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

Australian & New Zealand
GUIDELINES FOR FRESH & MARINE WATER QUALITY

Response to public submissions on draft default guideline values for zinc in freshwater

September 2026

Draft default guideline values (DGVs) for zinc in freshwater were published on the Water Quality Guidelines website for a 3-month public consultation period. During this period, comments for the draft DGVs for zinc in freshwater were received via public submission.

Responses to comments and any associated edits to the draft DGV technical brief are outlined in this report (Attachments A–C), de-identified for public record. In accordance with the formal post public comment DGVs approval process, the responses and revisions have been approved by the jurisdictional technical and policy oversight groups, and noted by the National Water Reform Committee.

The default guideline values for zinc in freshwater are now published as final. For additional information on the publication process, please refer to the pathway for toxicant default guideline value publication.

The Water Quality Guidelines Improvement Program thanks all submissions for their valuable contribution to the development of default guideline values for the protection of aquatic ecosystems.

Attachment A

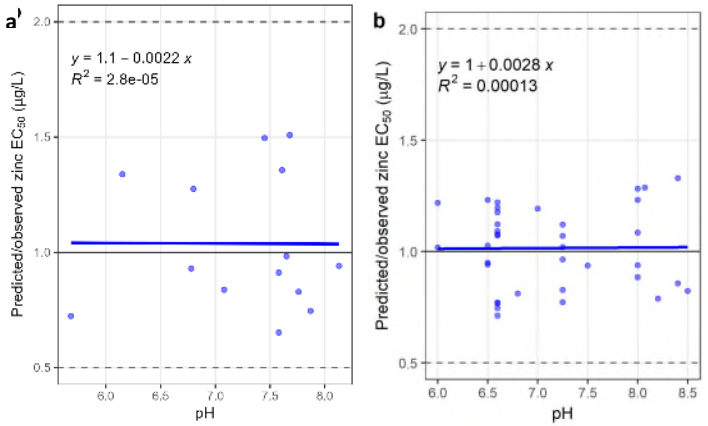
Response to public submissions on draft default guideline values

Toxicant: Zinc in freshwater

Technical comment	Response	Action taken
Submission 1 *(Note: The full text has not been reproduced here but, rather, only the key comments/criticisms have been extracted)*		
Executive summary (the issues noted below are revisited in subsequent comments extracted from the main body of the submission)		
Numerous comments were made in the Executive summary that reflect comments made later in the review report.	Comments addressed in later sections, below.	
Introduction		
1. The '2024 Draft Zinc Guidelines' draw heavily on CCME (2018) but have a necessary focus on "species found in Australia and New Zealand, including sensitive native species, and in water chemistry for Australia and New Zealand" (ANZG, 2024). This submission considers the project laudable, albeit with the obvious premise that technical aspects must be robust. Yet, this submission is made with no small degree of scepticism that the arguments herein will be seriously considered. In September 2023, several months before the call for submissions on the '2024 Draft Zinc Guidelines', key authors and technical advisors reported the draft DGVs "should be used as a replacement for values in ANZECC & ARMCANZ (2000) and reproduced at ANZG (2018). Any changes in the values are expected to be only minor" (NIWA, 2023). It is unclear where the authority to make such a statement comes from or the current regulatory status of the '2024 Draft Zinc Guidelines'. Noteworthy is that the April 2023 version of the 'Draft Zinc Guidelines' contained the disclaimer, "This publication is a draft only and is not considered an official DGV publication by Australian and New Zealand Governments and Australian state and territory governments"; in the final draft, from May 2024, the said disclaimer had been removed.	A draft version of the zinc freshwater DGVs was released with the permission of the New Zealand Ministry for the Environment and support of the Australian Government Department of Climate Change, Energy, the Environment and Water (DCCEEW). This was specifically in relation to a project on copper and zinc toxicity in New Zealand and an associated request to make the draft available to stakeholders. Regardless of any statements that were made at the time, the draft zinc freshwater DGVs were always going to be reviewed and approved through the same formal mechanism as any other toxicant DGV technical brief. This is what has occurred.	No action taken.
Factors affecting toxicity		

Attachment A

Technical comment	Response	Action taken
2. Hardness – The role of hardness on the amelioration of zinc toxicity has been well studied, most notably in fish. The effect of calcium has been referred to as "by far the most important modifier of chronic Zn toxicity" due to its binding at gill surfaces, for which the uncertainty of effect "is relatively small" (De Schamphelaere & Janssen, 2004). Yet, significantly, the '2024 Draft Zinc Guidelines' note that test water in some toxicity studies "with higher hardness also had higher alkalinity (and sometimes pH), thus confounding the protective effect of hardness". This is a crucial admission; while the '2024 Draft Zinc Guidelines' use three TMF coefficients (for pH, hardness, and DOC) on which they base four MLR models, the reality is there are strong TMF co-dependencies that must be accounted for in the development of all TMF coefficients, not just those confounded by hardness.	We agree that the role of hardness has been well-studied, particularly with fish. In older studies, for example from the 1970s and into the early 1990s, there was less attention to possible influences of alkalinity. Note that this quote by De Schamphelaere & Janssen (2004) is in respect of extrapolating their BLM to other fish species and life stages. They go on to say that "caution should still be employed to use the BLM developed in this BLM for predictions of zinc toxicity in natural surface waters." Regardless, hardness is identified in three of the MLR models as a key TMF. This is particularly true for the fish model, which has the highest slope with hardness. There is less evidence for its effect in mitigating toxicity to the freshwater algal species *R. subcapitata*. TMF co-dependencies are discussed further below under pH and alkalinity.	No revisions made.
3. pH – Due to factors such as confounding effects, even the relationship between zinc toxicity and pH, a relatively easy-to-understand TMF, can be misunderstood or incorrectly interpreted, and it comes as no surprise that the '2024 Draft Zinc Guidelines' confuse in their discussion of pH. On the one hand, it is stated that pH can influence zinc toxicity by influencing zinc speciation "with higher concentrations of Zn2+ occurring at low pH (increasing toxicity at low pH)." Yet, it is also reported that studies showed "the expected increasing toxicity with increasing pH." The authors are vague on this point and do not provide a further explanation when they note that "in other studies, the reverse or no effect has been found." Rather than equivocating, the '2024 Draft Zinc Guidelines' would do well to present readers with a clear overview of pH-driven zinc speciation in simple aqueous systems, which is important since it is stated: "the [aqueous] Zn^{2+} species is the most bioavailable and potentially toxic form". Yet, other than noting "hydroxide forms are less prevalent … in freshwaters" the '2024 Draft Zinc Guidelines' provide no discussion of the well-known 'zincates' , which become important to speciation within the pH range of both the data (pH 5.9–8.5) and MLR models used (pH 6.2–8.3) ; concentrations of aqueous Zn^{2+} begin to diminish relatively with respect to the 'zincates' at pH > 7.5.	The reviewer misrepresents the text in the technical brief, which after stating that pH can influence zinc toxicity via speciation, provides two processes by which pH influences zinc bioavailability. These both result in decreasing zinc toxicity at low pH, as clearly stated in the technical brief. The wording "expected increasing toxicity" has been amended in the technical brief in recognition of the multiple effects of pH, and we recognise that this wording suggested an overall expectation for the effect of pH, whereas differing effects for different studies and different species can be expected. No explanation is required when noting that the "reverse or no effect has been found" as the explanation was already provided in the three bullet points that describe how pH can affect zinc toxicity in multiple ways. The authors are not "equivocating" but simply aiming to show that the role of pH on zinc speciation (and presence or absence of zincates) is only one factor that influences zinc toxicity. In some cases, it is not the primary factor. The competition effect of hydronium ions can dominate under some circumstances (e.g., with low ligands) and at low pH. The citation of studies conducted with "varying pH and no ligands" is to isolate the role of pH, without the complicating effect of DOM. It is correct that De Schamphelaere et al. (2005) include notable DOC in their test solutions (5 mg/L) and that this should not have been included here. Similarly, Heijerick et al. (2003, 2005) report tests with DOC of 5 mg/L. This statement should have cited an earlier paper by the same laboratory	Amended wording from "*expected increasing toxicity*" to "*increasing toxicity*" Amended citations in technical brief. References have been added to the technical brief for interested readers. Amended text to clarify that it is not only Zn^{2+} that causes toxicity. Plots have been added to Appendix A to demonstrate the effect of pH on zinc toxicity with the four models used to develop the zinc GVs. Revised model developed for fish, based on *O. mykiss* data but excluding data at pH < 6.0.

Technical comment	Response	Action taken
The '2024 Draft Zinc Guidelines' confused position regarding the role of pH on zinc toxicity stems from a failure to interpret relevant key data. Cited are "many studies conducted with varying pH and no ligands", as if ligands are the only factors that can exert pH-dependent toxicity modification. While most of those studies cited utilised test solutions that were 'ligand' free (Two of the studies (De Schamphelaere et al., 2005 and Heijerick et al. 2003) are cited erroneously since test solutions also contained notable concentrations of DOC), most also had non-zero hardness concentrations, and calcium may exhibit a pH-dependent toxicity modification (De Schamphelaere et al. (2005) noted the protective effect of cations, such as calcium, may vary with pH, and clearly recognised the difficulties associated with incorporating a pH effect into a Biotic Ligand Model (BLM)). Of the cited 'no ligands' studies, the following comments are pertinent. Cusimano et al. (1986) undertook zinc toxicity testing on Rainbow trout (*Oncorhynchus mykiss)* at three pH values, 4.7, 5.7, and 7.0, at low hardness – the trend to higher toxicity at higher pH is meaningless to the '2024 Draft Zinc Guidelines' since only one pH value is relevant, and the trend is also unrelated to changes in zinc speciation, being attributed to hydronium ion "interference with metal uptake". In not dissimilar fashion, De Schamphelaere and Janssen (2004) also studied the toxicity of zinc on *Oncorhynchus mykiss* at three pH values, 5.7, 6.7, and 7.5, at low hardness and DOC. The authors noting the 30-day LC50 at pH 5.7 was more than twice that found at pH 6.7 (368 µg/L) and pH 7.5 (361 µg/L) – the higher pH results are unsurprisingly similar if one takes Zn^{2+} as the only species present. Schubauer-Berigan et al. (1993) reported acute zinc toxicity data for Fathead Minnow (*Pimephales promelas)* at three pH ranges (6.0–6.5, 7.0–7.5, and 8.0–8.5) and high hardness. Contrary to that reported in the '2024 Draft Zinc Guidelines', there was no trend to increased toxicity with increasing pH. Rather, the 96-h LC50 was 780 µg/L at pH 6.0–6.5, decreasing to 330 µg/L at 7.0–7.5, before increasing again to 500 µg/L at 8.0–8.5; perhaps evidence of a reduction in the hydronium ion effect, then offset by a speciation effect.	(Heijerick et al. 2002) where *R. subcapitata* was tested in the absence of notable DOC. These errors were due to some confusion in keeping track of the large number of papers on zinc toxicity by that specific research group. The information provided in the technical brief is not intended to be a complete discussion of the effects of pH on zinc toxicity, but an indication of the general trends and any complexity. We investigated further the models used, and the effect of pH - this is covered below within the response to point 9, TMFs. There are likely more complex relationships between pH and zinc toxicity than can be represented with an MLR model. However, more complex models, such as BLMs also simplify the relationships between TMFs and toxicity. A key issue is to assess whether the MLR models used are over- or under-predicting toxicity at high or low pH. This can be assessed using model residuals. Model residuals for neither the original fish nor invertebrate models demonstrate a clear relationship with pH (Figure 1) – this indicates that both models adequately addressed pH.  *Figure 1: Residuals (as ratio of predicted/observed) for the a) O. mykiss model used in the draft DGV technical brief and b) D. magna model against pH. The flat line in blue is the regression of the residuals and shows no apparent pattern with pH, indicating that the model predicts well with respect to pH.*	

