



An Australian Government Initiative



Australian & New Zealand

**GUIDELINES FOR
FRESH & MARINE
WATER QUALITY**

Toxicant default guideline values for aquatic ecosystem protection

Perfluorooctane sulfonate (PFOS) in freshwater

Technical brief

May 2023

Toxicant default guideline values for aquatic ecosystem protection: Perfluorooctane sulfonate (PFOS) in freshwater

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This publication is available at waterquality.gov.au/anz-guidelines/guideline-values/default/water-quality-toxicants/toxicants.

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Acknowledgements

These default guideline values (DGVs) were derived by Naomi Cooper, Kirsten Broadgate, Clare Papaleo and Carolyn Brumley of Golder Associates. The DGVs were peer reviewed by two anonymous reviewers, by the Contaminants Standards and Advice section of the Department of Climate Change, Energy, the Environment and Water and by two contracted technical advisors Dr Rick van Dam and Alicia Hogan. The DGVs were also reviewed and approved by jurisdictional technical and policy oversight groups and a National Water Reform Committee.

New Zealand Government



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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 ($\text{C}_8\text{F}_{17}\text{SO}_3^-$)) (NICNAS 2015). Common PFOS salts include the acid, potassium, lithium and ammonium salts. 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).

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 zebrafish *Danio rerio* was the most sensitive species, with a LOEC of approximately 0.7 µg/L, while the diatom *Navicula pelliculosa* was the least sensitive species, with an EC50 of 263 000 µg/L.

Very high reliability DGVs were derived using chronic EC10, NOEC, LOEC, and EC50 data for 35 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 0.0091 µg/L, 0.48 µg/L, 2.7 µg/L, and 17 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% DGV is recommended, biota in the water may have elevated tissue concentrations of PFOS that exceed the DGV (regardless of whether the water quality meets the DGV). Therefore, the 99% DGV alone may not be sufficient to protect the organisms that consume these biota (e.g. predators such as birds). Accordingly, assessments should consider the risk to higher consumers as well as the presence 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 ($\text{C}_8\text{F}_{17}\text{SO}_3^-$)) (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 the commercial production of PFOS, 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).

PFAS have been used extensively in metal plating, and in the manufacture of clothing and textiles, food wrapper coatings, paper and packaging, coating additives, cleaning products, stain repellents, pesticides, semi-conductors, surfactants, and firefighting foams (Bots et al. 2010, Buck et al. 2011). 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 (Bots et al. 2010, Brooke et al. 2004). 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).

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 were enacted, consistent with the convention (Parliament of Australia 2014, NICNAS 2015). Some state and territory governments have recently 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 2.0 (HEPA 2020) 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 2020).

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). 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 risk of PFOS contamination (OECD 2002, Li 2009). Long-range transport of PFAS is evidenced by concentrations reported in Arctic wildlife (UNEP 2006). For example, PFOS has been reported in:

- liver tissue of polar bears and ringed seals at concentrations of 3 770 ng/g and 96 ng/g, respectively
- whole fish for various species at concentrations ranging from 5.7 ng/g to 85.4 ng/g
- zooplankton at concentrations of 1.8 ng/g
- Herring Gull eggs (*Larus argentatus*) up to concentrations of 42 200 ng/g (OECD 2002, Swedish EPA 2004, ATSDR 2009, Fair et al. 2019).

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).

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 pK_a value (the acid dissociation constant), and readily dissociates in water (Moermond et al. 2010).

Bioconcentration factors (BCFs) for freshwater fish (whole body or tissue-specific) have been reported at approximately 3 000 L/kg (Qi et al. 2011, Lu et al. 2015). Bioaccumulation factors (BAFs) for PFOS in freshwater fish are reported to range from 2 500 L/kg to 95 000 L/kg (Moermond et al. 2010). 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, Hagenaaers 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. 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, and embryonic deformities) and via hepatotoxicity such as vacuolation of liver cells (Keiter et al. 2012, Rainieri et al. 2017, Sant et al. 2018, Fort et al. 2019, Zhang et al. 2019). 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)
- 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 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 30 species, with LC50 concentrations ranging from 700 µg/L for the nematode *Caenorhabditis elegans* (2 d LC50) (Chen et al. 2018) 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

Over 240 chronic toxicity data were identified for 35 species from nine taxonomic groups (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 0.734 µg/L for the zebrafish *D. rerio* (F2 generation growth LOEC, 90 d and 180 d post fertilisation) (Keiter et al. 2012) 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 3.5 µg/L (F6 generation, 150 d development LOEC for *Chironomus riparius*) (Marziali et al. 2019) 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)
- for crustaceans, values ranged from 8 µg/L (21 d growth LOEC for *Daphnia magna*) (Lu et al. 2015) to 50 000 µg/L (21 d reproduction LOEC for *D. magna*) (Boudreau et al. 2013a)
- 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 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 48 200 µg/L (4 d growth IC50 for *Raphidocelis subcapitata*) (Boudreau et al. 2013a) 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), *Oryzias latipes* (Ji et al. 2008), *Pimephales promelas* (Ankley et al. 2005)
- chironomid *C. riparius* (Stefani et al. 2014, Marziali et al. 2019)
- cladoceran *D. magna* (Jeong et al. 2016)
- rotifer *Brachionus 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.

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. 2018, Kim et al. 2020) and oligochaete *Limnodrilus hoffmeisteri* (Liu 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)
- snail *Lymnaea stagnalis* (Olson 2017)
- mussel *Dreissena polymorpha* (Fernandez-Sanjuan et al. 2013)
- frog *Lithobates pipiens* (Ankley et al. 2004, Hoover et al. 2017, Flynn et al. 2020)
- fish, including carp *C. carpio* (Inoue et al. 2012, Zhong et al. 2018), rainbow trout *O. mykiss* (Martin et al. 2003, Vidal et al. 2019), fathead minnow *P. promelas* (Ankley et al. 2005), salmon *Salmo salar* (Mortenson et al. 2011, Arukwe et al. 2013), and zebrafish *D. rerio* (Chen et al. 2013, Li et al. 2017, Gaballah et al. 2020).

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. stagnalis* (Olson 2017)
- *P. promelas* (Ankley et al. 2005).

Chen et al. (2013) observed increased mortality and PFOS bioaccumulation in embryos produced by adult *D. rerio* exposed for long periods (21–120 days post fertilisation (dpf) and 1–120 dpf) compared to control organisms. 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. 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 (Olsen 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) and using Burrlioz 2.0 software.

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 two-thirds 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 could 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 half of the assessed PFOS chronic aquatic toxicity data (>240 values), including data for 11 of the 35 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: Discussion of modality and concentrations for PFOS dataset.

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 2–5 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).

LOECs were selected for use in the current derivation for an additional five species where NOECs of the same concentration as the LOECs were also available. These LOECs were for the following species: a midge larva (*C. riparius*) (Marziali et al. 2019), two cladocerans (*D. magna*) (Lu et al. 2015) and (*Moina macrocopa*) (Ji et al. 2008), and two fish (*D. rerio* and *Xiphophorus helleri*) (Han & Fang 2010, Keiter et al. 2012). LOECs were selected in these cases because they represent true effects and, after conversion, represent a concentration below the available NOEC which achieves greater protection than if the NOEC were adopted.

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 wide spread 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 eight 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: two macrophytes (*M. spicatum*, *M. sibiricum*) (Hanson et al. 2005)), a crustacean (*Cyclops diaptomus*) (Sanderson et al. 2002), an insect (*E. cyathigerum*) (Bots et al. 2010), two fish (*D. rerio* (Keiter et al. 2012), *O. latipes* (Ji et al. 2008)), and two frogs (*L. pipiens* (Hoover et al. 2017) and *Xenopus laevis*) (Lou et al. 2013).

