Australian Government
Department of Climate Change, Energy, the Environment and Water

Independent Review of Key Issues Arising from Public Comments on the PFOS Freshwater Draft Guideline Values

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Report of the Independent Technical Commitee prepared for DCCEEW

Cataloguing data

This publication (and any material sourced from it) should be cited as: P. Dawson, C. Le-Steere, R. M. Mann, J. Stauber and S. Vardy (2024). Independent Review of Key Issues Arising from Public Comments on the PFOS Freshwater Draft Guideline Values. Report for DECCEEW, pp.

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Acknowledgement of Country

We acknowledge the Traditional Owners of Country throughout Australia and recognise their continuing connection to land, waters and culture. We pay our respects to their Elders past and present.

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1.1 Background

The draft Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZ Guidelines) toxicant default guideline values (DGVs) for aquatic ecosystem protection technical brief for Perfluorooctane sulfonate (PFOS) in freshwater (f) (ANZG 2023) was made available for public submissions in May 2023. The public comment period was open until 17 August 2023. Six public submissions were received.

1.2 Default Guideline Value Derivation

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 in the development of the DGVs, the zebrafish *Danio rerio* was the most sensitive species, with a LOEC of approximately 0.7 µg/L (F2 generation growth LOEC, 90 d and 180 d post fertilisation) (Keiter et al. 2012), while the diatom *Navicula pelliculosa* was the least sensitive species, with an EC50 of 263 000 µg/L (5-d EC50) (OECD 2002).

The draft PFOS (f) DGVs technical brief reported “very high” reliability DGVs based on chronic EC10, NOEC, LOEC, and EC50 data for 35 species from 11 taxonomic groups. The DGVs reliability rating was based on the high sample size (35 toxicity values), use of only chronic toxicity data and a good fit of the species sensitivity distribution (SSD) to the toxicity data, as per the reliability classification described in Warne et al. (2018). The DGVs for PFOS in freshwater for 99%, 95%, 90% and 80% species protection derived were 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 was recommended for application to slightly-to-moderately disturbed ecosystems.

The DGVs were derived in accordance with the method described in Warne et al. (2018) and using Burrlioz 2.0 software.

1.2.1 Toxicity data used in derivation

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

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).

According to Warne et al. (2018), Table 2, data from studies where the test concentrations differ by a large amount (for example ≥ 10-fold differences) should not be used, except where the data are of particular significance and a strong justification is provided. In the derivation of the draft PFOS (f) DGVs, 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.

1.3 Process for Addressing Public Comments

Key issues raised by the submitters during the public comment period were as follows:

1. Inclusion of the zebrafish value from the Keiter et al. (2012) study when a more recent and recently published US study (Gust et al., 2023) has reported very contrasting results
2. The large number of data selection decisions that deviated from the standard rules in the Warne et al. (2018) toxicant guideline value derivation method
3. Concerns over the guidance within the technical brief on how to deal with bioaccumulation of PFOS
4. Concerns over the use of Burrlioz over a better species sensitivity distribution (SSD) fitting approach such as shinyssdtools
5. The dataset should be treated as being bimodal, with algae/plants being less sensitive.

Given the importance and sensitivity associated with the draft PFOS (f) DGVs, it was necessary that the process for responding to public comments be rigorous and transparent to provide stakeholders with assurance and confidence in the process and final dataset and DGVs. Issues 1 and 2 were complicated and require that data and data selection decisions be revisited, and this required significant technical expertise and knowledge to address. Issue 3 also required expert consideration. Issues 4 and 5 were considered more straightforward and able to be addressed internally by the ANZG Technical Manager and Project Coordination Group. Consequently, it was agreed by the relevant ANZG Guidelines committees that issues 1, 2 and 3 should be considered by an independent review committee (IRC) comprising relevant experts that, together, meet all of the following criteria:

- Were not involved in the derivation of the draft PFOS DGVs
- Were not involved in the peer review of the draft PFOS DGVs
- Were not involved in a public response to the draft PFOS DGVs
- Have sound knowledge of PFAS fate/toxicity
- Have sound knowledge of the DGV derivation method.

The composition of the IRC was:

  - **Chair:** Suzanne Vardy, Principal Scientist, Queensland Department of Environment, Science & Innovation

- **Members:**
  - Chris Lee-Steere, Australian Environment Agency Pty Ltd
  - Jenny Stauber, Chief Research Scientist, CSIRO and La Trobe University
  - Peter Dawson, Principal Scientist, Environmental Protection Authority New Zealand
  - Reinier M. Mann, Science Leader, Queensland Department of Environment, Science & Innovation

This document presents the technically supported responses and proposed recommendations from the independent review committee relating to issues 1, 2 and 3 above arising from the public comments process for the PFOS (f) DGVs.

1.4 Approach to the Review

The IRC was provided with the following relevant materials:

- Draft PFOS freshwater DGVs technical brief (ANZG 2023)
- All submitted public comments
- Warne et al. (2018) toxicant GV derivation method
- Keiter et al. (2012) zebrafish study
- Gust et al. (2023) zebrafish study
- Pandelides et al. (2023) zebrafish PFOS toxicity meta-review

Additional relevant materials, including all the journal papers referred to in the public submissions, were also sourced and considered.