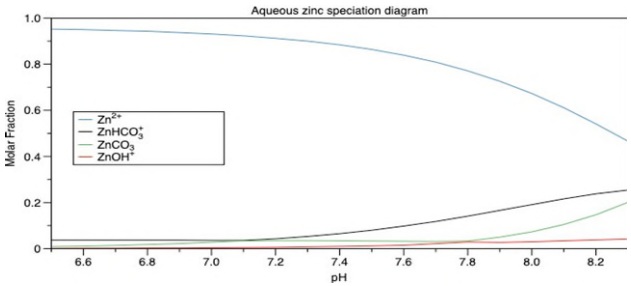
Attachment A

Technical comment	Response	Action taken
Hence, it is not accepted that in fish, there is a simple trend of increasing toxicity to zinc with increased pH. The '2024 Draft Zinc Guidelines' gloss over the complexity of pH effects, which are actually multi-modal. Hyne et al. (2005) considered acute zinc toxicity data to the water flea *Ceriodaphnia dubia* at pH 5.5, 6.5, and 7.5 and low hardness. They reported no significant difference in toxicity between pH 5.5 and 6.5 (48-hr EC50s, 382 µg/L and 413 µg/L, respectively) but a marked increase at pH 7.5 (200 µg/L). Yet, this does not evidence a trend to increased toxicity across the range of pH values relevant to the '2024 Draft Zinc Guidelines' (6.2–8.3), rather the study provides evidence for a reduction in the hydronium ion effect, but there is little ability to assess the effects of zinc speciation – the authors report the change in Zn^{2+} is from 93% at pH 5.5 to 87% at pH 7.5. The work of Heijerick et al. (2005) showed increased chronic toxicity (expressed as 21-d NOECs) to *Daphnia magna* with increasing pH, not "the reverse or no effect" stated by the '2024 Draft Zinc Guidelines'. The authors attributed the increase to a change in the hydronium ion competitive effect. However, the study did not isolate the effects of pH since it was conducted at moderate hardness. As with fish, this submission posits the effects of pH on zinc toxicity in crustaceans is probably multi-modal. Of the studies cited by the '2024 Draft Zinc, those on tropical green algae by Price et al. (2021) demonstrate the tightest control of aquatic parameters. The authors reported mixed trends: while 72-h EC50s showed a clear trend of increasing zinc toxicity with increasing pH (range 6.7–8.3) at moderate hardness, EC20s and EC10s (the latter being the preferred data for developing ANZG DGVs) showed a variable toxicity-pH relationship, with maximum toxicity occurring at pH 7.7. Hence, far from the simplistic (and incorrect) "increasing toxicity with increasing pH" stated in the '2024 Draft Zinc Guidelines', Price et al. (2021) evidences the complex relationship between zinc toxicity and pH in green algae, one which the authors acknowledge lacks mechanistic understandings. Another study cited by the '2024 Draft Zinc Guidelines' that also considered green algae (Wilde et al., 2006), which supposedly also showed the pH trend, summarised with due caution, "the derivation of a single stability constant for protons for	Despite the lack of relationship between residuals and pH for the *O. mykiss* model, further investigation suggested that this model could be being too highly influenced by a data point at pH 5.7, below the applicability range of other models. A revised model was developed which is described in detail in Appendix A of the technical brief. The effect of pH on molluscs has so far only been investigated for a single species, with two different pH values. Further tests need to be undertaken to understand if this trend to decreased toxicity at higher pH occurs across other molluscs, and at different hardness and DOC concentrations.	

Attachment A

Technical comment	Response	Action taken
zinc covering the whole pH range of natural waters, typical of the conventional Biotic Ligand Model (BLM) approach for fish and invertebrates, may not be appropriate for microalgae". Price and his co-workers further noted that in the presence of DOM, pH changes did not influence toxicity endpoints and concluded "pH may not influence zinc toxicity in the presence of DOM in the same manner as it would in the absence of DOM", again highlighting complexity and co-dependency (Price et al., 2023). The '2024 Draft Zinc Guidelines' state that when 'ligands' are present "the effect of pH is less clear", as if that were possible. But two studies are cited. For one, on *Ceriodaphnia dubia* (Belanger and Cherry, 1990), the '2024 Draft Zinc Guidelines' state an "increase in toxicity of only 1.7-fold was found with an increase in pH of 3 units". Yet, the study is irrelevant since it used two pH values, 6.0 and 9.0, which are outside the range relevant to the '2024 Draft Zinc Guidelines'. For the other (Heijerick et al., 2003), the authors reported "a significant decrease in chronic zinc toxicity with increasing pH" for *Daphnia magna*; more accurate is that stated by the '2024 Draft Zinc Guidelines'; there was no "clear pattern between pH and zinc toxicity". While this submission does not define pH-zinc toxicity relationships, it is noted the seemingly straightforward relationship for molluscs, which is a trend to decreased toxicity with increasing pH (De Schamphelaere & Janssen, 2010), has been ignored by the '2024 Draft Zinc Guidelines'. The authors state, "The individual effect of pH and calcium on chronic zinc toxicity to both species can easily be observed from our data … increases in pH and calcium result in increased EC10s and EC50s (decreased toxicity)" – the pH effect likely due to increased complexation of Zn^{2+} carbonates. If correct, there are two important considerations: (1) the pH effect in molluscs is the opposite of that in *Daphnia magna*, making the surrogate relationship advocated in the '2024 Draft Zinc Guidelines' void; and (2) alkalinity is an important TMF.		
4. Alkalinity – The '2024 Draft Zinc Guidelines' do not discuss the role of alkalinity at length, other than noting it can reduce zinc toxicity via carbonate complex formation, i.e., being a speciation effect. The '2024 Draft Zinc Guidelines' then follows with an odd narrative that seems to argue hardness is a factor that confounds alkalinity-	The authors agree that alkalinity influences zinc speciation, as alkalinity is a measure of dissolved inorganic carbon, and both HCO_3^- and CO_3^{2-} can complex with Zn. This may be an important factor when considering speciation, and therefore toxicity. Alkalinity is included in mechanistic	Amended wording by adding "uptake or" into statement "alkalinity had no influence on acute zinc uptake or toxicity to

Technical comment	Response	Action taken
mediated zinc speciation. Furthermore, the reference to two studies (Barron & Albeke, 2000; Bradley & Sprague, 1985) make in which "alkalinity had no influence on acute zinc toxicity to rainbow trout at or below pH 7" underlines a worrying lack of understanding: why would carbonate alkalinity be expected to markedly affect zinc toxicity at pH ≤ 7? Zinc carbonates constitute only approximately 5% of the molar fraction below pH 7.0 in low alkalinity systems. Not that '2024 Draft Zinc Guidelines' correctly interpret the said studies. Barron and Albeke (2000) make no comment at all on zinc toxicity, only zinc uptake. And Bradley and Sprague (1985) did not even investigate the effects of alkalinity on zinc toxicity at pH < 7. Rather, they reported *Oncorhynchus mykiss* LC50s at pH 5.6, at low and high hardness, and alkalinity < 1 mg/L (as CaCO3); hence, alkalinity could never have influenced acute zinc toxicity because there was none significantly present. The '2024 Draft Zinc Guidelines' also cites a study that considered how hardness and alkalinity "influenced" zinc toxicity in trout, but no further discussion is advanced. In fact, the study, by Holcombe and Andrew (1978) demonstrates a 23–31% reduction in trout toxicity at constant hardness, commensurate with a 47% reduction in Zn^{2+}, when alkalinity was raised from 43.0 to 170.2 mg/L (as CaCO3). The important role of zinc carbonates in waters, even of low alkalinity, is almost totally overlooked in the '2024 Draft Zinc Guidelines'; while not incorrect in stating, "at pH 4–7, the free zinc ion (Zn^{2+}) is by far the predominant form. At pH 8–9, zinc carbonate (ZnCO3) also becomes important", there comes the question, what about between pH 7 and 8? That answer can be understood with reference to Figure 1, which demonstrates the increasing status of zinc carbonates between pH 6.5 and 8.3 (Modeled by the ion-pair speciation model, PHREEQC. Similar software frequently cited in the '2024 Draft Zinc Guidelines' support documents (WHAM) does not accurately model the speciation of zinc carbonates at lower pH and alkalinities). The importance of not dismissing alkalinity when considering zinc toxicity can be further demonstrated via the estimation of zinc speciation in typical New Zealand waters (pH = 7.7 (New Zealand median: Julian et al., 2017); calcium = 7.7 mg/L, magnesium =1.6 mg/L, sodium 5.9 mg/L, potassium 0.8 mg/L, sulfate, 4.5 mg/L, chloride 5.1 mg/L, temperature 12.5°C, and	bioavailability models that include speciation e.g., BLMs, which have a speciation component. Many toxicity studies do not include independent measurements of zinc speciation, only of toxicity. Hence the discussion refers to effects of alkalinity on *toxicity*, which can be confounded by concurrent differences in pH and/or hardness. Note that Bradley & Sprague (1985) state in their abstract "*Thus, carbonate alkalinity is not an important factor at or below pH 7.0*." Whilst it is true that they did not test higher alkalinity at pH 5.6, they *did* test alkalinity at pH 7, with up to 24 mg $CaCO_3$/L (added as $NaHCO_3$). The "**at** or below" is an important part of their statement. Barron and Albeke (2000) assessed zinc uptake – uptake is an essential step for toxicity and therefore is relevant to this discussion, especially in the absence of other information on alkalinity. The text in the technical brief about alkalinity is kept brief as this was based on acute toxicity studies only, and is therefore of lesser relevance to the DGV derivation than studies assessing chronic toxicity. In using MLRs, which are further simplifications of BLMs, there is a need to reduce the correlated TMFs – such as pH and alkalinity. Including pH rather than alkalinity helps account for the effects of proton competition at low pH. Therefore, pH is the reasonable term to retain and alkalinity is the reasonable term to omit. Similarly, in natural waters, hardness and alkalinity are frequently correlated.	rainbow trout at or below pH 7" in Section 3.2. No changes to the TMFs included in models.

Technical comment	Response	Action taken
alkalinity 27 mg/L, as $CaCO_3$ (New Zealand medians: Smith & Maasdam, 1994)), in which Zn^{2+} in the absence of DOC contributes approximately only 80% of the molar fraction of total aqueous zinc. The position of the '2024 Draft Zinc Guidelines', i.e., not accounting for alkalinity appears to be a reproduction of CCME's position (2018), yet it underscores a clear inconsistency of approach. While the guidelines happily incorporate estimated DOC data, no such consideration appears to be afforded to alkalinity.  **Figure 1:** Key aqueous zinc species in carbonate only systems		
5. Dissolved organic carbon – DOC is a sub-set of Total Organic Carbon (TOC), being the fraction that passes through a 0.45µ filter, although other, less standard, filters might be used. TOC is an analytical parameter that is determined via methods that employ high-temperature combustion, heated chemical oxidation, high-pressure chemical oxidation, or UV irradiation, that convert organic carbon to carbon dioxide, which can then be readily determined by various means (Lipps et al., 2023). This manner of TOC analysis is indiscriminate, i.e., it "fails to distinguish the contaminant species" (Liu et al., 2008). In the context of the aquatic toxicology of metals, DOC has become synonymous with organic compounds that act as TMFs, and this assumption underpins the various guidance documents of authoritative regulators, such as USEPA and ECCC, i.e., all, or a high percentage of, DOC is due to humic acids that can an amenable metal. Such an assumption, therefore, necessarily runs through the	We agree with the submitter that the DOC reported in the tests with *E. menziesii* (Clearwater et al. 2014) is unlikely to act like humic acid, and the toxicity test results therefore should not have been normalised based on that DOC. The DOC for this test has been assumed to be negligible, as it would normally be in deionised water. A comprehensive and independent cross-check of the toxicity data and associated water quality parameters reported in the original studies has been undertaken. As identified by the reviewer, some anomalies in the DOC estimates were uncovered, and these have been corrected or clarified. Further, we have re-defined the circumstances for adopting estimated DOC values – see below. A detailed response for each species is presented in Attachment B which also addresses the specific comments raised here by the reviewer. The revised final dataset is provided in the revised Zn DGVs technical brief Table B1.	Several species were removed as outlined in Attachment B, below. Preferential approach described in Technical Brief.

