# Toxicant default guideline values for aquatic ecosystem protection

Chlorine in marine water

Technical brief

February 2025

© Commonwealth of Australia 2025

**Ownership of intellectual property rights**

Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Commonwealth of Australia (referred to as the Commonwealth).

**Creative Commons licence**

All material in this publication is licensed under a Creative Commons Attribution 4.0 Australia Licence, save for content supplied by third parties, photographic images, logos and the Commonwealth Coat of Arms.


Creative Commons Attribution 4.0 Australia Licence is a standard form licence agreement that allows you to copy, distribute, transmit and adapt this publication provided you attribute the work. See the [summary of the licence terms](https://creativecommons.org/licenses/by/4.0/) or the [full licence terms](https://creativecommons.org/licenses/by/4.0/legalcode).

Inquiries about the licence and any use of this document should be emailed to copyright@dcceew.gov.au.

**Cataloguing data**

This publication (and any material sourced from it) should be attributed as: ANZG 2025, *Toxicant default guideline values for aquatic ecosystem protection: Chlorine in marine water.* Australian and New Zealand Guidelines for Fresh and Marine Water Quality. CC BY 4.0. Australian and New Zealand Governments and Australian state and territory governments, Canberra, ACT, Australia.

This publication is available at [waterquality.gov.au/anz-guidelines/guideline-values/default/water-quality-toxicants/toxicants](http://www.waterquality.gov.au/anz-guidelines/guideline-values/default/water-quality-toxicants/toxicants).

**Contact**

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

GPO Box 858 Canberra ACT 2601

General enquiries: 1800 920 528

Email waterquality@dcceew.gov.au

**Disclaimer**

The author(s) of this publication, all other entities associated with funding this publication or preparing and compiling this publication, and the publisher of this publication, and their employees and advisers, disclaim all liability, including liability for negligence and for any loss, damage, injury, expense or cost incurred by any person as a result of accessing, using or relying on any of the information or data in this publication to the maximum extent permitted by law.

**Acknowledgements**

These default guideline values (DGVs) were derived by Dr Graeme Batley (CSIRO Land and Water). The DGVs were peer reviewed by two anonymous reviewers and by contracted technical advisors Dr Rick van Dam and Dr Melanie Trenfield. The DGVs have been reviewed and approved by jurisdictional technical and policy oversight groups.

Contents

Summary v

1 Introduction 1

2 Aquatic toxicology 3

2.1 Mechanism of toxicity 3

2.2 Toxicity 3

3 Factors affecting toxicity 5

4 Default guideline value derivation 6

4.1 Toxicity data used in derivation 6

4.2 Species sensitivity distribution 10

4.3 Default guideline values 11

4.4 Reliability classification 12

Glossary 13

Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values 15

Appendix B: Assessment of datasets for DGV derivation 20

Appendix C: Assessment of reliability of echinoderm toxicity data from Dinnel et al. (1981) 23

Appendix D: Modality assessment for chlorine 25

References 27

Figures

[Figure 1 Species sensitivity distribution, chlorine (CPO) in marine water 10](#_Toc185441656)

Tables

Table 1 Summary, single acute toxicity values, all species used to derive default guideline values for chlorine (CPO) in marine water 9

Table 2 Default guideline values, chlorine (CPO) in marine water, very high reliability 11

Appendix figures

[Figure B 1 SSD for chlorine (CPO) based on flow-through LC50 data, static EC50 data and water salinity ≥25‰ 21](#_Toc185441659)

[Figure B 2 SSD for chlorine (CPO) based on flow-through LC50 data and water salinity 15–<25‰ 22](#_Toc185441660)

[Figure B 3 Combined dataset SSD for chlorine (CPO) – based on flow-through LC50 data, static EC50 data and water salinity ≥15‰ 22](#_Toc185441661)

[Figure D 1 Histogram, ln-transformed chlorine marine acute toxicity data 25](#_Toc185441662)

[Figure D 2 Box plot, sensitivity of taxonomic groups to chlorine in marine water 26](#_Toc185441663)

Appendix tables

[Table A 1 Summary, acute toxicity data that passed screening and quality assessment processes, chlorine (CPO) in marine water, salinity ≥25‰ – used in DGV derivation 15](#_Toc185441664)