The IRC considered the technical merits of all the public submissions related to issues 1, 2 and 3 above, including by:

- Parallel consideration of the relevant decisions and justifications detailed in the draft PFOS (f) DGVs technical brief (ANZG 2023)
- Assessment of the consistency with the toxicant guideline value derivation method detailed in Warne et al. (2018)
- Consideration of any other technical information that might inform the issues.

In this report, the IRC has provided responses to the public comments related to issues 1, 2 and 3, supported by relevant technical analysis and justifications. Recommendations are presented to address each issue.

2. Specific concerns expressed in the public submissions

2.1 Concerns Over the Use of Keiter et al. (2012)

Five of the six public consultation submissions expressed concern about use of a LOEC for the zebrafish, *Danio rerio*, described in Keiter et al. (2012). For the purpose of DGV derivation, the DGV authors adopted a LOEC (F2 growth) from this study of 0.734 µg/L. The LOEC was converted to a NOEC equivalent of 0.294 µg/L by dividing the LOEC by 5 as recommended by Warne et al. (2018). This is the lowest value used in the SSD. It is also almost three times lower than the next lowest value (0.92 µg/L for *Chironomus tentans* development). As such, the *D. rerio* value plays a pivotal role in determining the shape of the SSD and thus the magnitude of the DGVs.

Appendix C of the technical brief provided the following rationale for the use of the Keiter et al. (2012) LOEC value:

- 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.

Concern raised by public consultation submissions revolve around the following main issues

1. **The study design used by Keiter et al. (2012) was not appropriate for use in DGV derivation, because:**
    a. The lowest exposure concentration was separated by more than an order of magnitude from the next highest exposure concentration (i.e. 0.6 and 100 µg/L (nominal)) which is inconsistent with the recommendations of Warne et al. (2018)
    b. Poor replication. Two replicate tanks were used for each test solution. All animals in one of the replicate tanks where F2 males were exposed to 100 µg/L experienced 95% mortality leaving a single replicate tank. One of the public consultation submissions suggested pseudo-replication; however, a reading of the methods section describing the statistical analyses used, is not detailed enough to infer which statistical tests were used in the various comparisons, and there is no indication how the loss of a whole replicate treatment (i.e. F2 exposed to 100 µg/L) was handled statistically; i.e. there is not a statement indicating that each individual fish was used as a replicate (pseudo-replication).
    c. Inadequate measurements of test concentrations during the 330 days of exposure for the three generations of fish. Exposure water PFOS concentrations were measured approximately once per month.
2. **The results did not demonstrate a concentration-response relationship.**

a. The lengths and weights of F2 males exposed to 0.6 µg/L for 30 days were significantly different from controls, but lengths and weights of F2 males exposed to 100 µg/L for 30 days were not significantly different to controls. Similarly, the weights of F2 females exposed to 0.6 µg/L for 30 days were significantly different from controls, but weights of F2 females exposed to 100 µg/L for 30 days were not significantly different to controls.

b. The lengths and weights of F2 males exposed to 0.6 µg/L for 30 or 90 days were significantly different from controls, but lengths and weights of F2 males exposed to 0.6 µg/L for 180 days were not significantly different to controls. Similarly, the weights of F2 females exposed to 0.6 µg/L for 30 or 90 days were significantly different from controls, but the weights of F2 females exposed to 0.6 µg/L for 180 days were not significantly different to controls.

c. The lengths of F1 males exposed to 0.6 µg/L for 90 days were significantly different from controls, but the lengths of F1 males exposed to 0.6 µg/L for 180 days were not significantly different to controls. Similarly, the weights of F1 males exposed to 0.6, 100 and 300 µg/L for 90 days were significantly different from controls, but the weights of F1 males exposed to all three concentrations for 180 days were not significantly different to controls.

3. Other toxicity data, of higher reliability, are available in the literature.

a. Five of the six submissions referred to the availability of the data published in Gust et al. (2023). Gust et al. (2023) was published after the public consultation responses were received, but the respondents asserted that data from that study had been readily available for some time. Gust et al. (2023) is referred to in public consultation submissions as the United States Army Corps of Engineer (USACE) study, or alternatively as the Strategic Environmental Research and Development Program (SERDP) study. The study was designed to address the design flaws of Keiter et al. (2012) and differs from the latter in that it:

   - used five PFOS exposure concentrations (0.1, 0.6, 3.2, 20, 100 µg/L) instead of three PFOS exposure concentrations (0.6, 100, and 300 µg/L), thus ensuring that all exposure concentrations were within a factor of 10 times that of adjacent exposure concentrations,
   - relied on five replicates per exposure concentration instead of only two replicates per exposure concentration, thus improving statistical power.
   - PFOS in water was measured weekly,
   - all the raw data were made available. The raw data provided in the supplementary materials would make possible the calculation of EC10 values or other statistics for use in DGV derivation.

The results of Gust et al. (2012) did not indicate any toxicological effects at concentrations below 1.0 µg/L and thus supported concerns raised about the reliability of the Keiter et al. (2012) results.

b. One submission provided a list of studies that examined the effects of PFOS in zebra fish. One in particular, Du et al. (2009) reported a maximum acceptable toxicant concentration (MATC) which was adopted for use in an SSD by Environment and Climate Change Canada (ECCC 2018).

c. One submission suggested that the availability of other zebra fish studies had permitted the US EPA to calculate a geometric mean in the development of Draft Aquatic Life Ambient Water Quality Criteria for PFOS, without using the Keiter data.