For the zebrafish *D. rerio* (Keiter et al. 2012) a LOEC of 0.734 µg/L (F2 generation, 90 dpf, growth in females) was selected for use in the DGV derivation when there were NOECs for other durations, generations and/or males of 0.734 µg/L. The LOEC was selected after a comprehensive review of

Keiter et al. (2012) and consideration of statistically significant effects reported at the concentration of 0.734 µg/L. LOECs of 0.734 µg/L were reported for the F1 generation at 90 dpf (length and weight in males) and at 180 dpf (weight in females), and for the F2 generation at 90 dpf (length and weight in males and females) and at 180 dpf (length in females). The per cent difference to controls as estimated from the figures presented in Keiter et al. (2012) indicates effects of greater than 10% relative to controls for exposure to 0.734 µg/L PFOS were limited to the following endpoints and exposure durations: F1 generation at 90 dpf (weight in males), F1 generation at 180 dpf (weight in females), and F2 generation at 90 dpf (length and weight in males and females). Thus, based on multiple statistically significant effects of >10% at the concentration of 0.734 µg/L, this concentration was considered to be the LOEC for *D. rerio* and was selected for use in the final dataset in preference to a NOEC (based on different durations, generations and or sexes) of the same concentration.

Despite having a diverse taxonomic diversity, the indoor microcosm study of Sanderson et al. (2002) was considered to not represent 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. In contrast, the outdoor study of Hanson et al. (2005) was conducted outdoors using a relatively large volume (12 000 L) per test chamber and did not include any exogenous food source; thus, it was considered to be a mesocosm study.

The hierarchy of statistical estimates of toxicity in Warne et al. (2018) preferences EC10s over NOECs, and NOECs over LOECs and EC50s, but allows for the use of professional judgement in making such decisions. There were eight EC/IC10s (from six taxonomic groups) 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 14 species (from seven taxonomic groups), 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. Two of the NOECs represented '≥' values (≥11 µg/L for the eel *Anguilla anguilla*, and ≥608 µg/L for the frog *X. laevis*). For both species, no other data were available. Moreover, review of the two '≥' NOECs indicated that they were within the existing data range for their respective taxonomic groups, and their inclusion in the DGV derivation did not have a large influence on the final DGVs. Based on Warne et al. (2018), these two values were acceptable for inclusion in the derivation. In addition to the EC/IC10s and NOECs, LOECs were available for an additional 13 species (from seven taxonomic groups), of which seven were lower than any of the EC/IC10s and NOECs. Thus, the exclusion of the LOECs was considered likely to result in a DGV 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). As EC50s and LOECs sit at the same level in the data hierarchy, EC50 values for two additional species (green alga *Desmodesmus communis* and fish *P. promelas*) were also included in the dataset (after being converted to 'negligible effect' concentrations by dividing by the default factor of 5). For *D. communis*, the EC50 was selected because no other data were available. For *P. promelas*, the EC50 (21 d F0 generation fecundity) of 230 µg/L was selected over a NOEC (24 d F1 generation growth) of 300 µg/L because the NOEC would have been under-protective of reproductive effects. Based on the above decisions, the dataset included chronic toxicity values for 35 species from 11 taxonomic groups, comprising eight EC/IC10s, 12 NOECs, 13 LOECs and two EC50s.

A modality assessment was performed on the dataset according to the method in Warne et al. (2018) and is provided in Appendix B: Discussion of modality and concentrations for PFOS dataset. The dataset did not exhibit bimodality or multimodality; thus, the chronic toxicity data for 35 species from 11 taxonomic groups were used to derive the DGVs (Table 1). These species included: one diatom, one cyanobacterium, four species of green algae, three macrophytes, one rotifer, one flatworm, six crustaceans, four insects, three molluscs, six fish and five amphibians. The toxicity values for these species span over five orders of magnitude. The dataset consisted of toxicity values from a mix of single species single generation studies and single species multigenerational studies as well as a mix of microcosm and mesocosm studies: 25 values were from single species, one generation (or less) studies; six values were from single species multigenerational (≥ 2 generations) studies (for *D. rerio*, *O. latipes*, *P. promelas*, *C. riparius*, *P. pomilia* and *B. calyciflorus*); two values were from a microcosm study (for *C. diaptomus* and *Cyclops canthocamptus staphylinus*); and two values were from a mesocosm study (*M. spicatum* and *M. sibiricum*). A comparison of the final dataset used for the current DGVs with that used for the interim guideline values reported in HEPA (2020) is presented in Appendix C: Comparison of datasets for current DGVs and interim guideline values reported in HEPA (2020).

A summary of the toxicity data (one value per species) used to calculate the DGVs for PFOS in freshwater are provided in Table 1; additional details are in Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values. 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) ^f
Amphibian	<i>Bufo gargarizans</i>	Tadpole	30	EC10 (survival)	2 000	2 000 ^b
	<i>Lithobates catesbeiana</i>	Tadpole	72	LOEC (growth)	144	57.6 ^{c, g}
	<i>Lithobates pipiens</i>	Tadpole	40	NOEC (development)	10	10 ^b
	<i>Xenopus laevis</i>	Tadpole	120	NOEC (growth)	≥ 608	608 ^b
	<i>Xenopus tropicalis</i>	Embryo	150	NOEC (growth)	590	590 ^b
Blue-green alga	<i>Anabaena flos-aquae</i>	–	4	EC10 (growth, biomass)	82 000	82 000 ^b
Crustacean	<i>Cyclops diaptomus</i>	–	28	LOEC (survival)	1 000	400 ^c
	<i>Cyclops canthocamptus staphylinus</i>	–	35	NOEC (survival)	1 000	1 000 ^b
	<i>Daphnia magna</i>	Neonate	21	LOEC (length, Intrinsic rate of population growth)	8	3.2 ^{c, g}

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freshwater

Taxonomic group	Species	Life stage	Duration (days)	Toxicity measure ^a	Toxicity value (µg/L)	Estimated chronic value (µg/L) ^f
	<i>Daphnia pulex</i>	Neonate	21	NOEC (survival)	6 000	6 000 ^{b, g}
	<i>Moina macrocopa</i>	Neonate	7	LOEC (reproduction)	313	125 ^{c, g}
	<i>Procambarus fallax f. virginalis</i>	Juvenile	28	NOEC (survival)	200	200 ^b
Diatom	<i>Navicula pelliculosa</i>	–	4	EC10 (growth, cell density)	<62 300	62 300 ^b
Fish	<i>Anguilla anguilla</i>	Adult	28	NOEC (growth)	≥11	11 ^b
	<i>Danio rerio</i>	Egg, F2 generation	90	LOEC (growth)	0.734	0.294 ^c
	<i>Oryzias latipes</i>	Embryo, F1 generation	24	LOEC (reproduction)	10	4 ^{c, g}
	<i>Pimephales promelas</i>	Adult, F0 generation	24	EC50 (reproduction)	230	46 ^d
	<i>Pseudorasbora parva</i>	Adult	30	EC10 (survival)	2 120	2 120 ^b
	<i>Xiphophorus helleri</i>	Fry	90	LOEC (growth)	100	40 ^{c, g}
Green alga	<i>Chlorella vulgaris</i>	–	4	IC10 (growth, biomass)	8 200	8 200 ^{b, g}
	<i>Desmodesmus communis</i>	Exponential growth phase	4	EC50 (growth, biomass)	89 340	17 868 ^d
	<i>Raphidocelis subcapitata</i>	–	4	IC10 (growth, biomass)	5 300	5 300 ^{b, g}
	<i>Tetradesmus obliquus</i>	Exponential growth phase	4	NOEC (growth, biomass)	25 000	25 000 ^{b, g}
Insect	<i>Aedes aegypti</i>	Larva, 1 st instar	40	NOEC (survival)	50	50 ^b
	<i>Chironomus riparius</i>	Larva, F6 generation	150	LOEC (development)	3.5	1.4 ^c
	<i>Chironomus tentans</i>	Larva	20	LOEC (development)	2.3	0.92 ^c
	<i>Enallagma cyathigerum</i>	Larva	120	LOEC (development)	7.95	3.18 ^{c, e, g}
Macrophyte	<i>Lemna gibba</i>	–	42	NOEC (growth)	300	300 ^b
	<i>Myriophyllum sibiricum</i>	–	42	EC10 (growth)	600	600 ^b
	<i>Myriophyllum spicatum</i>	–	28	EC10 (growth)	3 300	3 300 ^b
Mollusc	<i>Lampsilis siliquoidea</i>	Glochidia	36	LOEC (survival)	4.5	1.8 ^c
	<i>Lymnaea stagnalis</i>	Adult	21	NOEC (survival)	3 000	3 000 ^b
	<i>Physa pomilia</i>	Egg, F1 generation	44	NOEC (reproduction)	10 000	10 000 ^b