[Table A 2 Summary, flow-through acute toxicity data that passed screening and quality assessment processes, chlorine (CPO) in marine water, salinity ≥15‰–<25‰ – used in DGV derivation 17](#_Toc185441665)

[Table A 3 Summary, toxicity data that passed screening and quality assessment processes, chlorine (CPO) in marine water, salinity ≥15–<25‰ and ≥25‰ – not used in DGV derivation 18](#_Toc185441666)

[Table B 1 Summary, short-term protective concentrations for chlorine (CPO) in marine water – three datasets assessed 22](#_Toc185441667)

[Table C 1 Bioassay details for assessing chlorine toxicity in unfiltered seawater to two sea urchins, sourced from Dinnel et al. (1981) 23](#_Toc185441671)

## Summary

The DGVs and associated information in this technical brief should be used in accordance with the detailed guidance provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality website ([www.waterquality.gov.au/anz-guidelines](http://www.waterquality.gov.au/anz-guidelines)).

Chlorination is commonly used to control biofouling organisms; however, chlorine rapidly hydrolyses in seawater to hypochlorite which undergoes further reaction with bromide. Hypochlorite can also react with organic matter. These reaction products—collectively termed chlorine-produced oxidants (CPO)—can be toxic to marine biota. Because the residence times of the most toxic forms are limited to several days, appropriate water quality guideline values must be based on short-term (acute) toxicity tests, rather than chronic tests. Flow-through toxicity tests are the most appropriate, whereas static-renewal tests generate variable results dependent on the renewal rate.

Literature data for acute CPO toxicity from flow-through tests, combined with values from two sensitive 15 min static tests, (data for 29 species from five taxonomic groups) passed the quality assessment and screening processes and were used to derive default guideline values (DGVs). The fit of the species sensitivity distribution (SSD) to the data was good, resulting in very high reliability DGVs. The CPO DGVs for 99%, 95%, 90% and 80% species protection are 4.3 µg/L, 10 µg/L, 16 µg/L and 26 µg/L, respectively. The 95% species protection DGV for CPO is recommended for application to slightly-to-moderately disturbed ecosystems.

These are the first marine DGVs for chlorine to be derived using SSDs; all other formal international guideline values are based on using assessment factors applied to data for the most sensitive species. In applying these DGVs, it must be demonstrated that CPO concentrations would be reduced to below the DGV within an acceptable mixing zone both through dilution and dissociation.

The DGVs can be applied to the use of chlorine in the biocidal treatment of heat-exchanger pipes or other systems. This treatment is often continuous; however, where the discharge is into the marine environment, the impacts of the discharge are intermittent because of tidal currents and wave action. The DGVs in this document are conservative because they are mostly based on toxicity testing where the toxicant is continuously renewed, and not on static-renewal or static tests. The DGVs can be applied to all discharges, both continuous and intermittent.

## Introduction

Chlorination, either by the addition of sodium hypochlorite (NaOCl) or electrolysis of seawater, is one of the most effective approaches for the control of biofouling organisms in seawater (Nguyen et al. 2012; Rajagopal 2011). When discharging chlorine-treated waters, there are concerns for the impacts of chlorine and its decomposition products on the health of non-target aquatic biota. This treatment is often continuous; however, where the discharge is into the marine environment, the impacts of the discharge are also influenced by varying rates of dilution of chlorine-produced oxidants (CPO) due to tidal currents, wave action and losses through volatilisation. CPO include free chlorine and a number of chlorine reaction products that are rapidly formed as a result of its strong oxidising property (Section 3). The derivation of water quality guideline values for chlorine is complicated because chlorine is highly reactive in seawater, first hydrolysing to hypochlorite and then rapidly oxidising bromide (UK Marine SAC 2019). Since these reactions are rapid (Wallis and Chidgey 2022), chlorine or hypochlorite are not expected to pose a direct toxicity threat; however, toxicity from their reaction products remains and can be assessed in the laboratory. On that basis, it is possible to generate guideline values that relate to the original chlorine or hypochlorite concentration.