Technical comment	Response	Action taken
'2023 Draft Zinc Guidelines', where it most often finds expression as the default DOC concentration of 0.5 mg/L. The origin of a default 0.5 mg/L DOC as an estimate of baseline DOC is USEPA (2007), and stems from their consideration of what level of DOC contamination reduction typical laboratory water systems could achieve. While it is noted that modern laboratory water systems can achieve TOC concentrations markedly lower than 0.5 mg/L, yet there is no acceptance that TOC contamination in older, less capable water purification systems is predominantly due to humic acids. Rather, only a small fraction of TOC in laboratory water is comprised of humic acids – the majority of TOC comes from ion-exchange resin, which is comprised of organic polymers with functional groups such as sulfonic acids and amines that are "inevitably released" during day-to-day operation (Lee et al., 2020). In contrast to the difficulty controlling TOC contamination from ion-exchange resins, humic acids present in feed to laboratory water systems are readily eliminated (Zhao et al., 2019). An example of ion-exchange resin contamination, which provides much-needed cause for reflection, is found in the article 'Acute Toxicity of Copper, Zinc, and Ammonia to Larvae (Glochidia) of a Native Freshwater Mussel (*Echyridella menziesii)* in New Zealand' (Clearwater et al., 2014), in which reconstituted test water (20% dechlorinated tap water and 80% nanopure water) was found to have an "unexpectantly high" DOC (2.0-2.9 mg/L). The authors considered the DOC was "leached from the resin acid purification column for the nanopure water". It is little wonder the authors found "reconstituted water … did not mitigate [zinc] toxicity"' compared with the "careful studies" that used added humic acid as a source of DOC. The potential contamination of laboratory water with non-humic DOC, meaning that toxicity data which relies on DOC measured in test water that did not have humics added is more likely due to small particles of shed, or leached, plastic, is overlooked in the '2024 Draft Zinc Guidelines', despite it bringing into question the data the Guidelines heavily rely on. . A recurring theme, with the '2023 Draft Copper Guidelines' is the high degree of estimation of DOC concentrations, due to either DOC not being measured or being less than the detection limit. This	We disagree that 0 mg/L is an appropriate concentration for DOC when below detection in synthetic waters. There are multiple sources of DOC in chronic toxicity tests, including: - Dilution water from the purification system - Faecal matter from the living organisms - Detritus and debris (i.e., POM and DOM) from the food (which is a requirement in all chronic toxicity tests with fish and invertebrates) - Exudates produced by plants or algae used as test organisms. Based on this, we consider it appropriate to include a low concentration of DOC in waters. A preferential method for selecting DOC data for individual toxicity values was adopted. This was based on that used for the ANZG DGVs for copper in freshwater and, information provided in CCME (2018) and USEPA (2007) water quality criteria for copper (specifically Appendix C that identifies specific water quality parameters to adopt when they were not reported in the original paper). In summary, primary DOC data (measured and reported in the original paper) were prioritised over secondary DOC data that was sourced from other studies using the same waters or estimated as low DOC. Where a primary or secondary DOC value could not be sourced, the toxicity value was not used in the derivation of the Zn DGVs. **Ranking of data:** For each toxicity value, identify the data as primary or secondary based on the source of the DOC concentration. • Primary data (1°): DOC was measured and reported in the original study • Secondary data (2°): DOC was sourced from other studies that measured DOC in the same waters, or where DOC was estimated as being low. DOC concentrations from the secondary paper can be adopted under the following circumstances: o Measured DOC concentrations provided by original/primary authors of the original/primary study via direct communication can also be considered. These were provided in some cases in the CCME dataset.	

Attachment A

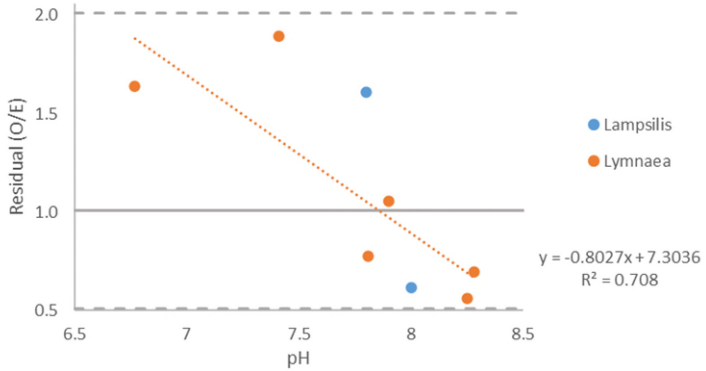
Technical comment	Response	Action taken
greatly weakens the zinc toxicity dataset and that opinion not only expressed in this submission, but is that held by the USEPA (2007), who noted, "if DOC measurements for the surface water cannot be obtained from a reliable source, then the toxicity test should not be included". While there is robust DOC data associated with the zinc toxicity data presented in the '2024 Draft Zinc Guidelines', approximately 25% of the data used is invalid due to DOC concentrations being either totally unreported, based on (variable) detection limit estimates, or other factors, such as measurement being made only at the start of toxicity testing, or contamination. That invalid DOC data affects more than half of the 31 species used in the Species Sensitive Distribution (SSD) and would effectively remove twelve species from inclusion in the database. Yet, an 'estimate approaches' to analytical data may be useful when there are data deficiencies, although they must be used consistently for all key parameters and in a manner that makes sense. When calculating summary statistics on analytical data, it is common practice to use 'half-detection limit data' when the concentration of an analyte is less than the detection limit. However, such an approach is invalid when compiling a toxicity dataset when there is zero expectation DOC is present, such as in laboratory water. Hence, having multiple concentrations of 0.5 mg/L (derived from < 1 mg/L) throughout the dataset used in the '2024 Draft Zinc Guidelines' makes no sense. The most appropriate concentration when DOC is below the detection limit in synthetic waters is 0 mg/L. Hence, the DOC concentrations used in the development of TMF coefficients and subsequent normalisations of zinc toxicity data in the '2024 Draft Zinc Guidelines' should be re-evaluated.	o For natural waters (such as well water, groundwater, surface water, or any other natural water) where DOC was estimated from other data (e.g., previous measurements in the same laboratory, other papers using that water, USGS monitoring) **and** DOC is <2 mg/L, the DOC estimate is adopted. o For test waters reported as synthetic or reconstituted water prepared from deionised or distilled water, a low DOC value of 0.5 mg/L is adopted. DOC values of ≥2 mg/L reported in other papers were not accepted as secondary values. This is because DOC in natural waters can fluctuate significantly (e.g. 2-fold or more), especially seasonally, leading to greater variability in toxicity and hence greater uncertainty in normalised toxicity values. In contrast, at low DOC concentrations (<2 mg/L) differences of 0.5 mg/L or less make little difference to the normalised toxicity values. These primary and secondary rankings were then used as follows: 1. If primary data are available for a species, use only those toxicity values and no other data for that species. 2. If no primary data are available for a species, use secondary data for that species. 3. If there are no primary or secondary data available, exclude that species from the derivation.	
The compiled toxicity data		
6. The toxicity data in the '2024 Draft Zinc Guidelines' (Appendix A) has been reviewed, and summary comments are provided in Table 1. Some expanded commentary is provided here. Unsubstantiated estimates of DOC are present throughout the toxicity dataset. For example, the DOC concentration cited alongside the *Dreissena polymorpha* toxicity data from Kraak et al. (1994), who made no DOC measurement whatsoever, is 6.7 mg/L. Similarly, the DOC	A comprehensive and independent cross-check of the toxicity data and associated water quality parameters reported in the original studies was undertaken. As identified by the reviewer, some anomalies were uncovered, and these have been corrected or clarified. A detailed response for each species is presented in Attachment B (Table B1), below, which also addresses the specific comments raised here by the	Table B1 (Appendix B) has been updated with revised/corrected values, an additional column detailing the test medium, and footnotes for improved identification of data sources,

Attachment A

Technical comment	Response	Action taken
concentration cited with the freshwater snail (*Potamopyrgus antipodarum*) toxicity data from Dorgelo et al. (1995) is 4.3 mg/L. And a more recent example of the commonality of poor DOC estimation can be demonstrated from Stauber et al. (2022), in which recorded DOC concentrations of < 0.3 mg/L and 0.4 mg/L are replaced with 0.5 mg/L in the toxicity table. Problematic is the frequency of data presented not matching what is found in the sources cited, and some citations are incorrect. Regarding the former, it is evident that some data has been reworked, but neither the data analysis nor the source of the work is provided so that it can be verified. For example, the '2024 Draft Zinc Guidelines' report an EC10 (mortality) for *Cottus bairdi*, citing Brinkman & Woodling (2005), yet while those authors report a No Effects Concentration (NOEC) and Low Effects Concentration (LOEC), they do not report an EC10 or, for that matter, DOC. The 'data entry' spreadsheet that accompanies the '2024 Draft Zinc Guidelines' is helpful because it points to CCME (2018) as the source of the EC10 and DOC estimates – yet CCME provides no basis for the EC10 estimate and no reference at all is made to DOC. Another example is for *Pimephales promelas*; an IC10 and DOC are attributed to Norberg & Mount (1985), who infer a NOEC, but no DOC. The 'data entry' spreadsheet again points to CCME, who report a 7-day IC10, but no DOC. The data for brown trout (*Salmo trutta)* demonstrates an important point concerning poor data selection, which, in this case a conservative bias. The data selected for use in the 2024 'Draft Zinc Guidelines' is from Davies et al. (2002), while the analogous data of Brinkman and Woodling (2014) was discarded. The former was chosen for the SSD since the endpoint zinc concentrations were "lower" even though DOC was not even measured; in contrast, the latter measured DOC. There are also examples of ill-considered data selection. For example, some of the data for *Raphidocelis subcapitata* from Stauber et al (2022) derived from "zinc-spiked natural waters" has been accepted even though the effect concentrations are, in practical terms, the same as, or even lower, than control concentrations. But perhaps the best example of poor data reproduction in the '2024 Draft Zinc Guidelines' is for *Orconectes virilis*, for which a LC10	reviewer. In addition, the revised final dataset is also provided, which is presented in Appendix B of the revised Zn DGVs technical brief (Table B 1). Outcomes from the data review included providing supporting information on the source and quality (e.g. measured value) of toxicity data and associated water quality parameters for improved transparency. *Additional supporting information* The CCME (2018) water quality guidelines for zinc supplemented the review of data used for the ANZG Zn DGVs because it provided a relatively recent assessment and compilation of zinc toxicity (and guideline derivation) to freshwater organisms. This can be found at https://ccme.ca/en/chemical/229, along with the detailed Excel compilation of toxicity data located under "Resources" Zinc - Summary of toxicity data for short-term benchmark concentration and long-term Canadian water quality guideline derivation (2018). These resources where used to supplement information in the ANZG Zn DGVs, or to provided leads to other relevant sources of supplementary information. Upon re-review of the toxicity data, the test media were added to Table B1 of the Technical Brief to provide additional context on the water quality parameters and TMFs for each toxicity value. Comprehensive footnotes were also included to identify the source and quality of the toxicity values, water quality parameters, and relevant experimental factors that may differ between studies. For the most sensitive species, *Chlorella* sp. (PNG isolate), we acknowledge that the normalised Zn IC10 value did not align well with the measured IC10 values for waters with similar pH, hardness and DOC. The TMF models have been amended (details described in responses to other queries) and the revised normalised IC10 value at the reference water chemistry for Australian and New Zealand conditions (6.2 and 4.2 µg Zn/L, respectively) now align more closely with the measured IC10 values under similar water chemistry. In summary, the final dataset was revised from 31 species to 25 species that consisted of 4 fish, 5 crustaceans, 2 insects, 10 molluscs, 1 rotifer and 3 green microalgae.	quality and associated experimental factors. Related data/text within the technical brief have been updated to reflect the changes made to the final dataset used to derive the DGVs (for example, normalised toxicity values, Section 2 (Aquatic Toxicology), Table 4, SSDs and DGVs). Text describing the preferential selection rules for DOC measurements has been added.