Taxonomic group	Species	Life stage	Duration (days)	Toxicity measure ^a	Toxicity value (µg/L)	Estimated chronic value (µg/L) ^f
Flatworm	<i>Dugesia japonica</i>	Fragment	10	LOEC (reproduction)	500	200 ^{c, g}
Rotifer	<i>Brachionus calyciflorus</i>	Neonate	28	LOEC (population)	250	100 ^c

a The measure of toxicity being estimated/determined: EC/ICx: 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/IC10).

c Default conversion from chronic LOEC to chronic negligible effect value: chronic LOEC ÷ 2.5 = chronic NOEC.

d Default conversion from chronic EC50 or IC50 to chronic negligible effect value: chronic EC/IC50 ÷ 5 = chronic NOEC.

e Exposure concentrations converted from PFOS tetraethyl ammonium salt to PFOS anion.

f Estimated chronic values are reported to no more than three significant figures.

g Nominal concentration.

– : not stated/no data.

4.2 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 35 chronic PFOS freshwater toxicity data reported in Table 1 is shown in Figure 1. The SSD was plotted using the BurrIioz 2.0 software. The model was judged to provide a good fit to the data.

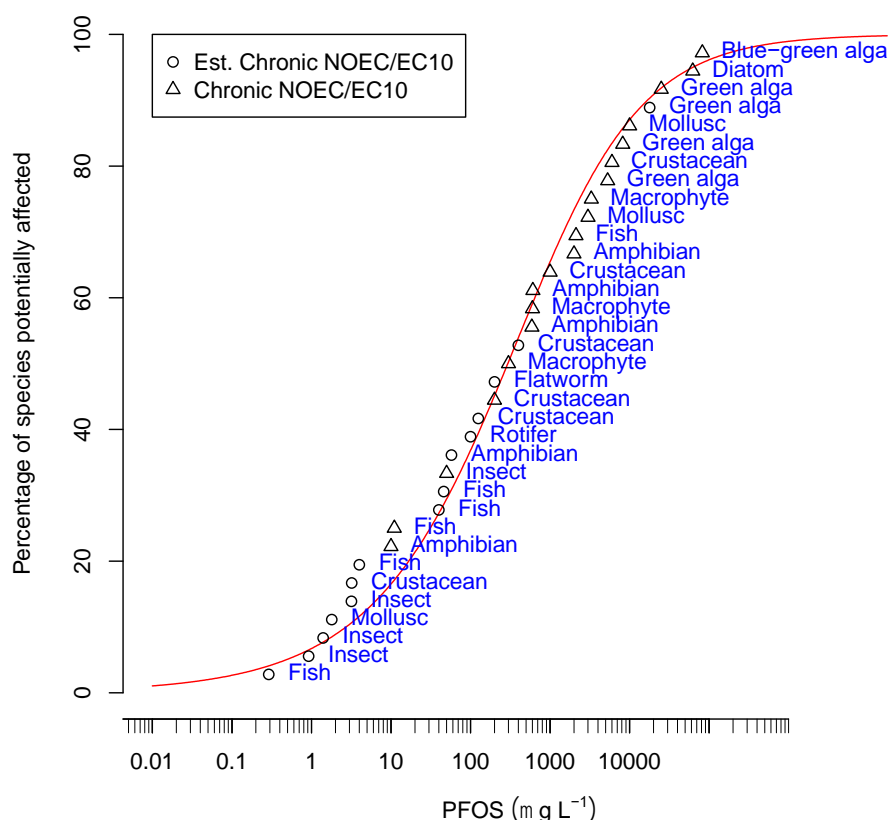


Figure 1 Species sensitivity distribution, PFOS in freshwater

4.3 Default guideline values

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).

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 eight (or 23%) of the 35 species represented in the final dataset. Therefore, it is recommended that a more conservative approach 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).

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
99	0.0091
95	0.48
90	2.7
80	17

^a Default guideline values were derived using the Burrlioz 2.0 software, and have been rounded to two significant figures.

The DGVs were compared with the freshwater chronic toxicity data that were compiled from the literature review and passed the quality assessment (i.e. 241 chronic values for 35 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.

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. However, 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 which 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 Canadian Federal Environment Quality Guidelines (ECCC 2018, HEPA 2020)) in waters where the concentrations were below the PC99 (median 0.0017 µg/L).

In relation to assessments for bioaccumulation, the PFAS NEMP (HEPA 2020) notes, on the basis of observations by the contributory jurisdictions, that a point-in-time water concentration of PFAS below an LOR of 0.001 µg/L should not be assumed to mean that there is minimal risk to aquatic ecosystems and does not mean that there is no need to sample aquatic biota. The recommended approach is to sample and analyse aquatic biota to account for bioaccumulation and comparison with relevant criteria. Environmental regulators or local catchment managers may be able to provide additional jurisdiction-specific information and guidance.

Environmental assessments should also consider the presence of PFOS precursors in water (HEPA 2020), 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.

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—35 (preferred)
- type of toxicity data—chronic negligible effect and estimated negligible effect values
- SSD model fit—good (Burr Type III).

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.

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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	<i>Bufo gargarizans</i>	Tadpole	30	EC10 (survival)	Dechlorinated tap water	22±2	7.0±0.5	2 000 ^b	Yang et al. 2014
	<i>Lithobates catesbeiana</i>	Tadpole, Gosner stage 25	72	LOEC (growth)	Filtered well water	21	–	144 ^d	Flynn et al. 2019
	<i>Lithobates pipiens</i>	Tadpole	40	NOEC (development)	UV irradiated well water	20±2	–	10 ^b	Hoover et al. 2017
	<i>Xenopus laevis</i>	Tadpole, NF stage 46/47	120	NOEC (growth)	Charcoal-filtered tap water	20–24	6.5±7.0	≥608 ^b	Lou et al. 2013
	<i>Xenopus tropicalis</i>	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	<i>Anabaena flos-aquae</i>	–	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	<i>Cyclops diaptomus</i>	–	28	LOEC (survival)	Natural pond water	10–18 (increased 2°C per week to 18°C)	8.28–8.37	1 000 ^{d, f}	Sanderson et al. 2002
	<i>Cyclops canthocamptus staphylinus</i>	–	35	NOEC (survival)	Natural pond water	10–18 (increased 2°C per week to 18°C)	8.28–8.37	1 000 ^{b, f}	Sanderson et al. 2002

Toxicant default guideline values for aquatic ecosystem protection: Perfluorooctane sulfonate (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
	<i>Daphnia magna</i>	Neonate, <24 h old	21	LOEC (length, intrinsic rate of population growth)	Culture medium as per OECD (2002)	20±1	7.5±0.3	8 ^d	Lu et al. 2015
	<i>Daphnia pulicaria</i>	Neonate, >24 h old	21	NOEC (survival)	Moderately hard clean well water	21±1	–	6 000 ^{b, h}	Sanderson et al. 2004
	<i>Moina macrocopa</i>	Neonate, <24 h old	7	LOEC (reproduction)	Moderately hard clean well as per USEPA (2002a)	25±1	–	312.5 ^{d, h}	Ji et al. 2008
	<i>Procambarus fallax f. virginalis</i>	Juvenile	28	NOEC (survival)	Moderately hard water	20±1	–	200 ^b	Funkhouser 2014
Diatom	<i>Navicula pelliculosa</i>	–	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	<i>Anguilla anguilla</i>	Adult	28	NOEC (growth)	Tap water	20±2	–	≥11 ^b	Roland et al. 2014
	<i>Danio rerio</i>	Egg, F2 generation	90	LOEC (growth)	Deionised water and tap water	26±1	8.25–8.75	0.734 ^d	Keiter et al. 2012
	<i>Oryzias latipes</i>	Embryo, F1 generation	24	LOEC (reproduction)	Dechlorinated tap water	25±1	–	10 ^{d, h}	Ji et al. 2008
	<i>Pimephales promelas</i>	Adult, F0 generation	24	EC50 (reproduction)	Lake Superior water	–	7.3	230 ^c	Ankley et al. 2005
	<i>Pseudorasbora parva</i>	Adult	30	EC10 (survival)	Dechlorinated tap water	22±2	7.0±0.5	2 120 ^b	Yang et al. 2014
	<i>Xiphophorus helleri</i>	Fry	90	LOEC (growth)	Dechlorinated tap water	27±1	–	100 ^{d, h}	Han & Fang 2010
Green alga	<i>Chlorella vulgaris</i>	–	4	IC10 (growth, biomass)	Bristol's algal growing media in laboratory-grade distilled water	23±1	–	8 200 ^{b, h}	Boudreau et al. 2003a