Guideline values for chlorine and its reaction products have already been derived by a number of jurisdictions (USEPA 1985; CCME 1999; ANZECC/ARMCANZ 2000; Sorokin et al. 2007). However, with improvements in guideline value derivation methods (e.g. Batley et al. 2018), and the availability of newer toxicity data, there was an opportunity to derive more robust default guideline values (DGVs) for Australia and New Zealand.

In evaluating the toxicity data from experiments with reactive chemicals, there is the option to use either static tests (to simulate one-off discharges) or flow-through tests that model continuous discharges over several days and avoid decay of toxic reaction products. Flow-through tests are more appropriate for the derivation of DGVs for ecosystem protection. Further, given that toxicity is time-sensitive, it is appropriate to derive DGVs based on short-term (acute) data rather than on long-term (chronic) data.

The oldest marine chlorine guideline value is from USEPA (1985), which recommended that ‘except possibly where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of CPO does not exceed 7.5 µg/L more than once every three years on the average and if the one-hour average concentration does not exceed 13 µg/L more than once every three years on the average’.

CCME (1999) noted that the four most sensitive species endpoints in its database were 5-min EC50 reduced egg fertilisation successes for sand dollars and green sea urchins (both echinoderms) of 2 µg/L as CPO and 5 µg/L as CPO, respectively (Dinnel et al. 1981), the 48-h LC50 for the eastern oyster larvae of 5 µg/L and the 48-h EC50 for hard clam larvae of 6 µg/L (Roberts et al. 1975). These were not considered acceptable by CCME (1999) due to the analytical methods and testing protocols used. The CCME (1999) default acute guideline value (termed a short-term guideline value) was derived by applying an ‘application factor’ of 0.05 to the 10 µg/L LC50 for the next most sensitive species, which included the blue crab (Patrick and McLean 1971), American oyster (Capuzzo 1979), rotifer *Brachionus plicatilis* (Capuzzo et al. 1976) and phytoplankton (Eppley et al. 1976), giving a guideline value of 0.5 µg/L.

A risk assessment report for the UK Environment Agency (Sorokin et al. 2007) identified the lowest reliable short-term toxicity data point as a 24-h LC50 of 5 µg/L as free available chlorine for the freshwater crustacean *Ceriodaphnia dubia*. A standard assessment factor of 100 was applied resulting in a predicted no effect concentration (PNEC) in saltwater of 0.05 µg/L. This was recommended as a replacement for the existing environment quality standard (EQS) as part of the European Water Framework Directive. The existing EQS for total residual oxidants (TRO) (Lewis et al. 1994) was based on an assessment factor of ~2 applied to an acute LC50 value of 28 µg/L for both plaice and sole. This resulted in an EQS of 10 μg/L, substantially higher than the proposed PNEC in saltwater.

In Australia and New Zealand, the absence of sufficient toxicity data for marine species led to the adoption of a moderate reliability freshwater chronic DGV of 3 µg/L as a low reliability environmental concern value for marine water (ANZECC/ARMCANZ 2000). Although ANZECC/ARMCANZ (2000) noted that the marine chlorine 95% species protection value was relatively close to the acute toxicity value for the most sensitive species, it was considered sufficiently protective due to its decomposition rate in seawater, the narrow difference between acute and chronic toxicity, and the lower sensitivity of other data for this species.

CPO analysis of environmental samples must be undertaken in the field, where possible, due to the reactivity and volatility of chlorine and the reaction products. Colorimetry is the most common analysis method that can be used in the field as well as in the laboratory. Laboratory methods (e.g. amperometry) have lower detection limits, but analysis must be undertaken immediately to avoid loss of analyte. Laboratory detection limits are as low as 2 μg/L, depending on the method used, while most field test kits are unable to reach this limit. However, common analytical methods can also be affected by various interferences (e.g. other oxidising agents, manganese compounds, bromide and turbidity (Harp 2002)). The N,N-diethyl-p-phenylenediamine (DPD) colorimetric method (APHA 4500-Cl G (APHA 2017)) has been used for freshwater and, according to the method, can be used for seawater (APHA 2017). A range of newer methods was reviewed by Wilson et al. (2019) and, of these, the 2,2’-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid-diammonium salt (ABTS) colorimetric method was the most promising, with a detection limit of 2 µg/L. Compared to DPD, the greater stability of the colour of the ABTS means that samples could be prepared in the field and measured in the laboratory. Using the ABTS method, chloramine and total residual chlorine can be determined separately, and free chlorine, if required, can be determined by the difference. However, the application of the ABTS method to seawater has yet to be described. A sensitive iodometric method for use in seawater has been described by Wang et al. (2008).