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(mortality) and DOC are attributed to Borgmann et al. (1993). Here, not only is the citation incorrect – the data is drawn from Mirenda (1986), but the endpoint is an LC50, and no DOC concentration is reported; CCME (2018) informs neither. And it is apparent little attention has been given to reflecting on *Chlorella* data under conditions that are close to normalisation: at hardness concentrations of 11 mg/L and 31 mg L (CaCO3), DOC concentrations of < 1 mg/L and 0.5 mg/L, and pH values of 7.5 and 7.6, the reported EC10s were 6.6 µg/L and 2.1 µg/L, respectively (Stauber et al., 2022; Price et al. (2022), compared with 0.9 µg/L used in the SSD. There are other examples that serve to demonstrate poor data handling. If such occurrences were infrequent, it could be accepted that 'mistakes happen', even in a document stated to have been subject to peer review by four individuals. Yet, the consistently erroneous reproduction of data undermines the '2024 Draft Zinc Guidelines'. An at times poor data reproduction was also a feature of the '2023 Draft Copper Guidelines'; the submission on that document noting data "may not have been visited first-hand; rather secondary sources have been used" (Ecological Solutions, 2023); an ill-advised practice but not one difficult to remedy.		
TMF coefficients		
7. As noted, the '2024 Draft Zinc Guidelines' utilise up to three TMF coefficients (pH, hardness, and DOC) across three taxa (fish, crustacea, and two species of green algae). No justification is provided for why the various TMF coefficients were selected, some in preference to others, aside from reference to an assessment by Gadd et al. (in prep). This submission contends that the '2024 Draft Zinc Guidelines' cannot cite work that is *in preparation* or not make it that such works are unavailable for review. Hence, the TMFs able to be reviewed were those for *Oncorhynchus mykiss* and green algae, but not *Daphnia magna*. The *Oncorhynchus mykiss* data are taken from CCME (2018), although the CCME report cited in the '2024 Draft Zinc Guidelines' does not detail how the TMF coefficients were derived, only pointing to a spreadsheet of data used in the derivation, which makes a review of the TMF coefficients difficult. A total of fourteen	The reference to Gadd et al. (in prep) was intended to be updated prior to publication with a link to the report describing model evaluation. The *D. magna* model was described in the supporting information of Stauber et al. (2023) and publicly available, but was not correctly referenced in the draft technical brief. These references have been amended in the revised technical brief. The three TMFs were selected on the basis of a review of information showing the factors that affect zinc toxicity, including those key factors in biotic ligand models and used in simplified BLMs. These three TMFs are also those identified by other jurisdictions (e.g., Canada, the European Union) and experts in the field of aquatic toxicology (see for example van Sprang et al. 2009; DeForest et al. 2023). Section 9.3 of the CCME report describes the development of the *O. mykiss* model and includes the MLR model parameters.	Reference to Gadd et al. (in prep) has been amended to Gadd (2023). New model developed for fish, which does not include a pH term. Model development and validation included in the technical brief (Appendix A).

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data were used from De Schamphelaere and Janssen (2004) and De Schamphelaere et al. (2005). It is evident there should be a reworking of the data: one data point (at pH 5.68) should be removed altogether and a further eight data should replace 0.3 mg/L DOC with 0 mg/L. The '2024 Draft Zinc Guidelines' cites Price et al. (2023) as the source of its Chlorella sp. TMF coefficients. The article provides a clear explanation of how coefficients and associated MLR models were derived. Even so, there is a disconnect between the coefficients selected for use in the 2024 'Draft Zinc Guidelines' and zinc toxicity data used in calculating DGVs, to which the TMFs are applied. The selected TMFs derived are based on EC50 data, yet the toxicity data are EC10s, or similar. As previously alluded to, the EC50s show a linear trend of increased zinc toxicity with increasing pH, yet EC10s show a non- linear relationship. TMF coefficients for another green algae (*Raphidocelis subcapitata*), which are the basis for the in-depth development and analysis of zinc toxicity MLR models for the species, have been reported by DeForest et al. (2023), and these are used in the '2024 Draft Zinc Guidelines'. However, in an identical fashion to the Chlorella sp. TMFs, the '2024 Draft Zinc Guidelines' incorporate the coefficients based on E50s – this despite the fact the slope correlation coefficients were notably worst for EC50 model, compared with the EC10 and EC20 models. It is worthwhile considering the opinions of scholars in the field of developing MLRs relating to zinc toxicity since this provides some important context concerning confidence. While these experts broadly agree that MLR validation requires modelled data be within two to three times the observed data, this brings pause for reflection on the need to avoid conservative bias when normalising toxicity data. The seeming inherent inability of MLRs to closely model observed data is found across all MLR models utilised in the '2024 Draft Zinc Guidelines', accepting that it is not possible to review the MLR developed for *Daphnia magna*, but this is unsurprising given some of the aforementioned issues of incorrect interpretations of TMF-effect and poor data selection. Hence, for example, it is little surprise that Stauber et al. (2022) concluded that "Zinc toxicity to Chlorella sp. is not predicted with sufficient accuracy by any of the tested microalgal MLRs".	However, this model has been replaced in the final zinc DGVs with a model based on EC50 values and excluding the data at pH less than 6. The DOC values of 0.3 mg/L are retained. This new model is described in an Appendix to the Technical Brief. <u>*Chlorella* model and EC50s</u> EC50s were preferentially used for the model development and then applied to the EC10 data. There is a very clear rationale for this: EC50 values have much less uncertainty than EC10 values as a result of the shape of concentration-response curves and the model fitting processes. The uncertainty in EC10 values is frequently around a factor of two, and sometimes an order of magnitude. Conversely, the uncertainty for EC50 values is more often around ± 20% of the EC50, rarely more than a factor of two. Added uncertainty in these values results in greater uncertainty in the models and in any bioavailability adjustments. Note that the *Chlorella* model has been examined at length in response to submitters (see response to point 14 below), and the EC50 model determined to be adequate for the DGV derivation. <u>Ability of MLRs to model observed data</u> A reanalysis of the MLR models used for the derivation has led to a change in the model for fish. While still based on *O. mykiss*, the model data set does not include a test at pH 5.7, which was somewhat leveraging the model and resulting in a stronger slope for pH. With this data point removed, the effect of pH is only weakly significant and was removed from the final model. The updated model is now described in Appendix A of the DGVs technical brief.. Predictiveness of the MLRs is assessed in each of the documents reporting the MLR, however some additional information has been added to Appendix A of the technical brief. <u>Molluscs</u> The available data suggests that pH may affect molluscs in a different way to other invertebrates. However, a closer look at the mollusc data shows there are insufficient data to develop a model – with six data points only and three TMFs to consider. We agree that the decrease in toxicity with higher pH indicates a need to develop mollusc models in the future, especially given the number of molluscs in the SSD. However, there would need to be more toxicity testing to develop this – ideally for an Australian or NZ species – which is outside the scope of the current derivation..	

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There is an obvious weakness in how the '2024 Draft Zinc Guidelines' handle mollusc data. Molluscs feature strongly in the SSD and several species are among the most sensitive. Yet the TMFs used to normalise mollusc data are derived from *Daphnia magna*, the basis for which is difficult to fathom, not being helped by the fact that the rationale behind the development of TMFs for *Daphnia magna* is unpublished.	Of the multiple molluscs included in the derivation, there are only 2 species with tests in different water chemistry conditions, for which model applicability can be assessed: *Lymnaea stagnalis* (6 tests) and *Lampsilis siliquoidea* (2 tests). Plots of the residuals (predicted/observed) against pH (below) provide some evidence for a relationship with pH for *L. stagnalis*. However, all predictions are within a factor of two of the observed values.  As there are insufficient data to develop a mollusc model, and the existing model predicts mollusc toxicity sufficiently well (within a factor of two), no changes are required.	
Relevance of zinc toxicity data		
8. The relevance of using all of the '2024 Draft Zinc Guidelines' compiled zinc toxicity data to derive generic DGVs across all regions of Australia and New Zealand also requires reflection. The species that is by far the major driver of the DGVs sensitivity is a green algae from Papua New Guinea, which on the basis of that presented in the '2024 Draft Zinc Guidelines', Table 4, is eight times more sensitive than the next most sensitive species. The argument for including the Papua New Guinean Chlorella in the draft guidelines is, that "there are no clear differences between species native to New Zealand and those found elsewhere" (NIWA, 2023) holds no weight. Rather, the Papua New Guinean Chlorella exhibits "unique high sensitivity" that is "likely due to the very low	*Chlorella* are a "moderately common" genus of algae (Matthews 2016) found in freshwaters globally. In line with the derivation of DGVs for other toxicants, the selection of freshwater species for Zn was not limited to those found in Australian and/or New Zealand. Sensitivity of freshwater species to Zn is not a criterion for excluding toxicity data. The exclusion of toxicity data was assessed based on the quality of the toxicity data reported (as described in Warne et al., 2018, and toxicant specific information (e.g. measured toxicant and water quality parameters)), and atypical water quality characteristics (e.g. outside the boundary limits of the TMF models applied) (refer to technical brief Section 4.1, 4.2 and Table 2). In relation to the ammonia DGVs, the higher sensitivity of some species was attributed to very low ionic strength of the water used in the tests, not the	Changes to *Chlorella* data included and averaged (as described in the response).

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ionic strength of the waters". In a parallel derivation of ANZG DVGs for ammonia, the authors concluded that incorporating such species results in an overly conservative toxicity assessment versus that under more typical water quality conditions (ANZG, 2023). Therefore, there is clear inconsistency among ANZG scientists in how they approach atypical species. While it is important to protect the most sensitive species, this submission posits the development of generic DGVs cannot be dependent on species that very rarely occur across the jurisdictions. Hence, this submission supports the wisdom of a site-specific approach, such as that followed by Trenfield et al. (2022), to setting guidelines values for highly atypical environments, while generic DGVs should be based on SSDs for typical species. There is an argument that "it is relevant to include species found outside of New Zealand to increase the number of species included in the DGV derivation", which is flawed and even contradicts ANZG's own guidance on DGV derivation. That guidance states there are three factors used to determine the reliability of guideline values: (1) the number of species in the SSD; (2) the type of toxicity data; and (3) a visual assessment of fit of the SSD. Concerning the number of species, there are three sample-size bands, 5–7, 8–14, and ≥15 (Warne et al., 2018). Only the latter can result in very high reliability DGVs, the point being that 15 species is sufficient – according to ANZG's own guidance, having more than 15 species does not increase reliability. Yet, one wonders how 'quality of analytical data' is not a determining factor, as it is under USEPA methodology (Olker et al., 2022). After the Papua New Guinean Chlorella, the next most sensitive species is the mayfly, *Neocloeon triangulifer*, which is a suitable surrogate for New Zealand species of mayfly, followed by several species of equally appropriate species of mollusc. Mollusc data provide a strong understanding of what concentrations of zinc are toxic to the most sensitive New Zealand species and afford the ability to derive robust guidelines at the 90-99%-ile levels, also having valid associated DOC data. There is no fundamental requirement that an SSD have a long tail at low concentrations, i.e., somehow requiring the presence of a species such as the Papua New Guinean Chlorella. While the oddly qualitative ANZG guidance	species used. Given that the ammonia DGVs do not account for ionic strength, the data were excluded from the DGVs. For Zn, low hardness is accounted for, so the PNG *Chlorella* data were retained along with the Kakadu *Chlorella* data, which fell within the lower hardness limits of the TMF model boundaries. The site-specific GV approach presented in Trenfield et al. (2023) was influenced by World Heritage listing for the relevant aquatic ecosystem, and the low ionic strength of the waters, outside the range of generic DGVs. This site-specific GV included several species that were tested only in waters with water chemistry combinations outside the range of the generic DGV derivation (and so were not included in this derivation). The data for the PNG isolate of *Chlorella* have been reassessed with the result that several of the data points are no longer included, whereas some additional data are. The way the average across species has been calculated has also changed – in sum, these have resulted in a higher estimated EC10 value for *Chlorella* sp. (PNG isolate), which is now less than a factor of two lower than the next most sensitive species (Australian freshwater mussels). The DGV reliability classification is guided by specific criteria, but there is no evidence that increasing the number of species to greater than 15 does not improve the reliability of the DGVs. The USEPA WQ criteria methodology currently does not have a reliability classification for GVs/thresholds, or one that considers data quality. The ANZG recommended approach for generating SSDs was used to derive the Zn DGVs, and the technical brief makes no assertion that a long tail is required. Note that the recently approved SSD curve-fitting tool (shinyssdtools) was used to re-derive the Zn DGVs. The visual fit assessment is used because the fit of most plausible distributions will not be rejected by a statistical test for sample sizes commonly generated for toxicity SSDs. This means that statistical goodness-of-fit testing are not always informative for SSDs. For large sample sizes such tests might be appropriate, but the current derivation method does not provide guidance on the sample size threshold above which statistical testing can be used to assess goodness-of-fit. Regardless of this, the revised Zn SSD generated by shinyssdtools is the average curve-fit from weighted distributions with goodness-of-fit tests used to determine the relative fit of the individual distributions.	