Toxicant default guideline values for aquatic ecosystem protection: Perfluorooctane sulfonate (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
	<i>Desmodesmus communis</i>	Exponential growth phase	4	EC50 (growth, biomass)	M4 medium in dechlorinated tap water	22±2	7.0±0.5	89 340 ^c	Yang et al. 2014
	<i>Raphidocelis subcapitata</i>	–	4	IC10 (growth, biomass)	Bristol's algal growing media in laboratory-grade distilled water	23±1	–	5 300 ^{b, h}	Boudreau et al. 2003a
	<i>Tetrademus obliquus</i>	Exponential growth phase	4	NOEC (growth, biomass)	HB-4 culture medium	24	–	25 000 ^{b, h}	Zhang et al. 2012
Insect	<i>Aedes aegypti</i>	Larva, 1 st instar	40	NOEC (survival)	Moderately hard water deionised laboratory water	25	–	50 ^b	Olson 2017
	<i>Chironomus riparius</i>	Larva, F6 generation	150	LOEC (development)	Reconstituted water	18.7–21.2	7.9–8.2	3.5 ^d	Marziali et al. 2019
	<i>Chironomus tentans</i>	Larva	20	LOEC (development)	ASTM hard water	21±2	–	2.3 ^d	MacDonald et al. 2004
	<i>Enallagma cyathigerum</i>	Larva	120	LOEC (development)	Dechlorinated tap water	21	>7.5	7.95 ^{d, e, h}	Bots et al. 2010
Macrophyte	<i>Lemna gibba</i>	–	42	NOEC (growth)	Irrigation pond water	15.9–20.5	8.3–8.8	300 ^b	Boudreau et al. 2003b
	<i>Myriophyllum sibiricum</i>	–	42	EC10 (growth)	Irrigation pond water	–	–	600 ^{b, g}	Hanson et al. 2005
	<i>Myriophyllum spicatum</i>	–	28	EC10 (growth)	Irrigation pond water	–	–	3 300 ^{b, g}	Hanson et al. 2005
Mollusc	<i>Lampsilis siliquoidea</i>	Glochidia	35	LOEC (survival)	Natural pond water	14.6–16.1	7.6–8.5	4.5 ^d	Hazelton et al. 2012
	<i>Lymnaea stagnalis</i>	Adult	21	NOEC (survival)	Aerated synthetic fresh water	20±1	–	3 000 ^b	Olson 2017
	<i>Physa pomilia</i>	Egg, F1 generation	44	NOEC (reproduction)	Moderately hard water	22±1	–	10 000 ^b	Funkhouser 2014

Toxicant default guideline values for aquatic ecosystem protection: Perfluorooctane sulfonate (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
Flatworm	<i>Dugesia japonica</i>	Fragment	10	LOEC (reproduction)	Aerated tap water	22	–	500 ^{d, h}	Yuan et al. 2014
Rotifer	<i>Brachionus calyciflorus</i>	Neonate, <2 h old	28	LOEC (population)	USEPA (2002b) culture medium for algae	20	–	250 ^d	Zhang et al. 2013

a The measure of toxicity being estimated/determined: EC_x / IC_x: 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 EC50 to negligible effect value conversion factor of 5.

d 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.

e Exposure concentrations converted from PFOS tetraethyl ammonium salt to PFOS anion.

f Values taken from a microcosm study.

g Values taken from a mesocosm study.

h 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*), *Tetradasmus 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 0.429, which does not support bimodality.

Based on these lines of evidence, there is potential for the data to be bimodal or multimodal.

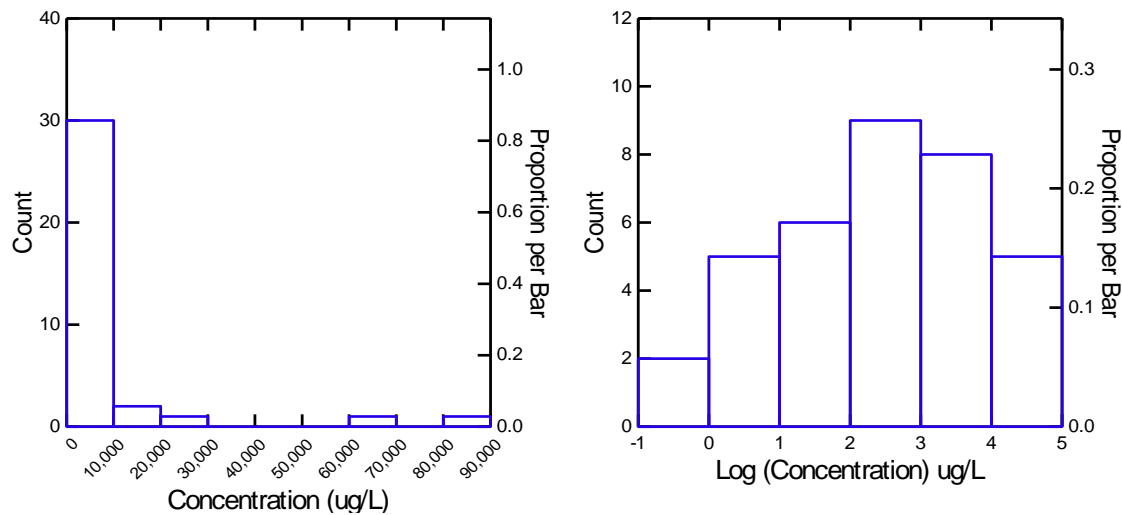


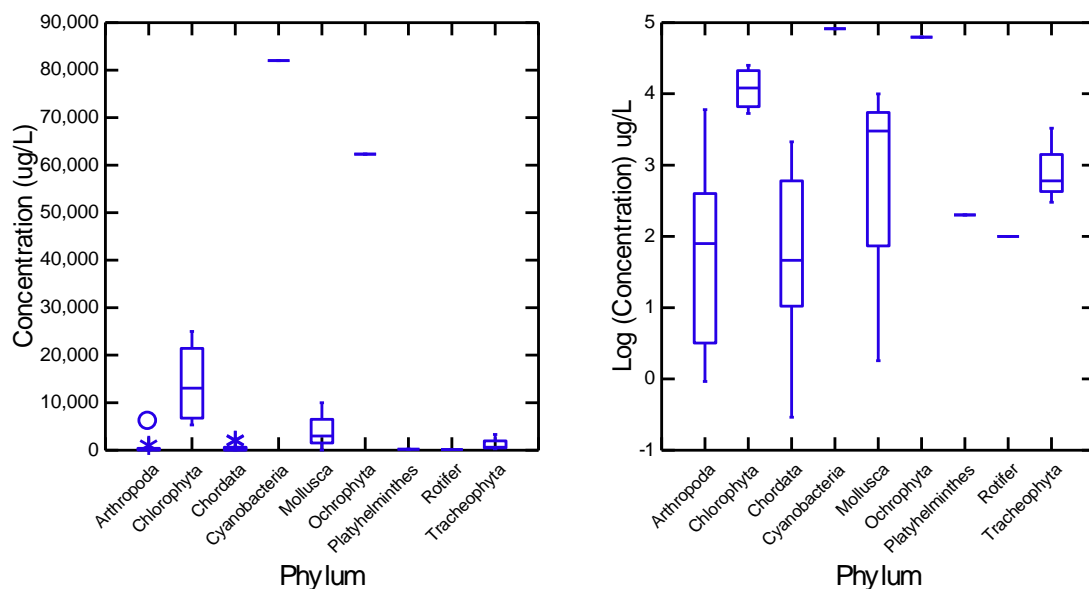
Figure B 1 Histogram, raw (left) and log transformed (right) PFOS data

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=10; Chordata, n=11; Mollusca, n=3; Platyhelminthes, n=1; Rotifera, n=1; Tracheophyta, n=3. In contrast, some phyla show reduced sensitivity to PFOS, namely: Ochrophyta, 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.