View of the IRC:

As noted by one of the submissions, despite the shortcomings of the Keiter et al. (2012) study design, the ANZG quality screening methodology still scored the Keiter study as a study of acceptable quality. The absence of a clear, monotonic concentration-response relationship should reduce the quality score for the study but would not necessarily preclude the use of the study for DGV derivation. The estimated differences in growth parameters for the F2 cohort are presented in Table 1. The estimates in Table 1 have been taken off the graphs in Keiter et al. (2012), and are likely to only approximate the data collected by the authors. The data do indicate substantial differences (19.7% to 50%) at the 30 day mark, but these differences largely disappear at the 180 day mark (5.7% to 7.7%) with PFOS-exposed males showing even greater growth than controls animals.

Table 1: Growth parameters for the F2 cohort based on visual estimates taken from Figure 4 in Keiter et al. (2012)

Cohort	Control	0.6 µg/L (nominal)	Per cent difference
F2 female length at 30 days	11.6 mm	9.3 mm	19.7% smaller (p<0.001)
F2 female weight at 30 days	13.3 mg	6.6 mg	50% lighter (p<0.001)
F2 male length at 30 days	11.6 mm	9.3 mm	19.7% smaller (p<0.001)
F2 male weight at 30 days	13.3 mg	6.6 mg	50% lighter (p<0.001)
F2 female length at 180 days	33.6 mm	31.7 mm	5.7% smaller (p<0.001)
F2 female weight at 180 days	337 mg	317 mg	6% lighter (insignificant)
F2 male length at 180 days	30 mm	32 mm	6.6% larger (insignificant)
F2 male weight at 180 days	300 mg	323 mg	7.7% heavier (insignificant)

The fact that such highly significant statistical results were obtained when the study only included two replicates, strongly suggests that each individual fish was used as a replicate. Such highly significant results would be unlikely if the statistical analyses had used only the two replicates. The loss of one of the replicates in the crucial F2 treatments compounds concerns over the validity of the statistics. Ignoring the apparent pseudo-replication, the statistics are persuasive. Nevertheless, the

inconsistency in results over time and with increasing exposure concentrations does raise concerns about the validity of the data, if not the statistics. The IRC are of the opinion the use of a more conventional statistical approach would be unlikely to support the significant results presented in Keiter et al. (2012) and the apparent lack of a monotonic concentration-response relationship reduces the utility of these data for the derivation of DGVs.

The fact that the exposure concentrations differed by more than a factor of ten was discounted by the DGV authors because the study represented the lowest toxicity result for the species, and therefore, adoption of the Keiter et al. (2012) LOEC would ensure that the DGVs were not under-protective. Again, the highly statistically significant results (mostly $P<0.001$) for the species would have provided some confidence in the study results. However, the IRC is of the opinion that the paper does not provide adequate detail on how and which statistics were applied, and that it should be treated with caution.

Overall, the IRC agreed that poor replication, the validity of the statistics applied and the poor spacing between exposure concentrations, are valid cause for the concerns raised in the public consultation process; concerns that are supported by other reviews (US EPA 2022, Pandelides et al. 2023). The SERDP study (Gust et al. 2023) was specifically designed to verify the statistically significant growth effect results reported by Keiter et al. (2012) at 0.6 µg/L (nominal). However, the results of Gust et al. (2023) were unable to confirm any toxicity at this low concentration, but rather, confirmed multiple other studies that indicated measurable impacts on survival and growth at much higher exposure concentrations. As other, more reliable data are available (i.e. from studies better designed for use in DGV derivation), including Gust et al. (2023), and as reviewed by Pandelides et al. (2023), the IRC recommends that the Keiter et al. (2012) data not be included in the PFOS SSD and that another toxicity value for *D. rerio* is used instead (see Section 2.2.4). Other *D. rerio* data will include those published in Gust et al. (2023), as these data have now undergone peer review. Selection of a different toxicity value for *D. rerio* will necessitate recalculation of the PFOS DGV.

2.2 Concerns over the use of the large number of data selection decisions that deviated from the Warne et al. (2018) DGV derivation method

Inclusion or exclusion of specific data in the PFOS SSD is based on best professional judgement (i.e. there is no correct or incorrect approach). For this report, the IRC reviewed data in each individual journal paper where public submission responses questioned the data decisions made by the DGV authors. The IRC reached consensus using best professional judgement and has recommended resolutions as below. This will necessitate follow up and recalculation of the PFOS DGVs.

2.2.1 Data from tests where test concentrations differed by 10-fold

One submission questioned the inclusion of data for 8 species in the SSD from studies with at least a 10-fold increase between test concentrations. The IRC carefully reviewed each of these studies and has recommended the following inclusions and exclusions:

1. *Myriophyllum spicatum* and *Myriophyllum sibiricum* (Hanson et al. 2005). This was the only study for these two species. The DGV authors used a 42-day EC10 (growth) of 600 µg/L for *M. sibiricum* and a 28-day EC10 (growth) of 3,300 µg/L for *M. spicatum*. The exposure concentrations for both species were 0, 0.3, 3.0, 10 and 30 mg/L. The two lower concentrations had a 10-fold difference, but this was not the case for the rest of the test concentration spacing. The response curves for both species show definite increasing effects with increasing concentrations. For *M. spicatum*, the DGV authors chose the EC10 (which was more conservative than the NOEC) for the most sensitive endpoint - 28-d dry mass, but no plots were given for this endpoint to check partial effects. Partial effects were only shown in plots of 42-d plant length and root length, which had higher EC10s of 9,900 and 10,000 µg/L respectively. Despite these limitations, the **EC10 of 3,300 ug/L for *M. spicatum* is acceptable and should be retained in the SSD**. For *M. sibiricum,* the DGV authors chose the EC10 (which was more conservative than the NOEC) for the most sensitive endpoints – 42-d wet mass (and 14-d root length). No plots of these were shown to check for partial effects; however, the **EC10 of 600 ug/L for *M. sibiricum* is acceptable and the IRC recommends that this value should be retained in the SSD.**

2. *Cyclops diaptomus* (Sanderson et al. 2002). This was the only study for this species. The DGV authors chose a 28-d LOEC for abundance of 1 mg/L. This is a microcosm study that aimed to determine a 35-d community no-observable-effect concentration ($NOEC_{community}$) for freshwater zooplankton and the fate of PFOS during the course of study. Results of these types of studies are open to a wide range of interpretations and can be difficult to interpret even when the full dataset is available rather than just a published paper. The tested concentrations were 1, 10 and 30 mg/L so while there is a 10-fold factor between the two lowest concentrations, there is a factor of 3 spacing for the middle and higher concentrations. *Cyclops diaptomus* was the most sensitive species in the zooplankton community. This organism was not found in the samples after one week at 30 mg/L and after two weeks at the 10 mg/L, whereas the abundance in the controls was more or less constant after 24 h. The IRC recommends that the **28-d LOEC for abundance of 1 mg/L is acceptable and should be retained in the SSD.**

3. *Enallagma cyathigerum* (Bots et al. 2010). This was the only study for this species. The DGV authors used the 120-day LOEC of 7.95 µg/L and converted this to a NOEC of 3.18 µg/L by dividing by 2.5 as per the Warne et al (2018) guidance. With a 10- fold dilution series (4 exposure concentrations and a control), this is really a range finder test using nominal concentrations only. Metamorphosis was inhibited at 10 µg/L (the lowest concentration tested, 75.5% compared to controls) therefore the NOEC was <10 µg/L. Bots et al. (2010) published a range of other NOECs for different endpoints, including long term NOECs of 10 µg/L for both larval survival and larval development time, and NOECs of 10,000 µg/L for egg hatching success and egg hatching time. According to Warne et al. (2018) less than (<) values should generally be excluded, so based on the weight of evidence, the IRC recommends that the NOEC for long term survival/development of 10 µg/L is better to use than the 120-day LOEC. With correction to the PFOS anion from the ammonium salt (as done by the DGV authors), a NOEC of 7.95 µg/L would be acceptable. However, because it was not clear in the paper if their PFOS concentrations were as the ammonium salt or corrected to PFOS anion, and noting that concentrations were only nominal, the IRC also recommends that the D**GV authors recheck with the study authors re. the PFOS anion corrections before inclusion of the NOEC of 7.95 µg/L.**

4. *Xenopus laevis* (Lou et al. 2013). This was the only study for this species. The DGV authors used a 120-d NOEC (growth) of 608 µg/L. However, this study tested 4 concentrations spaced by 1 or 2 orders of magnitude (0.1, 1, 100 and 1000 µg/L). The authors found no effect on either 2-month survival or growth of tadpoles at the highest concentration tested (measured as about 608 µg/L). The actual NOEC may therefore be much higher. Following Warne et al (2018), toxicity values expressed as greater than (>) should not be used if they are too far outside the existing data range. This is the case here, so the IRC **recommends removing this data from the SSD.**

5. *Danio rerio* (Keiter et al., 2012). This study had well documented concerns (see section 2.1) and a newer study (Gust et al, 2024) was performed to address those concerns. The IRC **recommends that the result from Keiter et al (2012) should not be used.**

6. *Oryzias latipes* (Ji et al. 2008). This is one of two studies with this species. The DGV authors used a 24-d LOEC (reproduction) of 10 µg/L adjusted to a NOEC by dividing by 2.5. Ji et al. (2008) exposed medaka to three nominal concentrations of 0.01, 0.10 and 1.0 mg/L. There did appear to be a reduction in fecundity (number of eggs produced) in all PFOS exposure groups with the reduction more pronounced at 0.10 and 1.0 mg/L from day 6 to day 14 (end of F0 exposure time). It does appear that the reduction in number of eggs at 0.01 mg/L was >10% to that of the control for most of the assessment periods from day 4 onwards, possibly up to 20% at the 14-day period. There were statistically significant reductions in total length and body weight of the F0 generation at all concentrations, however, the sample size at the higher two concentrations was quite small. At the lowest concentration, the % reductions in length and body weight were 4.3% and 12%, respectively. The weight changes may be considered biologically relevant at >10%. In the F0 generation eggs, hatchability was reduced at all concentrations (results only available graphically). From the graphs, it appears the reduction in hatchability at 0.01 and 0.10 mg/L are <10% so may not be considered biologically relevant. The F1 generation time to hatch parameter was clearly influenced by PFOS at 1.0 mg/L but was not statistically reduced at 0.10 or 0.01 mg/L. Given the potentially treatment-related effects on F0 body weight at the lowest concentration, it is probably still acceptable to maintain this concentration (0.01 mg/L) as the study LOEC. The very small (88% and 96% of control) but significant reduction in F1 growth after F0 adult exposure confirms a LOEC of 10 µg/L is acceptable, although the LOEC could actually be lower as this was the lowest concentration tested. The IRC recommends that, despite the limited number of test concentrations and given that no other toxicity data are available for this species, that the **LOEC of 10 µg/L is retained in the SSD.**