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is that "a panel of at least three relevant experts agree on the [visual SSD] fit" (Warne et al., 2018); this submission suggests SSD fitting and variation analysis is most reliably performed by software.		
Some practical considerations		
9. A significant oversight in the '2024 Draft Zinc Guidelines' is the practical need to take all precautions to ensure there is no contamination of water samples during the sample handling process, although the technically more detailed guidance provided by NIWA touches on some aspects of contamination risk, noting the situation when field staff should be trained in "field filtration methods" (NIWA, 2023). Yet, there is much more to consider than whether to field filter, which is always preferable but not always advisable. The zinc DGV concentrations once passed the draft stage, will be ultra-trace, but there is no point even analysing samples at the ultra-trace level unless sample handling methods are commensurate. There is a real risk, for example, that samples submitted for compliance monitoring will be found in exceedance of DGVs but not for any other reason than sample contamination – zinc being ubiquitous in the environment. While there is excellent guidance for avoiding such contamination risk, i.e., 'clean techniques' (USGS, 1999), there are very few practitioners in New Zealand. This brings into focus the additional need for strong QC procedures, such as the use of field blanks, which are not currently a common feature of monitoring programmes in New Zealand, mainly on account of cost.	We note that the comment relates to the application of the DGVs rather than the derivation of the DGVs. We concur that zinc can be a common sample contaminant and that appropriate sampling guidance needs to be followed, but it is not the responsibility of the DGV technical briefs to provide such guidance. The ANZG zinc DGV concentrations, even at the 99% species protection level, are above the detection limits required by the National Environmental Monitoring Standard (NEMS 2019). This means they are above detection limits widely used around New Zealand by commercial laboratories and in routine monitoring programs. While guidance on collecting, storing and analysing water samples is described elsewhere and is not within the scope of DGV technical briefs, some comment can be made here regarding the risks of sample contamination, based on the results of those routine programs. Sample results are regularly at or below detection limits for sites with no obvious zinc sources (for example streams in native forest catchments, rural streams, see Gadd et al. 2023). This suggests that those collecting samples in these routine locations are able to do so without contaminating samples.	No revisions made.
The Look-Up table approach		
10. It is considered that the look-up table approach (Appendix C of the '2024 Draft Zinc Guidelines') is dated and overly burdensome for users. Moreover, look-up tables are limited because they do not allow DGV calculations at intermediate TMF values. However, such is the nature of the zinc toxicity SSD that species-specific formulae can easily be derived, affording the calculation of DGVs at the appropriate level of protection directly across the range of TMFs dispensing with the need for look-up tables.	These look up tables have now been supplemented with an online tool/calculator. However, the look up tables have been retained in the technical brief to illustrate how the guideline values change with different water chemistry. In the new tiered assessment framework, an on-line tool will enable calculation of Zn bioavailability-adjusted GVs (BAGVs) at the matching water chemistry of the sample. This tool includes the need to input data for pH, DOC and hardness as these parameters affect Zn bioavailability. Guidance	No revisions made.

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For example, and assuming good sense prevails concerning the exclusion of the Papua New Guinean Chlorella, it is clearly evident that zinc toxicity data from a mollusc species could be used directly as 95%-ile DGV – the look-up tables confirm as much – although this would require the development of mollusc TMFs rather than having a dependency on (unpublished) TMFs for *Daphnia magna*.	and training materials have been developed to assist users with the new tool and framework.	
Summary and recommendations		
11. This submission recommends the '2024 Draft Zinc Guidelines' undergo a comprehensive revision. Rather than being referred to as a *fait accompli* requiring no more than 'minor changes', this submission considers 'major changes' are required, the most important of which are: • Correctly accounting for pH effects, which can demonstrate multi-modality; • Incorporating alkalinity, rather than it being totally ignored; • Discarding poor DOC data; • Developing a multiple linear regression model, or models, for molluscs; • Ignoring toxicity data that is irrelevant to generic guidelines.	All addressed above, summarised here: • Acknowledgement that pH effect is complex, adjustment to fish model, no other changes • Alkalinity not included in MLR models as correlated to pH and hardness • Data with poor DOC estimates discarded • Insufficient data to derive mollusc MLR • All species are relevant to generic guidelines	See earlier Actions.
Submission 2 *(Note: The full text has not been reproduced here but, rather, only the key comments/criticisms have been extracted.)*		
Process and transparency		
12. Despite the comprehensive nature of the ecotoxicological database, there is one flaw that hampered the review process. The lack of information pertaining to the development of bioavailability models relating the effects of toxicity modifying factors (TMFs) on dissolved Zn effect concentrations to arthropods/invertebrates (e.g., for *Daphnia magna* – which will be described in Gadd et al. (*in prep.*)) makes it difficult to critically review the potential invertebrate MLR models (and therefore to understand the rationale for choosing one model over another) for normalization of effect concentrations, and therefore the DGVs. To that end, it is very difficult to understand the performance of any of the MLR models described in Table 1 for the organisms to which they will be applied for normalization purposes.	The reference to Gadd et al. (in prep) was intended to be updated prior to publication with a link to the report describing model evaluation. The *D. magna* model was described in the supporting information of Stauber et al. (2023) and publicly available but was not correctly referenced in the draft technical brief. These references have been amended in the revised technical brief.	Technical brief updated to include correct references. Additional evaluation of the MLR models has been added to the technical brief (Appendix A).

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Model Choice for *Chlorella* sp. and Related Data Questions		
13. The most sensitive species in the SSD is the green microalga *Chlorella* sp. (Papua New Guinea isolate), with a "normalized" EC10 of 0.9 µg/L at the index condition. This species appears to be 8- to 10-fold more sensitive than the next sensitive species, *Neocloeon triangulifer* (a North American mayfly species). The current version of the chronic *N. triangulifer* test is suspected as not being optimized with regard to feeding during tests, so its inclusion in the SSD is questionable, as some effects could potentially be attributed to the nymphs not getting enough or adequately nutritious food (see Soucek et al. 2022).	The paper referenced (Soucek et al. 2022) assessed feeding and proposed the same feeding regime as that used in the papers with the zinc toxicity tests (Besser et al. 2021).	No revisions made.
14. Adequacy of the MLR model for microalgae (based on model from Price et al. 2023 for *Chlorella* sp.) – see Attachment B for details.	Further evaluation of the MLR model for *Chlorella* sp. has demonstrated that the original EC50 model fits the data adequately when using study-specific intercepts (or sensitivity). When comparing the predicted vs observed concentrations, an EC10 model developed had a slightly higher r^2 (0.86 vs 0.76), however given the higher uncertainty in EC10 estimates, EC50 values are preferred for model development.	No change to the MLR model. Amendment to sensitivity for each species due to change in the calculation method (based on study and a mean rather than median).
Specific comments		
15. Minor comment: In Section 2.3, it is described that the US EPA (1996) used "an average ACR of 1 resulting in its chronic water-quality criterion being equal to its acute water-quality criterion." The general idea is true, but the details are incorrect. The US EPA actually uses an average ACR = 2 for zinc, but it is applied to the final acute value – which is the 5th percentile of the acute genus sensitivity distribution to provide the chronic criterion. The FAV is also divided by 2 to determine the acute criterion, so because the ACR = 2, and the acute 'safety factor' = 2, the acute and chronic criteria are the same.	Text updated as follows (amended text in italics): US EPA (1996) used an average ACR of *2, which when combined with the acute 'safety factor' of 2*, *resulted* in its chronic water-quality criterion being equal to its acute water-quality criterion.	Text updated in the technical brief.
16. In Section 2.3, page 4, last paragraph before Section 3: It is mentioned that "Chronic toxicity data for fish species include EC10s of 19-228 ug/L (all data normalized to pH 7.5, hardness 30 mg/L $CaCO_3$ and 0.5 mg/L DOC: references in Appendix A)." It is unclear if the data in the preceding paragraphs are also normalized to the index condition. Please explain, or present in a consistent manner.	For this paragraph, all data were normalised to allow for comparisons between species. However, as this differs from the preceding paragraphs, this has been amended to report the original data for consistency.	Paragraph edited in the technical brief.

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17. Minor comment: In Section 3, first paragraph: If you mention biotic ligand models for justification for TMFs, why not use BLMs directly? Is it because they require too many inputs? If so, then why not use a BLM with simplified inputs (i.e., specify only pH, DOC, and hardness, then use ion ratios to derive a full BLM input file)? An R package is currently being developed for the BLM and will likely be available within the next few months.	There are several reasons for use of MLRs: - Although there are multiple BLMs available for zinc, none have been validated for Australian or New Zealand species. - More variables are required to use a BLM and although simplified packages can be developed, to do so would require data for the major anions and cations across both countries to develop ion ratios between the simplified inputs and the required variables. Such data are not currently widely available and the existing data indicates the ion ratios may be very different between locations and/or countries.	No revisions made.
18. Minor comment: In Section 3, "The pH of a waterbody can influence zinc toxicity by…"; why the change in format when initially summarizing pH effects? None of the discussion for the TMFs includes a bulleted summary. Also, it seems that the second and third bullets are communicating the same competitive effect of protons.	The bullets in this section are to more clearly indicate the multiple different effects of pH related to toxicity, whereas all other TMFs act in a singular manner. The second bullet communicates the competitive effect of protons, bullet three describes the changes to the binding sites.	Text added to third bullet to clarify (new text in italics): modifying the affinity between zinc and membrane binding sites *due to conformational changes in binding sites* (decreasing toxicity at low pH).
19. Section 3.1: The hardness equation is still used in the US for all metals except for Cu and Al. Also, even though the national recommendation for Cu changed to BLM in 2007, and for Al changed to MLR model in 2019, many states still use the hardness equations. So, the hardness function is still the basis for US EPA ambient water quality criteria for most metals.	The text around the hardness equation was specific to zinc.	Text added in Section 3.3 to clarify (new text in italics): The inverse relationship between water hardness and toxicity was the basis of a hardness function in the US EPA ambient water-quality criterion *for zinc* from 1984 to 2007….
20. Section 3.1: Check to confirm the following statement: "An MLR approach has been suggested as a simpler method than BLMs for deriving guideline values (Brix et al. 2017, DeForest et al. 2018) and was used by Environment and Climate Change Canada in developing zinc (and more recently, copper) guidelines for freshwater (CCME 2018)." I believe that the ECCC guideline for Cu finalized in 2021 is based on the BLM, so too is the British Columbia Cu guideline.	Agreed. This has been amended to include the aluminium derivation by Environment and Climate Change Canada as a further example of MLR use.	Text amended in Section 3.3 (new text in italics, below) and reference added to reference section: "…and *has been* used in Canada in developing zinc *and, more recently, aluminium guidelines* for freshwater (CCME 2018; *ECCC 2022*)."