Recently in Australia and New Zealand, there have been at least two efforts to derive guideline values for chlorine in marine water. Batley and Simpson (2020) derived short-term exposure guideline values for chlorine in marine water based on the derivation method of Warne et al. (2018). They reported 99%, 95%, 90% and 80% species protection values (as CPO) of 2.2 µg/L, 7.2 µg/L, 13 µg/L and 22 µg/L, respectively. Subsequently, Wallis and Chidgey (2022) challenged some of the data used in the derivation by Batley and Simpson (2020) and, using a slightly modified dataset, reported 99% and 95% species protection values (as CPO) of 12 µg/L and 18 µg/L, respectively.

The DGVs derived and reported in this document are largely based on the work of Batley and Simpson (2020), taking into consideration the work of Wallis and Chidgey (2022), and they supersede the ANZECC/ARMCANZ (2000) DGVs. To ensure that the DGVs account for the rapid formation of chlorine reaction products, they are expressed as the concentration of CPO. However, as CPO measures the oxidising power of a solution, methods for CPO analysis may also measure oxidants that are not covered by the DGVs. Nevertheless, the use of CPO as a measure of chlorine and its reaction products is considered the most appropriate approach for the DGVs.

## Aquatic toxicology

### Mechanism of toxicity

The toxicity of chlorine is strongly related to its oxidising capacity (ATSDR 2010). It is a well-known respiratory toxicant and, although the mechanism by which it affects respiratory tissues is not fully known, it is thought to be due to CPO interacting with functional groups in components from cells in the respiratory epithelium (ATSDR 2010). Other reported mechanisms of CPO toxicity include chlorination of amino acids and the inhibition of biological processes such as enzyme function. In fish, oxidative damage to the gills affects respiration, while oxidation of haemoglobin is also possible, affecting oxygen transport (Sorokin et al. 2007). For algae and aquatic plants, El-Sherbiny et al. (2021) hypothesised that chlorine may damage chloroplasts or interfere with enzymes.

### Toxicity

The toxicity of CPO to aquatic organisms is dependent on the species, the exposure duration and how they are exposed. Acute LC50s reported in the literature for over 30 species of marine organisms range from <5 µg/L to almost 3 000 µg/L.

Of the species assessed for CPO toxicity, the most sensitive are the echinoderms *Strongylocentrotus droebachiensis* and *Dendraster excentricus* (Dinnel et al. 1981). In this study, sperm viability for the echinoderms was assessed by quantifying egg fertilisation after 15 min of incubation with sperm that had been exposed to chlorine (as hypochlorite) for 5 min. Prior to exposing the sperm, the chlorine solutions were subject to several different reaction times from 1 min to 48 h depending on the species, where reaction time refers to the time allowed for chlorine in the test solution to react with the seawater diluent before adding the sperm. The EC50s (expressed as TRO, which is analogous to CPO) for *D.* *excentricus* and *S. droebachiensis* in unfiltered seawater ranged from approximately 2 µg/L to 13 µg/L and 5 µg/L to 6 µg/L, respectively (Dinnel et al. 1981). For *D. excentricus* in filtered seawater, the EC50s (after 1–2 h reaction time) were higher, at 0.018–0.020 µg/L (Dinnel et al. 1981). For both species, reaction time did not appear to affect toxicity, suggesting that reaction products other than CPO were causing toxicity (Dinnel et al. 1981). Wallis and Chidgey (2022) also recognised that the reaction times used by Dinnel et al. (1981) were long enough for no CPO to remain in solution, and that the sensitive responses of the echinoderms must have reflected some other stressor in the test water. As the chlorine exposure duration was only 5 min in these fertilisation experiments, the tests are classified as acute (Warne et al. 2018); chronic tests with this species and endpoint require ≥1 h exposure duration to chlorine. The implications for the inclusion of the Dinnel et al. (1981) echinoderm data in the DGV derivation are addressed in Section 4.1.