7. *Lithobates pipiens* (Hoover et al. 2017). This is one of two studies with this species. The DGV authors used a 40-d NOEC (development) of 10 µg/L. The tested concentrations were 10, 100 and 1000 µg/L. The exposure and observation period for survival, growth and development was 40 days. Only sublethal effects were observed for tadpoles, as survival for all treatments was above 90%. Length and development trended below controls for nearly all exposures and was deemed statistically significantly different at the two highest concentrations at 40 days. There was a definite reduction also in the 10 µg/L treatment (nominal). In this case, the draft DGV authors note this is the most sensitive of the data available for frogs and exclusion of this toxicity value may result in DGVs that are under-protective. This argument appears

justified and the IRC recommends that the 4**0-d NOEC of 10 µg/L from this study be retained.**

2.2.1 Studies with only one exposure concentration

One submission questioned the use of data for two species where only one PFOS concentration was tested. The IRC carefully reviewed these studies and has recommended the following inclusions and exclusions:

1. *Chironomus riparius* (Stefani et al. 2014, Marziali et al. 2019). The DGV authors used a LOEC (development) of 3.5 µg/L converted to a NOEC of 1.4 µg/L by dividing by 2.5. However, only one concentration (10 µg/L nominal) was used (and measured as 3.5 µg/L) and very small effects were only found on growth/development time in females in only 2-4 of 10 generations at 3.5 µg/L (not survival, reproduction). Given that the study authors concluded that population effects were unlikely, the IRC recommends that **this data is excluded from use in the SSD.**

2. *Xiphophorus helleri* (Han et al. 2010). The DGV authors used a 90-d LOEC (growth) of 100 µg/L, converted to a NOEC of 40 µg/L by dividing by 2.5. Three concentrations (0.1, 0.5, 2.5 mg/L) and a control were tested for the first generation, while a single concentration (0.1 mg/L) and control were tested for the second generation. At the end of the exposure period for the first generation, the survival rates among the adult females were 100%, 88.9%, 89% and 33.3% in the 0, 0.1, 0.5 and 2.5 mg/L groups (Chi-square test, $p < 0.01$), respectively.) While there was no statistical difference in the average number of offspring in the treatment groups, there was a difference ($p < 0.01$) in the 14-day survival rates of the offspring (98%, 96% and 43% in the 0, 0.1, and 0.5 mg/L PFOS groups, respectively). The effects of growth in the second generation were only considered for a single treatment concentration (0.1 mg/L), but given the effects observed earlier, this should be considered acceptable. Growth (90 days) based on both body length and weight in females was statistically significantly different at this concentration and inhibition compared to the control for both measurements was ~13%. Therefore, the IRC recommends that the **100 µg/L LOEC should be retained.** When converted to a NOEC of 40 µg/L this NOEC is within the range of other values for fish.

2.2.2 Data selection decisions inconsistent with the hierarchy of acceptable toxicity estimates

One submission raised questions around the possible inconsistency with the preferred hierarchy of toxicity estimates applied in several species. Some studies have been addressed already in the above sections and are not reported further within this section. The IRC has reviewed the other studies and recommends the following inclusions and exclusions:

1. *Chironomus tentans* (MacDonald et al., 2004). The DGV authors selected the lowest exposure concentration (2.3 µg/L) from the chronic test as a LOEC because of the statistically significant reduction in emergence at the lowest concentration. The chronic test was undertaken with measured concentrations of 2.3, 14.4, 21.7, 94.9, 149.0 µg/L. With respect to emergence (results only provided graphically), there is an apparent concentration-response by virtue of the fact that the highest inhibition was observed at the highest concentration. Correcting emergence in the treatment groups for the control emergence at the end of the study (~73%), the inhibition in emergence in the 2.3, 14.4, 21.7 and 94.9 µg /L was 31.5, 41.1, 35.6 and 84.2%, respectively as read from the graph (Figure 4 in the literature paper). There was 100% inhibition at the highest treatment. At this level (149 µg/L), a few larvae were observed in the emergence/reproduction replicates, but none survived through

pupation to emerge. The study authors report a calculated EC50 and EC10 for total emergence of 94.5 and 89.3 µg /L, respectively. Relatively tight confidence intervals are associated with these values, but without the raw data, it is not clear how these values are possible given the apparent >30% effect at the lowest concentration and >80% effect at the measured 94.9 µg /L. These values are not consistent with the data shown in Figure 4 in the publication as described above where >30% inhibition was observed at the lowest exposure concentration. The IRC considered that, without the raw data, the ECx values reported in this study should not be relied on for emergence results. It cannot be reconciled how the study authors calculated an EC10 for total emergence of 89.3 µg /L with such tight confidence intervals, yet the study NOEC is listed as being <~40 times lower than this. Despite these inconsistencies, the study appears valid. **Given the concerns above, the DGV authors should obtain the concentration-response curve to ensure reliability of the EC10 prior to its inclusion. If the curve is deemed unreliable, the current choice of applying the LOEC with the appropriate assessment factor should be maintained in the SSD. In addition, one of the submissions noted that there is another recent study available for this species (McCarthy et al,. 2021) that was not available at the time the draft DGVs were derived. This study has not been reviewed by the IRC, but the IRC recommends that it is obtained and considered for use in the selection of the final toxicity value for this species.**