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21. Section 3.1, last paragraph: Gadd et al. (in prep.) is mentioned, and it would seem to be critical for evaluating the appropriateness/adequacy of the various bioavailability models. This paper should have been provided as a resource for reviewers of this draft DGV document.	The reference to Gadd et al. (in prep) was intended to be updated prior to public release with a link to the report describing model evaluation but, unfortunately, this was missed prior to release. The *D. magna* model was described in the supporting information of Stauber et al. (2023) and publicly available but was not correctly referenced in the draft technical brief. These references have been amended in the revised technical brief.	References corrected in the technical brief.
22. Section 4.1, last paragraph on page 11: Relative to the statement about "studies that did not report pH and either hardness or the concentrations of calcium and magnesium…" Unfortunately, lack of information on TMFs in published studies is common, but the rule here seems a bit backwards, given the importance of DOC relative to Ca+Mg or hardness. If you have some information on the dilution water source or the reconstituted water recipe, it seems that you should also be able to reasonably estimate Ca and Mg concentrations for use in the normalization process.	Agree that it would be possible to estimate Ca and Mg concentrations, however, the preference is for measured data. Also see further amendments to exclude data using estimated DOC.	No revisions made.
23. Section 4.2, page 14, last paragraph: "The predicted negligible-effect values for the index condition were summarized to single species values for use in the SSD,…" This sentence accurately describes the values in the column of Table 4 and Table A1 described as "Normalized toxicity value (ug/L Zn)". In other words, these are actually predictions, not normalizations. Table A1 most clearly demonstrates this, because with the ranges in Zn effect concentrations and TMF values, the actual "normalized" values should exhibit some variability for each species/endpoint. So, for Table A1, it would be nice to see a normalized value, and then the geometric mean of the normalized value. Table 1 could simply describe this value as the prediction at the index condition (or alternatively, the geometric mean normalized value).	The description in section 4.2 (now section 4.3) has been amended to better describe the process of calculating sensitivity coefficients for each species. While it could be useful to compare each reported value to the predicted or normalised value, Table A1 (now Table B1) needs to show the data used in the SSD, rather than a slightly different value calculated from the geometric mean of normalised values. The additional information has been added to the Excel spreadsheet as a supplementary sheet.	Change to description in section 4.2 to better describe the process of calculating sensitivity coefficients for each species. Table A1 (now Table B1) has been updated to indicate that predicted concentrations are only made for the mean for each species, rather than displaying the data. Additional information added to the Excel data spreadsheet.
24. Section 4.5: Potentially a new classification needs to be added here: applicability and/or predictability of the bioavailability-based models for the taxonomic groups of interest?	The reliability classification of guideline values is in accordance with Warne et al. (2018) and additional classifications to assess reliability cannot be added to the technical brief. The applicability of the bioavailability models was assessed in other publications and in the Appendices.	Comment added in Section 4.7 on model applicability with reference to other publications for further details.

Attachment A

Technical comment	Response	Action taken
Submission 3 *(Note: The full text has not been reproduced here but, rather, only the key comments/criticisms have been extracted.)*		
25. A direct comparison of the existing DGVs (ANZG 2018) and the proposed DGVs for zinc, and New Zealand's current National Policy Statement for Freshwater Management (NPS-FM) ….illustrates that at standard conditions, **the new guideline values are significantly more stringent** and future compliance with these conservative values could impact discharge consenting and compliance …. going forward.	The ANZG website provides guidance on how toxicant DGVs should be applied, but it also acknowledges that the DGVs are not mandatory and that it is up to local jurisdictions to determine if and how they will be implemented. Relevant jurisdictions were aware of the lower DGVs well before final publication, and they will implement any necessary measures as part of the DGV publication and implementation process. Also, see response to the following comment, in relation to the ability to tailor the DGV to local conditions.	No revisions made.
26. The proposed zinc DGVs in ANZG (2024b) employ "bioavailability models at the species level and trophic level, developed to account for the influence of pH, hardness and DOC on the toxicity of zinc". The proposed DGVs apply to the dissolved fraction of zinc in waters with a pH of 6.2-8.3, hardness of 20- 440 g/m3 as CaCO3 and DOC concentrations of ≤0.5-15 g/m3. For water bodies with measurements outside these ranges, the bioavailability models used "may not be suitable and, therefore, the DGVs may not be reliable" (ANZG 2024b). This uncertainty is very concerning.	Few, if any, bioavailability models can account for ALL water chemistry conditions. This is because the applicability ranges of the models are determined in no small part by the tolerances of the test species that are used for the ecotoxicity tests. While some water chemistry conditions will fall outside the model applicability ranges, the ranges do cover the large majority of natural waters in Australia and New Zealand (based on available data) Guidance is provided by ANZG (see website and Stauber et al. 2026) on what to do when waters fall outside the applicability ranges. In some cases, there may be a need to derive site-specific guideline values, for example, where there are discharges into acidic waters. In such cases, both the bioavailability models and the species used to derive the DGVs may not be relevant. The zinc DGVs should be used within the tiered assessment framework, a decision tree to guide the user through the process of accounting for zinc bioavailability when comparing monitoring data with guideline value (Stauber et al. 2026, ANZG 2018).	No revisions made.
27. Table 2 compares the existing zinc DGVs (ANZG 2018) and the proposed DGVs (ANZG 2024b) at different levels of species protection. The proposed DGVs are presented at the index water quality condition (a pH of 7, hardness of 30 g/m3 as CaCO3, and a DOC concentration of 0.5 g/m3) which is representative of conditions where zinc would be highly bioavailable. The comparison indicates that the proposed DGVs are much more stringent at index/standard conditions. Expert advice …. indicates that the more conservative nature of the proposed DGVs means future compliance	We note that the comment relates to the application of the DGVs rather than the derivation of the DGVs. Firstly, note that the DGVs are now based on a "real" high bioavailability water chemistry condition, termed the reference condition, rather than the previous index condition. This change has been explained in Gadd et al. (2026). Using DGVs based on a high bioavailability condition is intentional, so as to ensure they are conservative (i.e. protective) in the first instance (i.e. where local TMF data are not being measured, the default approach is	No revisions made.

Technical comment	Response	Action taken
with this guideline could impact discharge consenting and compliance …. going forward. Again, this points to relevance and usefulness of site-specific assessments rather than reliance on general ANZG guidelines. *Table 2. Comparison of existing and proposed DGVs for dissolved zinc in freshwater.* Species protection level (%) / ANZG (2018) DGV* / ANZG (2024b) DGV** 99 / 0.0024 / 0.0015 95 / 0.0080 / 0.0041 90 / 0.0150 / 0.0068 80 / 0.031 / 0.012 **Notes:** Units are g/m³; *at a standard hardness of 30 g/m³ as $CaCO_3$; **at index water quality conditions (pH of 7.5, hardness of 30 g/m³ as $CaCO_3$ and DOC of 0.5 g/m³).	to assume high bioavailability conditions until otherwise known). This represents an incentive for operators/dischargers to measure the relevant TMFs (pH, hardness, DOC) and calculate an appropriate bioavailability adjusted guideline value (BAGV) that is more relevant to the local receiving water chemistry. In most cases, the BAGVs will be higher than the DGVs. However, where local water chemistry is outside the applicability ranges for the zinc BAGVs, then site-specific GVs may need to be developed, or the conservative DGV could be adopted instead. The available guidance on using the tiered assessment framework (ANZG 2018, Stauber et al. 2026) and applying DGVs (ANZG 2018) should allow users to move through the process in a transparent and appropriate manner. Finally, operators/proponents should always consult with their local jurisdiction with regards to regulatory matters.	
28. Finally, there can be practical difficulty distinguishing between naturally occurring levels of zinc in freshwater, and zinc from mine discharges, therefore it may be that zinc site-specific guideline values should be derived rather than general reliance on the proposed DGVs.	We note that the comment relates to the application of the DGVs rather than the derivation of the DGVs. See response to previous comment, and also see guidance in ANZG (2018) related to DGVs and background concentrations. We agree that, in some cases, site-specific guideline values may be more appropriate. Also, monitoring programs should be robust enough to be able to distinguish anthropogenic versus natural zinc.	No revisions made.
Importance of certainty in regulation		
29. The concept of continual improvement is stated as a guiding principle in the guidelines. In line with its own operating philosophy, ------- *[entity deleted for anonymity]* is also committed to continual improvement. At the same time, we need to operate within practical and commercial realities, and in this regard, it is vital that regulatory frameworks provide a high degree of predictability and are reasonable in their approach, so as to enable significant investments to be made with confidence. The new guideline values for nitrate and zinc are significantly more stringent than the existing values, within the context that over the coming years …. will need to renew several discharge consents for existing operations. Independent expert advice …. indicates that mining operations may have an issue with future compliance if the more stringent proposed DGVs for nitrate and zinc are applied by default, rather than the site-specific guideline values that are	We note that the comment relates to the application of the DGVs rather than the derivation of the DGVs. See response to the previous two comments. We again emphasise that the DGVs for zinc in freshwater can be adjusted to account for local water chemistry, and that a tiered assessment framework has been developed to help implement this. Importantly, the zinc freshwater DGVs technical brief and associated supporting documentation (i.e. ANZG 2018, Gadd et al. 2026, Stauber et al. 2026) explicitly embed the bioavailability adjustment approach within the implementation of the zinc DGVs. Notwithstanding this, the ANZG Guidelines are just guidelines and local jurisdictions have the flexibility to adopt them as they see fit.	No revisions made.

Technical comment	Response	Action taken
required to afford the protections needed. Given the significant value of existing investment at … mine sites, and the closely managed environments at each, it is important that regulation remains focused on achieving outcomes appropriate for the specific locations in which it applies. We believe that there is a risk, in taking the "default" approach proposed, that regional councils would take the data provided in the new guidelines and apply it broadly without consideration for local context, in a way that was unnecessarily conservative or aspirational. The onus would then be on consent holders or applicants to challenge overly stringent levels applied by default. Similarly, active anti-development groups may misinterpret the nature of the default levels and seek unnecessary conservatism in the DGVs to be applied without due consideration of the particular context in a given circumstance.		
Importance of applying the water quality management framework		
30. … it is important that the context of the DGVs being part of the wider Water Quality Management Framework is emphasised in the technical briefs themselves. In particular, the preference to use locally derived site-specific guideline values (**SSGV**s) should be highlighted. The management framework itself talks in several places about the preference for SSGVs We submit that this principle is of significance such that it should be emphasised in the opening to the technical briefs. In this regard, we suggest the following amendment to the opening statement of the technical guidelines (additions underlined): *The default guideline values (DGVs) and associated information in this technical brief should <u>only</u> be used in accordance with the detailed guidance provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality website (www.waterquality.gov.au/anz-guidelines). <u>In particular, it should be noted that use of locally relevant site-specific guideline values (SSGVs) will be preferable where these are available or may be derived in accordance with the guidelines.</u>*	We note that the comment relates to the application of the DGVs rather than the derivation of the DGVs. The DGV technical briefs clearly state in two locations (Executive summary and Section 4.3 Default guideline values) that the DGVs should be used in accordance with the detailed guidance provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality website (www.waterquality.gov.au/anz-guidelines). While there is some merit in adding more specific information related to how DGVs should be applied, it is difficult to determine what information should be included and what should be excluded. Moreover, if national guidance changes, then any such specific guidance captured in a technical brief will be outdated and incorrect. Consequently, it is preferred that key guidance on the application of DGVs is only retained on the ANZG website. All users are encouraged to use the ANZG Guidelines website and understand the process of the water quality management framework (or at least the elements that are relevant to them). However, the ANZG Guidelines provides guidance only, and it is up to each jurisdiction to decide if and how the DGVs and associated guidance are implemented.	No revisions made.