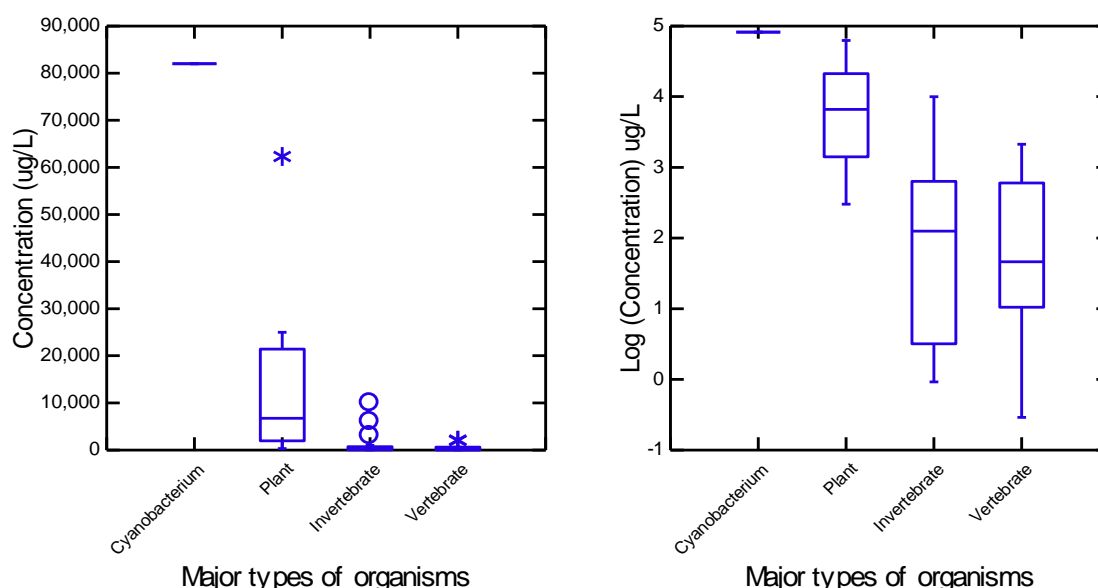


Note: asterisk represents an outlying value >1.5x the interquartile range; open circle represents an outlying value >3x the interquartile range.

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 (n=15) and vertebrates (n=11) are generally more sensitive to PFOS

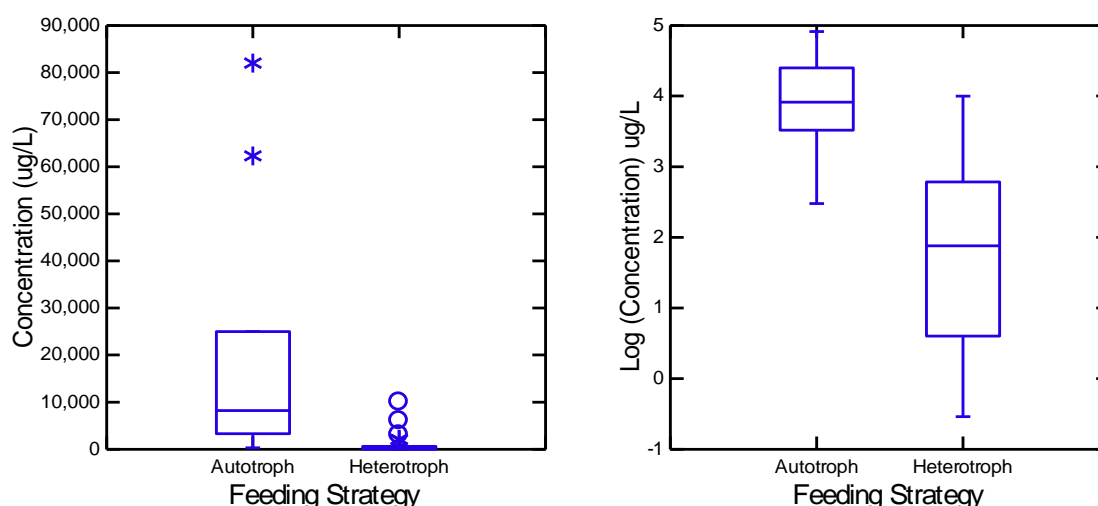
than cyanobacteria (n=1) and plants (n=8). However, the sample size for cyanobacteria is too small to draw conclusions regarding differences in sensitivity and, without a confirmed mode of action, the reason for the apparent differences in sensitivity between the types of organisms is unknown.



Note: asterisk represents an outlying value $>1.5\times$ the interquartile range; open circle represents an outlying value $>3\times$ 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=26) is larger than for autotrophs (n=9), and the inclusion of the less sensitive planktonic plants (Chlorophyta, Ochrophyta, 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 $>1.5\times$ the interquartile range; open circle represents an outlying value $>3\times$ the interquartile range.

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

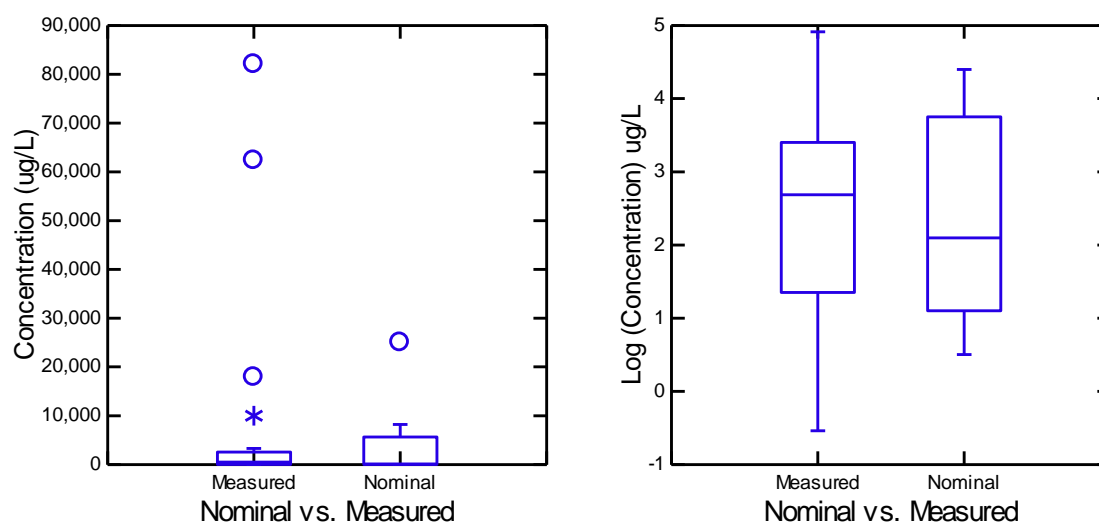
Is it likely that indications of bimodality or multimodality or distinct clustering of taxa groups are not due to artefacts of data selection, small sample size, test procedures, or other reasons unrelated to a specific mode of action?

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 35 species identified in preparation of the SSD.

Nominal and measured concentrations

Box plots of nominal (n=12) and measured (n=23) 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 range to measured concentrations and are within the maximum and minimum values of the measured concentrations. The mean for nominal concentrations is lower compared to the mean of the measured concentrations.



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

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

Appendix C: Comparison of datasets for current DGVs and interim guideline values reported in HEPA (2020)

Background

The Joint Steering Committee for the revision of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality agreed to develop DGVs for PFOS in freshwater in January 2015. An initial draft of the DGVs was prepared, peer-reviewed and circulated by the Commonwealth Government to the states and territories and New Zealand in December 2015 so the information could be used until the final DGVs were published. These 2015 draft DGVs were also reported as interim guideline values in HEPA (2020).