2. *Lampsilis siliquoidea* (Hazelton et al., 2012). The DGV authors applied a LOEC based on survival of 4.5 µg/L justified because it was the only available toxicity result for this species. There was contamination in the control group (2.11 µg/L), and only two other measured concentrations tested (4.52, 69.5 µg/L). Control contamination was quite high. Further, viability of controls decreased over time, and it is likely no established acceptability limits for control survival were met. While there was a highly significant ($p<0.0005$) difference in survival after 36 days (7 weeks) between 2.11 µg/L (control) and 4.52 µg/L, there was no clear concentration-response relationship as the viability at 69.5 µg/L produced a similar decrease in viability. The IRC has concerns about the validity of this study given the poor survival in the controls noting the contamination within this group and recommends that **this data is excluded from use in the SSD.**

3. *Daphnia magna* (Lu et al., 2015). The DGV authors adopted a LOEC based on length and rate of population growth of 8 µg/L. The study provided biological results for 6 parameters, namely, adult body length, time to first pregnancy, time to first brood, number of first brood per female, number of offspring per brood per female, and the intrinsic rate of population growth (r). Two of these, adult body length and r had statistically significant effects compared to the control even at the lowest concentration tested, resulting in 8 µg/L being deemed the study LOEC. For adult body length, no tested concentration resulted in ≥10% inhibition despite all concentrations being deemed statistically significant. For r, there was a concentration-response relationship apparent, although the slope of the relationship was relatively shallow. The study was performed to OECD Test Guideline 211. As noted in the OECD test guideline, the ecologically most relevant response variable is the total number of living offspring produced per parent animal. There is a validity criterion related to reproduction in this guideline such that, for the study to be valid, the mean number of offspring produced per parent animal at the end of the test should be ≥60. This was not met in the Lu et al. (2015) study (mean 45.1 based on reported mean number of brood/female 6.53; mean number of offspring per brood 6.91). The reproduction rate influences the intrinsic rate of population growth, so failing the validity criterion for reproduction means the study should not be relied on. The IRC **recommends that this study NOT be used in the SSD.**

There is a second *D. magna* 21-d reproduction study that followed the same OECD test guideline (Ji et al., 2008 (summary results in Table 2 below)). It is unclear why the results of that test were not included in the DGV authors' assessment. It also measured the rate of population growth, and like the results in Lu et al. (2015), this was the most sensitive. However, the results overall were less sensitive in this study than in the Lu et al. study. This study also followed OECD 211 and used appropriate replication. The study was conducted under static renewal conditions with exposure to 5 concentrations. Validity criteria for adult mortality and reproduction performance were met with 100% adult survival and a mean 83.2 juveniles per female. **The IRC recommends the DGV authors apply the mean replicate results from Table 1 below to calculate an EC10. In doing this, it is noted that there are limitations in using mean replicate results usually available in literature papers to calculate ECx values as individual replicate results are not available, so factors such as variability and confidence intervals are not able to be calculated. Nonetheless, the IRC is of the view that it is still preferable to calculate an ECx where possible as these are considered more useful than lower reliability endpoints such as a NOEC or LOEC.**

Table 2: Measured parameters and % inhibition to Daphnia magna, Ji et al. (2008)

Concentration (µg/L)	Adult length	No. young/adult	No offspring/brood	Intrinsic rate of population growth (r)
0	3.61	83.2	16.33	0.403
312.5	3.58	80.7	16.14	0.388
625	3.55	78.3	16.01	0.371
1250	3.41	78.25	16.57	0.35
2500	3.34	56.57	12.88	0.291
5000	3.19	42.4	11.08	0.196
% inhibition relative to control:				
312.5	0.8	3.0	1.2	3.7
625	1.7	5.9	2.0	7.9
1250	5.5	5.9	-1.5	13.2
2500	7.5	32.0	21.1	27.8
5000	11.6	49.0	32.1	51.4

From these results, the EC10 for the intrinsic rate of population growth was calculated to be ~900 µg/L (to be confirmed by DGV authors) and the result did not need to be extrapolated.