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Technical comment	Response	Action taken
Inclusion of this statement (or words to similar effect) at the start of the technical briefs would lessen the danger them being read in isolation, and in turn lessen the risk of inappropriate application of the default values without regard for the local context. We also think that other key principles of the management framework could usefully be reproduced in the technical guidelines. Again, this inclusion would serve to emphasise the significance of the context in which the DGV sit. In particular, the following statements from the guidance are important and merit emphasis by reproduction in the technical guidelines: *The Water Quality Guidelines aim to facilitate the productive and sustainable use of water resources while still maintaining the biological communities and ecological processes that the resource supports, consistent with the principles of ecologically sustainable development.* *(ANZG Philosophy and Guiding Principles: Sustainable Use)* This statement serves as a pertinent reminder that sustainable development is about use of resources as well as environmental protection. *Planning and management of water quality should be coordinated with management of other stressors, such as water quantity and habitat quality (for aquatic ecosystems). This will require close alignment of water quality planning with other planning initiatives that deal with the management of water resources and ecosystem health.* *(ANZG Philosophy and Guiding Principles: Integrated planning and assessment)* Again, this is a very significant point. Inappropriate application of DGVs in a regulatory context might be counterproductive in achieving optimal environmental outcomes. For instance, it may be more environmentally beneficial in a given circumstance to prioritise riparian planting, rather than to allocate substantial resources towards research, development, and implementation of technologies or strategies aimed at meeting stringent DGVs, which may not actually reflect the most important values of the area in question. Due consideration of local environmental priorities enables industry to focus on a long-term legacy of environmental		

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Technical comment	Response	Action taken
improvement rather than mechanically pursuing compliance with limits that are not locally derived and may not actually be the most important outcomes in real world terms. In a similar vein, we think it is important to reproduce in the technical guidelines the statement that "The Water Quality Guidelines' DGVs have no formal legal status, and their use is not mandatory, unless specified as being so by the relevant state, territory or local jurisdictional authority." Again, this serves to emphasise that the DGVs are guidelines and not rules that may be applied mechanically without further consideration of actual effects.		

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Attachment B

Additional information associated with submission 1

Table B1: Comments on zinc toxicity data in the 2024 draft zinc default guideline values technical brief – associated with Submission 1.

Species	Endpoint	Comments	Responses/changes (as of 22 December 2025)
Bufos boreas	NOEC (development)	DOC is not reported.	Species was removed from the final dataset. DOC was not reported and there was a lack of secondary data.
Cottus bairdi	EC10 (mortality)	DOC is not reported. EC10 is not reported although a NOEC is.	Species was removed from the final dataset. DOC was not reported and there was a lack of secondary data.
Oncorhynchus clarkii	EC20 (mortality)	DOC is not reported. EC20s are not reported although NOECs are.	Species was removed from the final dataset. DOC was not reported and there was a lack of secondary data.
Oncorhynchus mykiss	LC10 (mortality)	DOC is OK in De Schamphelaere et al. (2005a). DOC is not reported in De Schamphelaere et al. (2004).	Data set was revised. Data from Cairns et al (1982) was retained. A secondary DOC value of 1.6 mg/L was used (reported in USEPA 2007, Appendix C). Data from DeSchamphelaere et al. (2004) was retained. Secondary DOC data of 0.3 mg/L from De Schamphelaere & Janssen (2002) was adopted based on primary data that was "generally below 1 mg/L". Data from DeSchamphelaere et al. (2005a) was unchanged. Data from Mebane et al (2008) was removed due to uncertainty in the measured DOC concentrations of the natural waters used, and alternative primary and secondary DOC values were available.
Pimephales promelas	IC10 (growth)	DOC is not reported. IC10 is not reported although a NOEC is.	The species was retained, and the data revised. The DOC was not reported, but a secondary DOC value of 1.1 mg/L was adopted from USEPA (2007) Appendix C. The 7-d growth IC10 of 84 µg Zn/L calculated and reported by CCME (2007) was originally adopted here. However, comparison with the original data reported in Table 3 of Norberg and Mount (1985) indicates that at this concentration there was an increase in fish growth (weight), not a decrease. Therefore, toxicity value for *P. promelas* was revised to be the 7-d mortality NOEC of 85 µg Zn/L.
Prosopium williamsoni	IC10 (growth)	DOC is OK. IC10 is not reported although a NOEC is.	Data set was revised. A 90-d growth IC10 of 380 µg Zn/L was calculated and reported by CCME (2007) and originally used here. Review of the original study identified more sensitive toxicity values that could be used instead (30-d fry survival NOEC of 208 µg Zn/L, and 30-d fry survival IC20 of 30 µg Zn/L). The IC20 was selected here in preference over the NOEC value.
Salmo trutta	NOEC (growth)	DOC is not reported. Data from Brinkman & Woodling (2014) should be used.	The data set was revised. DOC was not reported in the original study of either Brinkman & Woodling (2014) or Davies et al. (2002). Secondary DOC data was adopted from Brinkman and Johnston (2012) for the Brinkman & Woodling

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Species	Endpoint	Comments	Responses/changes (as of 22 December 2025)
			(2014) study. Data from Davies et al. (2002) was removed due to a lack of available secondary DOC values.
Ceriodaphnia dubia	EC10 (reproduction)	DOC is OK.	No changes
Ceriodaphnia reticulata	NOEC (reproduction)	DOC is not reported.	Species was retained in the final dataset and the DOC value revised. DOC was not reported but a secondary DOC value of 1.1 mg/L was available for the same water source from USEPA (2007) Appendix C.
Daphnia magna	EC10 (reproduction)	One DOC concentration in De Schamphelaere et al. (2005a) is not reported. One data point is for a 14-d exposure and should be removed.	Data set was revised. Data from Munzinger and Monicelli (1991) was removed because the DOC was not reported and there was a lack of secondary values for this natural water. De Schamphelaere et al. (2005a) – toxicity data for tests with a DOC of 5.37 mg/L were not used because an EC10 could not be calculated. While a NOEC was reported, the data used here for *D. magna* (and other species) prioritises EC10s over NOECs (as per Warne et al., 2018). Data for the 14-d exposure duration was removed.
Daphnia thomsoni	EC10 (reproduction)	DOC is OK.	No changes
Hyalella azteca	NOEC (mortality)	DOC is not reported.	Species was retained and the DOC value revised. DOC was not reported in the original study but a secondary DOC measurement (1.1 mg/L) for the same test water was reported in Borgmann et al., 2005. The secondary DOC value was based on 3 years of measurements (n=69).
Orconectes virilis	LC10 (mortality)	The reference is Mirenda (1986) not Borgmann et al. (1993). DOC is not reported. Neither an LC10 nor a NOEC are reported but an LC50 is.	Species was removed from the final dataset. DOC was not reported and there was a lack of secondary data. The Borgmann et al. (1993) reference was added in error.
Rhithrogena hageni	EC10 (development)	DOC is not reported. EC10 is not reported although a NOEC is.	Species was retained and the DOC value revised. DOC was not reported but a value of 1.9 mg/L was adopted by CCME (2018) based on tests reportedly in the same water. However further review indicated differences in the waters, and a DOC value of 1 mg/L was adopted as recommended by US EPA (2007) for well water. The EC10 was reported by CCME (2018) in the supporting information Excel datafile and calculated using raw data provided from the author. While further details of the derivation of the EC10 were not provided, the EC10 (2.069 mg Zn/L) was considered a reasonable toxicity value to use because it aligned with a visual inspection of the concentration-response relationship presented in Figure 1 of the original paper (where at 10 days, the treatment of 5.3 mg Zn/L was approximately 80% compared to the control). The EC10 provided a more conservative toxicity value than the NOEC (5.3 mg Zn/L) and is preferred over

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Species	Endpoint	Comments	Responses/changes (as of 22 December 2025)
			NOECs.
Neocloeon triangulifer	EC20 (growth)	DOC is OK.	No changes
Alathyria profuga	NEC (mortality)	DOC is OK.	No changes
Cucumerunio novaehollandiae	NEC (mortality)	DOC is OK.	No changes
Dreissena polymorpha	LC10 (mortality)	DOC is not reported. LC10 is not reported although an EC50 is.	Species was removed from the final dataset. DOC was not reported and there was a lack of secondary data.
Echyridella menziesii	EC20 (mortality)	Data is invalid; DOC is resin contamination.	Species was retained and DOC values revised. DOC was reported but it doesn't reflect the DOM that would mitigate zinc toxicity. A value of 0.5 mg/L used as DOC expected to be low in laboratory waters.
Hyridella australis	NEC (mortality)	DOC is OK.	No changes
Hyridella depressa	NEC (mortality)	DOC is OK.	No changes
Hyridella drapeta	NEC (mortality)	DOC is OK.	No changes
Lampsilis siliquoidea	IC10 (growth)	DOC is not reported.	Species was retained and DOC values revised. While DOC was not reported in the original study, the author provided CCME a measured DOC of 0.3 mg/L for the reconstituted softwater used in the study. The laboratory-based reconstituted water isn't expected to change over time, and this value is in line with other DOC measurements for lab-based reconstituted waters (e.g. Markich, 2017; Price et al., 2021; Stauber et al., 2022; De Schamphelaere et al., 2005a). Hence, a secondary DOC value of 0.3 mg/L was adopted here.
Lymnaea stagnalis	EC10 (growth)	DOC is OK.	No changes
Physa gyrina	NOEC (mortality)	DOC is not reported.	Species was retained and DOC value was revised. While DOC was not reported in the original study, a secondary DOC value of 1.1 mg/L was adopted from USEPA (2007) Appendix C.
Potamopyrgus antipodarum	NOEC (mortality)	DOC is not reported.	Species was removed from the final dataset. DOC was not reported and secondary data showed DOC estimate would be uncertain.
Velesunio ambiguus	NEC (mortality)	DOC is OK.	No changes
Brachionus calyciflorus	EC10 (growth)	DOC is OK.	No changes
Chlorella sp. (PNG	EC10 (growth)	DOC is not reported in Johnson et al.	Data set was revised.

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Species	Endpoint	Comments	Responses/changes (as of 22 December 2025)
isolate)		(2007). Some DOC data from Price et al. (2022) is misreported, even allowing for rounding.	Data from Johnson et al., (2007) was tested in laboratory water and so a low DOC concentration was assumed. The DOC values for Price et al. (2022) were reported as <1 mg/L. Considering that these test waters were synthetic waters and unlikely to change over time, a low DOC of 0.5 mg/L was adopted. This low DOC value is similar to the measured DOC reported in the same lab-based synthetic water when a lower detection limit was used (Price et al. (2021, 2023a); 0.7, 0.4, 0.6, 0.6, 0.7 & 0.5 mg/L).
Chlorella sp. (Kakadu isolate)	EC10 (growth)	DOC is OK.	No changes
Raphidocelis subcapitata	EC10 (growth)	The DOC data from Stauber et al (2022) is invalid since it was measured only at the start of the testing.	DOC measurements at the start and end of the toxicity test were not a requirement of accepting DOC values associated with toxicity values. Few studies reported the exact timing of obtaining a subsample for measurement of DOC and in many instances measured DOC was most likely obtained from a bulk source of test/dilution water prior to dispensing into individual test vessels. Hence, there was no reason to dismiss the data presented in Stauber et al. (2022). The DOC values associated with three toxicity values were revised to those reported in the Stauber et al. (2022) (0.3, 0.3 and 0.45 mg/L and where relevant identified as 'less than' values in the footnotes).

Attachment C

Additional information associated with submission 2

Details of issue related to comment #14 – adequacy of MLR model for microalgae

More surprising than the apparent ultra sensitivity of *N. triangulifer* is the range in sensitivity of two different *Chlorella* sp. entries in the SSD. Why would one isolate be the most sensitive organism to zinc, yet the other isolate is one of the most tolerant organisms to zinc? This raises concerns immediately, given that these results are for the same genus of organisms (and could potentially represent the same species). These results appear to come from similar types of toxicity tests. Despite the apparent wide range in sensitivity of these two similar microalga, it also appears that the same MLR model was used to normalize them to the index condition.