A revised *Method for Deriving Australian and New Zealand Water Quality Guideline Values for Toxicants* (Warne et al. 2018) was issued in October 2018. In 2018, the 2015 draft DGVs for PFOS were updated in accordance with the revised derivation method. The updated draft DGVs and associated data package were peer reviewed by three experts and by the Water Quality Guidelines Technical Manager. Subsequently, the review comments were addressed and a new literature review was undertaken to identify aquatic toxicity data (i) published since or (ii) that may not have been originally considered in the derivation of the draft DGVs. This process resulted in the DGVs reported in this technical brief, which represent an update of the interim guideline values reported in HEPA (2020).

The information provided below compares the derivation of the current DGVs (presented in this technical brief) with the 2015 draft DGVs that were reported as interim guideline values in HEPA (2020).

Key changes made to current PFOS DGVs

Consistent with the approved derivation method, professional judgement was applied in the decision to use all acceptable chronic toxicity data (i.e. EC/IC10, NOEC, LOEC and EC/IC50) to generate the updated SSD, including data for autotrophs and heterotrophs. The reasons for using all the acceptable chronic toxicity data for autotrophs and heterotrophs were as follows.

- The data appeared to be generally unimodal and normally distributed.
- The data included several chronic partial-generation, full-generation or multi-generation studies that were considered key for inclusion.
- The reduced sensitivity of autotrophs is largely driven by a small number of planktonic species. For most of the remaining autotrophs included in the DGVs (i.e. three macrophyte species and two of the four green alga species), the effect concentrations were within the range of the heterotroph data.

Consequently, a dataset of 35 species was used in the derivation of the current DGVs, compared with 18 species for the 2015 draft DGVs. Table C 1 provides a comparison of the current DGVs and 2015 draft DGVs.

Table C 1 Current DGVs and 2015 draft DGVs, PFOS anion in freshwater

Level of species protection (%)	Current DGV (µg/L)	2015 draft DGV (µg/L) ^a
99	0.0091	0.00023
95	0.48	0.13
90	2.7	2.0
80	17	31

a As reported in HEPA (2020).

A comparison of the key elements of the current DGVs and the 2015 draft DGVs is presented in Table C 2. This comparison covers the following aspects:

- details of species used in the SSD
- professional judgements used in including or excluding data
- modality checks
- species protection levels
- theoretical protection of the species protection levels
- reliability classification.

Table C 2 Comparison of key aspects of current DGVs and 2015 draft DGVs

Key aspect	2015 draft PFOS DGVs	Current PFOS DGVs
Number of species used in the SSD	<p>Chronic studies for 18 species comprising 10 heterotrophic organisms and eight autotrophic organisms from eight taxonomic groups.</p> <p>The heterotrophic species comprised:</p> <ul style="list-style-type: none"> • four fish: <ul style="list-style-type: none"> ○ <i>Danio rerio</i> ○ <i>Oryzias latipes</i> ○ <i>Pimephales promelas</i> ○ <i>Xiphophorus helleri</i> • one amphibian: <ul style="list-style-type: none"> ○ <i>Lithobates pipiens</i> (formerly <i>Rana pipiens</i>) • two insects: <ul style="list-style-type: none"> ○ <i>Chironomus tentans</i> ○ <i>Enallagma cyathigerum</i> • three crustaceans: <ul style="list-style-type: none"> ○ <i>Daphnia magna</i> ○ <i>Daphnia pulicaria</i> ○ <i>Moina macrocopa</i>. <p>The autotrophic species comprised:</p> <ul style="list-style-type: none"> • three macrophytes: <ul style="list-style-type: none"> ○ <i>Lemna gibba</i> ○ <i>Myriophyllum sibiricum</i> ○ <i>Myriophyllum spicatum</i> • one cyanobacterium: <ul style="list-style-type: none"> ○ <i>Anabaena flos-aquae</i> • one diatom: <ul style="list-style-type: none"> ○ <i>Navicula pelliculosa</i> • three green algae: 	<p>Chronic studies for 35 species comprising 26 heterotrophic organisms and nine autotrophic organisms from 11 taxonomic groups.</p> <p>The species are listed below; species that were not included in the 2015 draft DGVs are underlined.</p> <p>The heterotrophic species comprised:</p> <ul style="list-style-type: none"> • six fish: <ul style="list-style-type: none"> ○ <u><i>Anguilla anguilla</i></u> ○ <i>Danio rerio</i> ○ <i>Oryzias latipes</i> ○ <i>Pimephales promelas</i> ○ <u><i>Pseudorasbora parva</i></u> ○ <i>Xiphophorus helleri</i> • five amphibians: <ul style="list-style-type: none"> ○ <u><i>Bufo qaraqarizans</i></u> ○ <u><i>Lithobates catesbeiana</i></u> ○ <i>Lithobates pipiens</i> ○ <u><i>Xenopus laevis</i></u> ○ <u><i>Xenopus tropicalis</i></u> • four insects: <ul style="list-style-type: none"> ○ <u><i>Aedes aegypti</i></u> ○ <u><i>Chironomus riparius</i></u> ○ <i>Chironomus tentans</i> ○ <i>Enallagma cyathigerum</i> • six crustaceans: <ul style="list-style-type: none"> ○ <u><i>Cyclops canthocamptus staphylinus</i></u> ○ <u><i>Cyclops diaptomus</i></u> ○ <i>Daphnia magna</i> ○ <i>Daphnia pulicaria</i>

Key aspect	2015 draft PFOS DGVs	Current PFOS DGVs
	<ul style="list-style-type: none"> ○ <i>Chlorella vulgaris</i> ○ <i>Raphidocelis subcapitata</i> (formerly <i>Pseudokirchneriella subcapitata</i> and <i>Selenastrum capricornutum</i>) ○ <i>Tetrademus obliquus</i> (formerly <i>Scenedesmus obliquus</i> and <i>Acutodesmus obliquus</i>). 	<ul style="list-style-type: none"> ○ <i>Moina macrocopa</i> ○ <i>Procambarus fallax f. virginalis</i> • one rotifer: <ul style="list-style-type: none"> ○ <i>Brachionus calyciflorus</i> • one flatworm: <ul style="list-style-type: none"> ○ <i>Dugesia japonica</i> • three molluscs: <ul style="list-style-type: none"> ○ <i>Lampsilis siliquoidea</i> ○ <i>Lymnaea stagnalis</i> ○ <i>Physa pomilia</i>. <p>The autotrophic species comprised:</p> <ul style="list-style-type: none"> • three macrophytes: <ul style="list-style-type: none"> ○ <i>Lemna gibba</i> ○ <i>Myriophyllum sibiricum</i> ○ <i>Myriophyllum spicatum</i> • one cyanobacterium: <ul style="list-style-type: none"> ○ <i>Anabaena flos-aquae</i> • one diatom: <ul style="list-style-type: none"> ○ <i>Navicula pelliculosa</i> • four green algae: <ul style="list-style-type: none"> ○ <i>Chlorella vulgaris</i> ○ <i>Desmodesmus communis</i> ○ <i>Raphidocelis subcapitata</i> ○ <i>Tetrademus obliquus</i>. <p>Of the 18 species that were used in the 2015 draft DGVs, the toxicity values remained unchanged for the following nine species:</p> <ul style="list-style-type: none"> • <i>D. rerio</i>: LOEC 0.734 µg/L • <i>O. latipes</i>: LOEC 10 µg/L • <i>X. helleri</i>: LOEC 100 µg/L