4. *Lithobates catesbeiana* (Flynn et al., 2019). The DGV authors applied a LOEC of 144 µg/L based on growth. The paper describes initially performed acute 96 hour toxicity studies to determine various LCx values. The final 96 h LC50 for PFOS to this test organism was 144 µg/L. Based on this, the chronic study was undertaken using exposure levels of 0.1% (144 µg/L) and 0.2% (288 µg/L) of LC50 concentration (nominal). Both concentrations resulted in highly significant ($p<0.001$) reductions in mass at 42, 56, 63,70 and 72 days. Considering the

highly significant result, and the consistency of observations over an extended (chronic) time frame, the use of a LOEC=144 µg/L is defensible, although the actual negligible effect concentration is potentially much lower. This is the only study available for this species and **the IRC recommends the LOEC should be retained for use in the SSD.**

5. *Brachionus calyciflorus* (Zhang et al., 2013). The DGV authors adopted a LOEC of 0.25 mg/L based on population density. Exposures were 250, 500, 1000, and 2000 µg/L. Based on an assessment of life history (where test duration was variable depending on when all individuals in each cohort died) (Table 1 in the literature paper), an EC10 could be calculated. The most sensitive indicators of toxicity were the net reproductive rate (14.6% reduction compared to control at the lowest test concentration; indicative EC10 of 220 µg/L) and the intrinsic rate of natural increase (11.8% reduction compared to control at the lowest test concentration; indicative EC10 of 290 µg/L). In the next phase, the long-term exposure (28 days) was only tested at the lowest two concentrations. The maximum population densities of rotifers were 14.34, 11.8, and 7.48 ind/mL in culture media containing 0.0, 250, and 2000 µg/L, respectively. The population densities decreased by 17.7% and 47.8% compared to the control, respectively. This does indicate a biologically relevant effect at the lowest concentration and appears more sensitive than results obtained in the life history tests. In the absence of a calculated EC10 (only 2 exposure concentrations), the use of the LOEC/2.5 is defensible considering three endpoints were affected consistently with relatively tight confidence intervals. The IRC **recommends the LOEC should be retained for use in the SSD.**

6. *Moina macrocopa* (Ji et al., 2008). The DGV authors adopted a LOEC of 312.5 µg/L based on reproduction. There was sufficient information in the paper to calculate EC10 values, which are preferable to the use of a LOEC. The most sensitive endpoint from this study was the number of young/adult with a statistically significant effect at the lowest concentration tested. There was a clear concentration-response and at the lowest concentration the % reduction compared to the control was 14.2%. This is shown in Table 3 where an EC10 of 260µg/L was calculated by one of the reviewers. The IRC **recommends the DGV authors calculate an appropriate EC10 for use in the SSD.**

Table 3: Data from Ji et al 2008

Concentration (µg/L)	No young per adult	% reduction to control
0	50.6	-
312.5	43.4	14.2
625	33.7	33.4
1250	29	42.7
2500	24	52.6
5	20.25	60.0
EC10 (Weibull model)		260 µg/L

7. *Dugesia japonica* (Yuan et al., 2014). The DGV authors adopted a LOEC of 500 µg/L based on reproduction. Exposure was to nominal concentrations of 0.5, 1.0, 5.0, 8.0, and 10 mg/L. There was good replication (10x3) in the study. Number of planarians with auricles after 10 days was the most sensitive endpoint and the lowest concentration (0.5 mg/L) significantly ($p<0.01$) inhibited regeneration. The LOEC/2.5 is defensible, and consistent with the

observations of biomarker effects despite the variability compared to controls. The IRC **recommends the LOEC should be retained for use in the SSD.**

8. *Pimephales promelas* (Ankley et al., 2005). The DGV authors adopted the EC50 (reproduction) of 230 µg/L. Their rationale for this value was that the 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 considered a true effect and the EC50 was selected for the DGVs because the growth NOEC was not sufficiently protective of reproductive effects. The trends with respect to fecundity seemed apparent even though the concentration-response relationship exhibited some non-monotonicity. With values just read from the graph in the report (Figure 1), the changes in egg production in the 30, 100, 300 and 1000 µg/L groups compared to the control were +22% (increased production), -49%, -28% and -86%, respectively. It is hard then to say the results at 100 µg/L are entirely treatment-related, but given all results ≥100 µg/L are >10%, a NOEC of 30 µg/L appears justified. The use of the EC50 is preferred to the NOEC,. Consequently, the IRC **recommends the EC50/5 (46 µg/L) should be retained for use in the SSD.**

9. *Desmodesmus communis* (formerly *Scenedesmus quadricauda*) (Yang et al., 2014). The DGV authors adopted the EC50 of 89.34 mg/L. The authors applied the default assessment factor of 5 to the calculated EC50 to approximate a NOEC. This is the lowest preference, with an EC10 or other negligible effect concentrations being more desirable. Supplementary material indicates that only the 50 mg/L and 185.65 mg/L concentrations were analysed. It is not clear if the effect concentration is calculated from nominal or corrected/measured concentrations. Also, there is no indication of concentration in controls. The 96-h *D. communis* test is described as an acute test by the study author, but as a 96-h proliferation test it can be considered chronic. The supplementary material for exposure to nominal 0, 50, 65, 84.5, 109.85, 142.81 and 185.65 mg/L resulted in growth inhibition of 3%, 10%, 17%, 53%, 57%, 83% and 93%, respectively). The IRC **recommends the DGV authors apply the mean replicate results from the supplementary information (including information on measured exposure concentrations if available) to calculate an EC10.**

10. *Tetradesmus obliquus* (formerly *Scenedesmus obliquus*) (Zhang et al., 2012). The DGV authors applied a NOEC of 25 mg/L. The report does not provide sufficient information to calculate a concentration-response curve and confirm, for example, an EC10. However, there were sufficient concentrations (6 plus control), and replication (3 per treatment). The EC50 based on cell density is reported as 126 mg/L and the NOEC is the lowest treatment level of 25 mg/L. The IRC **recommends the NOEC should be retained for use in the SSD.**

2.2.3 Rules for calculation of geometric means for toxicity data

One submission considered that the rules for calculation of geometric means for toxicity data had not been followed. Warne et al. (2018, Section 3.4.4) states that geometric means for toxicity values should be calculated where there are multiple toxicity values for the same species, endpoint and test duration. Species for which this might be applicable but for which geometric means were not calculated were *D. rerio* and *D. magna*.