However, before applying the model of Price et al. (2023b), which was developed for the effects of TMFs on zinc EC50s, a performance evaluation of the model should be conducted with the *Chlorella* sp. data in Table A1 to predict EC10s (e.g., and auto-validation). Here, I have conducted such an evaluation, and the results clearly demonstrate that by applying the Price et al. (2023b) model to the *Chlorella* sp. data in Table A1, the result is that the model introduces additional variability, rather than clearly explaining it. For example, in Figure 1 (below), application of the Price et al. (2023b) EC50 model to the EC10 data in Table A1, the r^2 (calculated as 1-residual sum of squares/total sum of squares) in the upper left panel is -0.51. This indicates that the model residuals exhibit more variability than the actual data. As a first reaction, this might indicate that an MLR model should not be used to normalize the *Chlorella* sp. data. Rather, a mean or geometric mean would be a more satisfactory model. In other words, by applying the *Chlorella* sp. EC50 model from Price et al. (2023b), the TMFs (i.e., pH, DOC, and hardness), increase the variability in the EC10s. This is not a desired result for the bioavailability models supporting derivation of DGVs.

However, we can further examine these data to determine what is causing this behaviour. The first place to look is at the residuals (here shown as predicted EC10/observed EC10, and plotted on a log10 scale), and examine any trends in the residuals across the ranges of TMFs considered. The residual panels in Figure 1 (i.e., top right panel [pH], lower left panel [DOC], and lower right panel [Hardness] indicate that the residuals are quite flat for several of the data series. The only apparent exception to this is for hardness, and the only data series that exhibits a trend with hardness (i.e., under-predicting EC10 at low hardness and over-predicting EC10 at high hardness) is the data series from Price et al. (2022). However, if the data are taken as a whole, the same observation appears to be true. Another important point from the residual panels is that it appears that the *Chlorella* sp. sensitivity varies from study to study (i.e., the data series appear stratified vertically), most clearly in the pH residual panel. This could indicate that the tests from the various studies were conducted differently, that the *Chlorella* sp. in the tests actually had different sensitivities, or that an important TMF that varied among studies is not being properly considered.

Attachment C

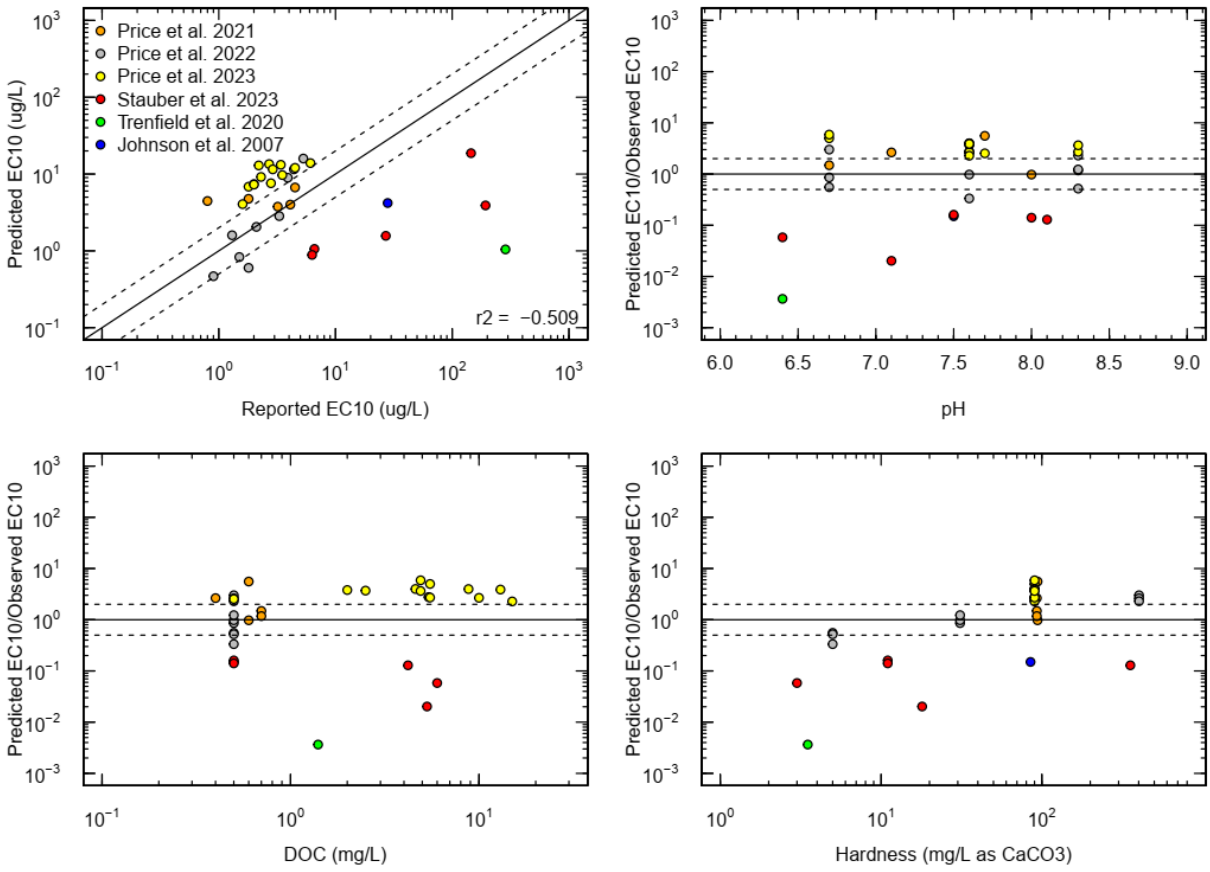


Figure 1. Application of the *Chlorella* sp. EC50 model from Price et al. (2023b) to *Chlorella* sp., EC10 data in Table A1. The upper right panel shows predicted EC10s vs. observed EC10, the upper left panel shows residuals (i.e., predicted EC10/observed EC10) against pH, the lower left panel shows residuals against DOC concentration, and the lower right panel shows residuals against hardness. The r^2 in the upper left panel was calculated as 1 – residual sum of squares/total sum of squares.

After recognizing the issues that appear to be causing the model to under-perform with EC10s, two alternative models were evaluated here. Both models could be considered the MLR model equivalent of an analysis of covariance (ANCOVA), where a single model with identical TMF slopes is developed for this overall dataset (minus the two datasets that each contributed only a single point) while assuming that intercepts would be study-specific. The results of this modelling evaluation indicate that both hardness and DOC would be highly significant TMFs, but that study-specific intercepts are also highly significant (Figure 2). When the data are modelled in this fashion, pH is no longer an important TMF for *Chlorella* sp. Examining the residual panels in Figure 2, it is clear that the residuals vs. TMFs are flat for the overall dataset, and also for each of the individual studies. So, from a modelling perspective, we have just identified the most important TMFs for the EC10 data for *Chlorella* sp. in Table A1, but the difficulty is that to explain the data, the sensitivity of the *Chlorella* sp. had to be characterized on study-specific basis.

Perhaps a way to deal with this issue in the SSD is to normalize the results of each study separately using the model described in Figure 2, and then calculate a geometric mean for each study. If we consider each study in a manner analogous to a separate species, then in US EPA terminology, each value would be a “species mean chronic value” or SMCV. If we then take the geometric mean of these values, we get the “genus mean chronic value” or GMCV. In this case, the GMCV could be used rather than the geometric mean for all data, normalized with an inferior EC10 model. Alternatively, this can be skipped entirely, by using a non-normalized *Chlorella* sp. geometric mean EC10 for the SSD. This approach seems inadequate, because it was just demonstrated above that TMFs are indeed important for *Chlorella* sp. EC10s.

Attachment C

This type of analysis should ideally be repeated for each of the organisms that were used to develop the MLR models described in Table 1 (e.g., *Oncorhynchus mykiss*, *Daphnia magna*, and *Raphidocelis subcapitata*), using the data for the relevant species included in Table A1. If the models in Table 1 are not appropriately predictive of the relevant data in Table A1, then the models should be re-evaluated (i.e., potentially as described above).

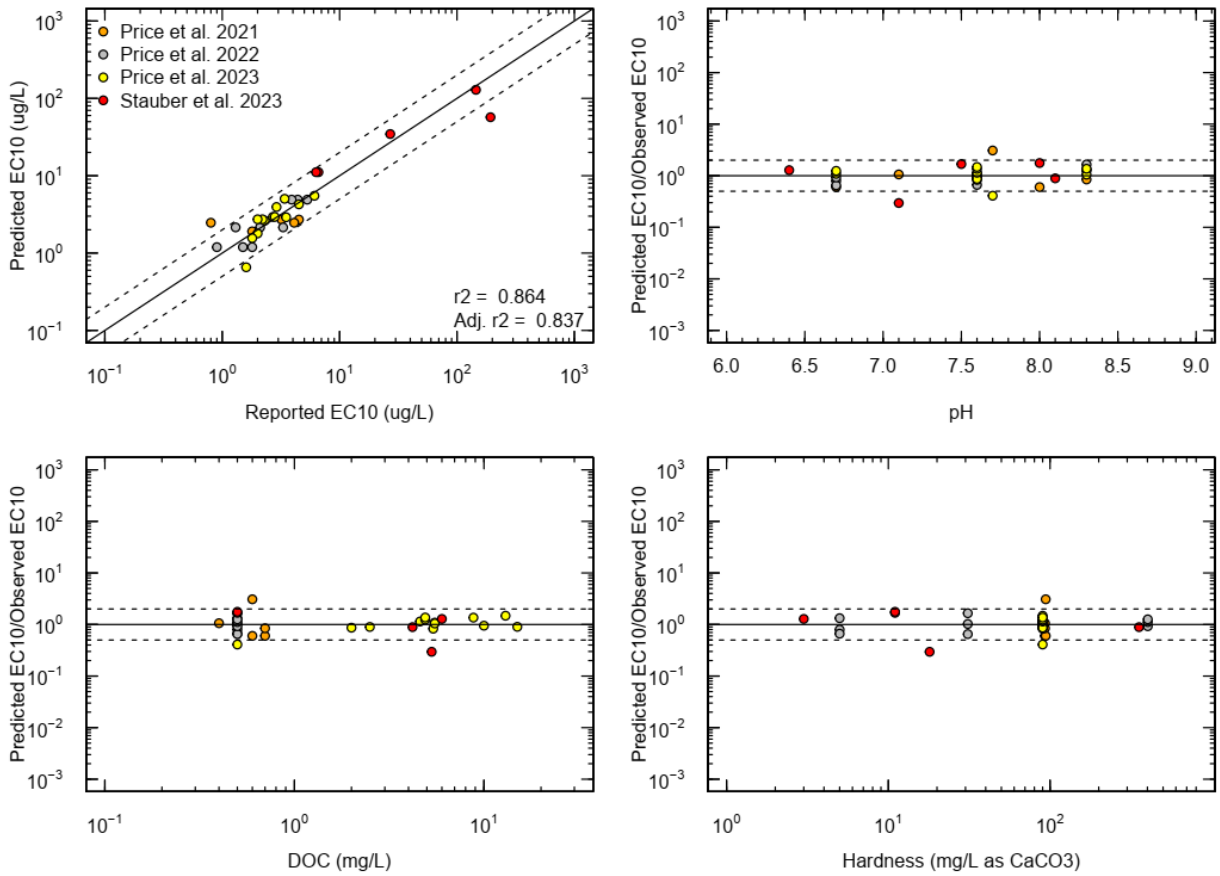


Figure 2. Development of a new *Chlorella* sp. EC10 model from *Chlorella* sp. EC10 data in Table A1. The upper right panel shows predicted EC10s vs. observed EC10, the upper left panel shows residuals (i.e., predicted EC10/observed EC10) against pH, the lower left panel shows residuals against DOC concentration, and the lower right panel shows residuals against hardness. The r^2 in the upper left panel was calculated as 1 – residual sum of squares/total sum of squares.