Key aspect	2015 draft PFOS DGVs	Current PFOS DGVs
		<ul style="list-style-type: none"> • <i>M. spicatum</i>: EC10 3 300 µg/L • <i>R. subcapitata</i>: NOEC 5 300 µg/L • <i>D. pulicaria</i>: NOEC 6 000 µg/L • <i>C. vulgaris</i>: NOEC 8 200 µg/L • <i>N. pelliculosa</i>: EC10 62 300 µg/L • <i>A. flos-aquae</i>: EC10 82 000 µg/L <p>For the following species available in 2015, lower toxicity values were available for the current DGVs:</p> <ul style="list-style-type: none"> • <i>E. cyathigerum</i>: NOEC 3.18 µg/L (2020) vs NOEC 7.95 µg/L (2015) • <i>D. magna</i>: LOEC 3.2 µg/L (2020) vs NOEC 8 µg/L (2015) • <i>C. tentans</i>: LOEC 0.92 µg/L (2020) vs EC10 49.2 µg/L (2015) • <i>P. promelas</i>: EC50 46 µg/L (2020) vs NOEC 300 µg/L (2015) • <i>M. macrocopa</i>: LOEC 125 µg/L (2020) vs NOEC 312.5 µg/L (2015) • <i>L. pipiens</i>: NOEC 10 µg/L (2020) vs LC50 1 242 µg/L (2015) • <i>L. gibba</i>: NOEC 300 µg/L (2020) vs NOEC 6 600 µg/L (2015) • <i>T. obliquus</i>: NOEC 25 000 µg/L (2020) vs IC10 51 000 µg/L (2015). <p>For one species available in 2015, a higher toxicity value was selected as follows.</p> <ul style="list-style-type: none"> • <i>M. sibiricum</i>: EC10 600 µg/L (2020) vs EC10 100 µg/L (2015). The endpoint (longest root) selected for use in 2015 was reconsidered and reclassified as not ecologically relevant. <p>The remaining toxicity values used for the current DGVs represent new species for which aquatic toxicity data were not available for the 2015 draft DGVs.</p>
Use of professional judgement to include studies in DGVs ^a	<p>Professional judgment was applied as described below.</p> <ul style="list-style-type: none"> • Studies for 12 species in the final SSD were nominal concentrations that were included without case-by-case consideration. • A multigenerational zebrafish <i>D. rerio</i> study (Keiter et al. 2012), which had the following limitations, was included. 	<p>Professional judgement was applied as described below.</p> <ul style="list-style-type: none"> • Studies with nominal concentrations were included. The modality assessment and SSD were undertaken using two datasets: one with all data (nominal and measured); and one with only measured data. The nominal data represented approximately half of the available data which would represent a substantial loss of data if they were to be excluded. The conclusion of this assessment was that the nominal and measured datasets were not significantly different, and the nominal concentrations were

Key aspect	2015 draft PFOS DGVs	Current PFOS DGVs
	<ul style="list-style-type: none"> ○ An unusual concentration series that spanned more than one order of magnitude (nominal concentrations of 0.6 µg/L, 100 µg/L and 300 µg/L). ○ Effects relative to controls were greater in some shorter duration exposures (90 d) compared to longer duration exposures (180 d) and were greater in the F2 generation compared to the F1 generation. • Adoption of a LOEC for zebrafish <i>D. rerio</i> (Keiter et al. 2012) multigenerational growth effect (90 d, F2 reduced length and weight) when NOECs (preferred toxicity values) were available for other durations and generations. LOEC was selected because it represented a true (i.e. statistically significant) effect, and after conversion represented a concentration below the available NOEC; hence, the converted LOEC was selected as it was more likely to achieve greater protection than if the NOECs were adopted. • The zebrafish <i>D. rerio</i> LOEC (Keiter et al. 2012) was the lowest toxicity value in the SSD. Inclusion of this toxicity value was considered more likely to result in protective DGVs. • An EC10 was selected for use in the DGV for <i>C. tentans</i> over a lower available NOEC based on the hierarchy of preferred toxicity values. • For <i>P. promelas</i> a NOEC was selected for use in the DGV when a lower EC50 concentration was available. The justification for use of the NOEC was not explicitly provided because the hierarchy of preferred toxicity values rank EC50s as the least preferred toxicity value (i.e. EC10 are preferred to NOECs, NOECs are preferred to LOECs, and LOECs are preferred to EC50s). • Studies with only one or two exposure concentrations were excluded without explicit professional judgement. These included studies undertaken on: <ul style="list-style-type: none"> ○ rotifer <i>Brachionus calyciflorus</i> ○ mollusc <i>Lampsilis siliquoidea</i> ○ fish <i>Xiphophorus helleri</i>. • The effect concentrations spanned over 4 orders of magnitude, and there was a statistically significant difference between the grouping of species into heterotrophic and autotrophic organisms; however, because of the 	<p>scattered throughout the dataset. Therefore, the nominal data were included in the DGVs.</p> <ul style="list-style-type: none"> • Studies with only one exposure concentration were included for species where no other data were available. The toxicity values selected for inclusion in the DGVs for the following species represent testing with one exposure concentration. <ul style="list-style-type: none"> ○ <i>C. riparius</i> (3.5 µg/L) (Stefani et al. 2014, Marziali et al. 2019). The toxicity value is at the more sensitive end of the range, and within the range of toxicity values for other insects used in the DGV derivation (0.92–50 µg/L). Exclusion of toxicity value may result in DGVs that are under-protective. ○ <i>X. helleri</i> (40 µg/L) (Han et al. 2010). The toxicity value is at the more sensitive end of the range, and not outside the range of toxicity values for other fish used in the DGV derivation (0.294–2 120 µg/L). Exclusion of toxicity value may result in DGVs that are under-protective. • Studies with test concentrations that differ by a large amount were included for the following species. <ul style="list-style-type: none"> ○ <i>E. cyathigerum</i> (exposure concentrations 0 µg/L, 10 µg/L, 100 µg/L, 1 000 µg/L and 10 000 µg/L) (Bots et al. 2010). <ul style="list-style-type: none"> ▪ This was the only study available for this species. ▪ The selected toxicity value (3.18 µg/L) is at the more sensitive end of the range, and is within the range of toxicity values for other insects used in the DGV derivation (0.92–50 µg/L). Exclusion of toxicity value may result in DGVs that are under-protective. ○ <i>O. latipes</i> (exposure concentrations 0 µg/L, 1 µg/L, 10 µg/L, 100 µg/L and 1 000 µg/L) (Ji et al. 2008). <ul style="list-style-type: none"> ▪ The toxicity value (4 µg/L) is at the more sensitive end of the range, and is within the range of toxicity values for other fish used in the DGV derivation (0.294–2 120 µg/L). Exclusion of toxicity value may result in DGVs that are under-protective. ○ <i>L. papiens</i> (exposure concentrations 0 µg/L, 1 µg/L, 10 µg/L, 100 µg/L and 1 000 µg/L) (Hoover et al. 2017). <ul style="list-style-type: none"> ▪ Concentrations were measured.

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	lack of a confirmed mode of action to explain the difference between autotrophs and heterotrophs, all data were used in the DGV.	<ul style="list-style-type: none"> ▪ The toxicity value (10 µg/L) is the most sensitive of the data available for frogs used in the DGV derivation. Exclusion of toxicity value may result in DGVs that are under-protective. ○ <i>D. rerio</i> (exposure concentrations 0.073 µg/L, 0.734 µg/L, 106.9 µg/L and 267.6 µg/L) (Keiter et al. 2012). <ul style="list-style-type: none"> ▪ Concentrations were measured. ▪ The toxicity value (0.294 µg/L, converted from a LOEC of 0.735 µg/L) is the most sensitive of the data available for fish used in the DGV derivation. Exclusion of toxicity value may result in DGVs that are under-protective. ○ <i>A. anguilla</i> (exposure concentrations 0 µg/L, 1 µg/L and 10 µg/L nominal (0 µg/L, 0.081 µg/L and 11 µg/L measured) (Roland et al. 2014). <ul style="list-style-type: none"> ▪ This was the only study available for this species. ▪ Concentrations were measured. ▪ The toxicity value (11 µg/L) is at the more sensitive end of the range, and within the range of toxicity values for other fish used in the DGV derivation (0.294–2 120 µg/L). Exclusion of toxicity value may result in DGVs that are under-protective. ○ <i>C. diaptomus</i> and <i>C. canthocamptus staphylinus</i> (exposure concentrations 0 µg/L, 1 000 µg/L, 10 000 µg/L and 30 000 µg/L) (Sanderson et al. 2002). <ul style="list-style-type: none"> ▪ This was the only study available for these species of rotifer. ▪ Concentrations were measured. ○ <i>L. gibba</i> (exposure concentrations 0 µg/L, 300 µg/L, 3 000 µg/L, 10 000 µg/L and 30 000 µg/L) (Boudreau et al. 2003b). <ul style="list-style-type: none"> ▪ Concentrations were measured. ▪ This is the most sensitive (300 µg/L) of the three macrophytes available for use in the DGV (effects ranging 300–3 300 µg/L). ○ <i>M. sibiricum</i> and <i>M. spicatum</i> (exposure concentrations 0 µg/L, 300 µg/L, 3 000 µg/L, 10 000 µg/L and 30 000 µg/L) (Hanson et al. 2005). <ul style="list-style-type: none"> ▪ This was the only study available for these species. ▪ Concentrations were measured.