For *D. rerio*, Pandelides et al. (2023) reviewed 12 key studies examining long term, chronic exposures to PFOS including multigenerational exposures of 300 days or more. They estimated a screening level NOEC of 31 µg/L which was calculated from the geometric mean of NOEC and LOEC values from several datasets. When they only considered the most reliable studies (Gust et al. 2023 and Krupa et

al. 2022) which had good study designs with high replication and at least 5 measured treatment concentrations, the calculated geometric mean NOEC of 28 µg/L was similar to their recommended screening NOEC. **The IRC recommends that the GV authors carefully consider the Pandelides et al. (2023) review and calculate an appropriate geometric mean NOEC for *D. rerio* according to Warne et al. (2018) guidance.**

The biological findings from Lu *et al*, 2015 are described in point 2, Section 2.2.3

2.3 Policy around Bioaccumulation

Concerns were raised in two submissions relating to the application of the 99% species protection DGV to protect against bioaccumulation in slightly to moderately disturbed aquatic ecosystems. The Summary of the draft DGV technical brief states "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."

As elaborated upon in Section 4.3 of the draft DGV technical brief, the above text specifically refers to published data showing that meeting the 99% species protection DGV does not necessarily mean that tissue concentrations in aquatic biota will be below relevant wildlife protection values. Given this evidence is now available (i.e. Baddiley et al. 2020), it is considered that it is reasonable and valid to include statements that may differ from other DGVs where such evidence is lacking. Moreover, both ANZG (2018) and ANZECC/ARMCANZ (2000) clearly state that the guidance to use the 99% species protection DGV for bioaccumulative compounds is arbitrary and not based on any evidence of biological protection – e.g. from ANZG (2018): "The recommended approach has no mechanistic basis with regards to bioaccumulation, and is recommended solely as a precautionary measure to minimise the risks from bioaccumulative substances. "

ANZG (2018) is clear that guidance has not been developed to protect terrestrial and semi-terrestrial wildlife from impacts to animals from contaminated drinking water nor food around aquatic ecosystems (https://www.waterquality.gov.au/anz-guidelines/guideline-values/default). The guidelines acknowledge these gaps and state 'Terrestrial and semi-terrestrial wildlife linked to aquatic food chains are at risk from a suite of water-borne contaminants that can bioaccumulate in organisms and biomagnify along the food chain. In these instances, guideline values that protect aquatic species from waterborne contaminants may not convey safety for species that consume aquatic organisms'.

It is not within the scope of the ANZG (2018) Guidelines to address impacts to these organisms. To fill this data gap, the state and federal governments have provided guidance in the National Environmental Management Plan on PFAS (NEMP 3.0). **Further, the ANZG 2018 WQGs promote the use of multiple lines of evidence to assess potential impacts to aquatic ecosystems, rather than simply applying a DGV.**

ANZG (2018) cites sections 8.3.3.4 and 8.3.5.7 of ANZECC/ARMCANZ (2000) to address bioaccumulation, but acknowledges the information contained in these sections are dated (i.e. close to 25 years old). This is particularly the case for PFOS, where the mechanisms of bioaccumulation are still not completely understood. PFOS and other PFAS do not appear to behave like other organic compounds (De Silva et al 2021). It is known that PFOS impacts higher order consumers that are reliant on the aquatic food chain, particularly those that do not respire via gills (De Silva et al 2021). Although PFOS is rarely measured as a single PFAS in the environment, PFOS appears to be the dominant PFAS in Australian air breathing biota (e.g. Letthoof et al 2023; Sharp et al 2021; Vardy et al 2024). Further, the assessment of PFOS in aquatic ecosystems is confounded by the potential presence of PFOS precursors, and their transformation to PFOS within aquatic ecosystems and organisms (e.g. Kolanczyk et al 2023). Direct measurement of biota allows for direct assessment.

A concern that PFOS detected in organisms that have moved from an area with higher PFOS present to one with a lower concentration will confound assessments of PFOS from a particular site, has also been raised. This may apply to all biomonitoring programs that have been traditionally undertaken (e.g. mercury, pesticides, etc.). A well-designed monitoring program with a selection of species that are likely to be resident in an area will overcome this concern.

Note that the information in ANZG (2018) and ANZECC/ARMCANZ (2000) represents guidance only and does not reflect regulatory requirements. Further guidance on assessing bioaccumulation is likely to be provided via the NEMP as more information becomes available.

The IRC recommends **the guidance be retained.**

3. Conclusions

The IRC has carefully considered a number of key issues raised in the public submissions for the PFOS f DGVs technical brief. As discussed, inclusion or exclusion of specific data in the PFOS SSD is based on best professional judgement. The IRC reached consensus using this approach and has made a range of recommendations about the inclusion and exclusion of particular text in the technical brief and data in the SSD as above. The IRC recommends that, in the light of the substantive public comments received, the freshwater PFOS DGVs technical brief and calculated DGVs from the SSD are revised in accordance with the recommendations in this report.

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