of PFOS in macroinvertebrate samples from studies undertaken by Brase et al (2022) and Vardy et al (2025). Grey shaded area indicate samples collected that were above the screening threshold but lower than the mammalian biota guideline value (3.1 ug/kg w.w).

Overall, the data provided by studies in other jurisdictions aligns with the data provided in Vardy et al. (2025) and supports the biota screening threshold of 0.0005 µg/L, above which biota sampling should be undertaken to assess risk to air-breathing wildlife from consumption of aquatic organisms.

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