Key aspect	2015 draft PFOS DGVs	Current PFOS DGVs
		<ul style="list-style-type: none"> Studies with toxicity values expressed as greater than (\geq) values were included for two species. Warne et al. (2018) states that '\geq' values can be used, subject to professional judgement being applied to determine whether they: (i) are too far outside the existing data range and/or (ii) have an overly large influence on the final DGV. Both of the following species were within the range of toxicity values for other species in the same taxonomic groups (fish and amphibians, respectively) selected for the DGV derivation, and their inclusion did not have a large influence on the DGVs. <ul style="list-style-type: none"> <i>A. anguilla</i> NOEC of $>11 \mu\text{g/L}$ was selected for use in the DGVs (Roland et al. 2014). <ul style="list-style-type: none"> This was the only study available for this species. The toxicity value fell within the range available for other fish ($0.294\text{--}2\ 120 \mu\text{g/L}$). <i>X. laevis</i> NOEC of $>608 \mu\text{g/L}$ was selected for use in the DGVs (Lou et al. 2013). <ul style="list-style-type: none"> This was the only study available for this species. The toxicity value fell within the range available for other amphibians ($10\text{--}2\ 000 \mu\text{g/L}$). For the species listed below, a LOEC was selected for use in the DGVs where (i) it was the only available toxicity value and/or (ii) the effect concentration for the LOEC was lower than an available NOEC, and represents a true effect, whereas the NOEC has greater uncertainty because the test exposure concentrations used represented large differences between concentrations and/or between the lowest concentration and the control. Those species included: <ul style="list-style-type: none"> <i>D. rerio</i>—lowest available toxicity value following conversion to estimated EC10/NOEC <i>C. tentans</i>—lowest available toxicity value <i>C. riparius</i>—lowest available toxicity value following conversion to estimated EC10/NOEC <i>L. siliquoidea</i>—only available toxicity value <i>E. cyathigerum</i>—lowest available toxicity value following conversion to estimated EC10/NOEC

Key aspect	2015 draft PFOS DGVs	Current PFOS DGVs
Guidance relating to, and outcomes from performing, modality checks	Checks for modality were performed consistent with the Warne et al. (2015) guidance. A histogram was used to visually assess modality. Statistical tests were performed and concluded that the autotroph and heterotroph data were statistically significantly different. Heterotrophs showed greater sensitivity compared to autotrophs. Other lines of evidence considered included the span of the data, toxicant mode of action, and whether mode of action suggested taxa-specific sensitivity. Although the data spanned greater than 4 orders of magnitude, because greater precedent was placed on evidence of toxicity from mode of action rather than modality, and because the mode of action was not known, it was a professional judgement to include plant and animal data in the SSD.	<ul style="list-style-type: none"> ○ <i>D. magna</i>—lowest available toxicity value following conversion to estimated EC10/NOEC ○ <i>O. latipes</i>—lowest available effect concentration ○ <i>X. helleri</i>—lowest available toxicity value following conversion to estimated EC10/NOEC ○ <i>L. catesbeiana</i>—only available toxicity value ○ <i>B. calyciflorus</i>—lowest available toxicity value ○ <i>M. macrocopa</i>—lowest available toxicity value following conversion to estimated EC10/NOEC ○ <i>D. japonica</i>—lowest available toxicity value ○ <i>C. diaptomus</i>—lowest available toxicity value • For the following species, EC50 values were selected for use in the DGVs. <ul style="list-style-type: none"> ○ <i>P. promelas</i>. This study showed no effects on growth up to the highest concentration (NOEC of >300 µg/L) for the F1 generation. However, effects (EC50) on reproduction for the F0 generation were reported at 230 µg/L. As the growth NOEC is higher than the reproduction EC50, and the EC50 is a true effect, the EC50 was selected for the DGVs because the growth NOEC was not sufficiently protective of reproductive effects (Ankley et al. 2005). ○ <i>D. communis</i>. The EC50 was the only available toxicity value available for this species (Yang et al. 2014).
		<p>Checks for modality were performed consistent with the updated Warne et al. (2018) guidance. A histogram and box and whisker plots were used to make a visual assessment of modality. A statistical measure of bimodality (the bimodality coefficient) was calculated. The results of these assessments indicated that the data were consistent with a unimodal normal distribution. This is despite the data spanning greater than 4 orders of magnitude, which may indicate the potential for bimodality or multimodality. Additional lines of evidence considered included:</p> <ul style="list-style-type: none"> • toxicant mode of action (which remains unknown) • whether indications of bimodality or multimodality or distinct clustering of taxa groups were not due to artefacts of data selection (e.g. nominal versus measured concentrations, toxicity values (EC10 vs LOEC vs NOEC))

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		<ul style="list-style-type: none"> • small sample size within taxonomic groups • test procedures, or other reasons unrelated to a specific mode of action. <p>Although autotrophs were generally less sensitive to PFOS than heterotrophs, this did not result in a bimodal distribution of species sensitivity. The weight of evidence concluded that the assessment supported use of all 35 animal and plant species identified in preparation of the SSD, and that this would give comprehensive and robust coverage of the range of effects data.</p>
Reality check if the theoretical protection offered by the DGVs is adequate	The derived per cent protection levels were compared to the raw toxicity data (over 120 acute and chronic toxicity values). The following per cent of species in the raw toxicity data are protected for the calculated DGVs for 99%, 95%, 90% and 80% species protection levels, respectively: 100%, 100%, 98% and 84%. This check confirmed that the theoretical protection offered by the DGVs is likely to be adequate.	The derived per cent protection levels were compared to the converted chronic toxicity data (241 chronic toxicity values). The following per cent of species in the chronic toxicity data are protected for the calculated DGVs for 99%, 95%, 90% and 80% species protection levels, respectively: 100%, 96%, 92% and 85%. This check confirmed that the theoretical protection offered by the DGVs is likely to be adequate.
Reliability classification ^b	The number of chronic studies and shape of the SSD supported classification of the DGVs as ‘very high’ reliability according to the Warne et al. (2015) method. However, it was noted in the DGVs that because the data spanned greater than 4 orders of magnitude there was greater uncertainty and lower confidence in the 99% species protection level.	The number of chronic studies and shape of the SSD supported classification of the DGVs as ‘very high’ reliability according to the Warne et al. (2018) method.

a Studies with specific test characteristics should be excluded, namely:

- nominal concentrations
- large differences in test concentrations
- non-traditional endpoints (defined in Warne et al. (2018) as ‘*such as photosynthesis inhibition, in vivo biochemical and physiological endpoints, behavioral endpoints, and genotoxicity and mutagenicity*’ and noting that ‘[non-traditional endpoints] *may be used provided that their ecological relevance for the species, or closely related species, has been demonstrated*’)
- endpoints of unknown ecological relevance (defined in Warne et al. (2018) as ‘*an endpoint [that] negatively affects a species’ ecological competitiveness (that is its ability to increase the frequency of its genes in subsequent generations)*’. Endpoints that are ecologically relevant include lethality, immobilisation, growth, development, population growth, and reproduction).

b The number of species available, the type of data (chronic vs acute, or a mixture), and a visual assessment of the fit of the SSD to the toxicity data (that is good or poor) are used to assign a reliability classification to a DGV.

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