The next most sensitive species is the fish *Pleuronectes* *platessa*, with a 96-h LC50 of 24 µg/L (Alderson 1972). Crustaceans appear to exhibit a wide range of sensitivity to chlorine, with acute LC50s ranging from 29 µg/L for the copepod *Acartia tonsa* (Roberts and Gleeson 1978) to 1 420 µg/L for shore crabs *Hemigrapsus* spp. (Thatcher 1978) and to 2 890 µg/L for the lobster *Homarus americanus* (Capuzzo et al. 1976). Also, in contrast to the high sensitivity of the two echinoderms reported by Dinnel et al. (1981), Wallis and Chidgey (2022) reported an EC50 (1-h fertilisation success, no pre-exposure reaction time) of 133 µg/L for the echinoderm *Heliocidaris tuberculata*; however, this would generally be considered a chronic test for this life stage and endpoint.

There are toxicity data for only a few marine microalgal species. The available data suggest that algae are not the most sensitive species to chlorine. Lopez-Galindo et al. (2010) reported chronic 96-h IC15 values of 172 µg/L for *Isochrysis galbana* and481 µg/Lfor *Dunaliella salina*. Their respective IC50 values of 1 390 µg/L and 824 µg/L were the highest of any tests reported. The relatively low sensitivity might be attributed to the test methods employing static exposures. Flow-through tests with algae are difficult to undertake and are, therefore, rarely reported. However, Vannoni et al. (2018) assessed the toxicity of chlorine to two marine microalgae using both static and flow-through procedures. Based on static exposures, 72-h EC50s (fluorescence) for *Navicula pelliculosa* and *Achnanthes* spp. were 2 790 µg/L and 230 µg/L, respectively. Based on flow-through exposures, the toxicity of chlorine was increased, with 72-h EC50s (fluorescence) for the same two species being 430 µg/L and 80 µg/L, respectively. There appear to be no chlorine toxicity data available for marine plants such as macroalgae or seagrasses.

Ebenezer and Ki (2013) examined the effects of chlorine on the population growth of the marine dinoflagellate *Prorocentrum minimum* in static tests. Unfortunately, test salinity was not reported. They reported a significant (>50%) reduction in population growth at the lowest concentration tested: 0.1 mg/L. However, concentrations had decayed to below the detection limit of 0.01 mg/L over this duration, suggesting that it was the initial exposure that governed the impact.

The following are general observations of static and flow-through tests.

* Static tests with regular renewal (24 h) show lower toxicity (higher LC50 values) than continuous flow-through tests because of the reactivity and volatility of chlorine (hypochlorite). For example, a 30-min flow-through test with the rotifer *Brachionus plicatilis* had an LC50 of 90 µg/L (as CPO, Capuzzo et al. 1979) compared to a 24-h static test LC50 of 586 µg/L (as CPO, Lopez-Galindo et al. 2010).
* In flow-through systems, short-term exposures (30 min) generally show lower toxicity than 96-h exposures for the same species. The former may better reflect discharge conditions and the high reactivity of chlorine and its reaction products in seawater. For some species in flow-through tests, LC50 values decreased significantly as exposure duration increased from 24 h to 96 h as shown by Wan et al. (2000) for two marine amphipods. Although, for studies on *Menidia beryllina* fish embryos, Fisher et al. (1994) found little difference between 24-h and 48-h LC50 values.

There have been few studies that have examined the toxicity of reaction products. The oxidation products from bromine were found to be less toxic than those from chlorine (Dinnel et al. 1981). With regard to the toxicity of chloroform and bromoform (both produced by reactions with organics), a recent review showed that—at least for chloroform—effects on algae and fish are typically seen at mg/L concentrations, orders of magnitude above those for hypochlorite toxicity (UK Marine SAC 2019). The LC50 values for larval survival for the oyster *Crassostrea virginica*, estimated from the published dose-response curves (Stewart et al. 1979), were 2 mg/L, 1 mg/L and 0.1 mg/L respectively for chloroform, bromoform and bromate. They noted that chloroform and bromoform were both lost from solution by volatilisation. The toxicity of chloramine and bromamine products that are formed only when ammonia concentrations are elevated in the seawater was not considered.

## Factors affecting toxicity

The rapid hydrolysis of chlorine (Cl2) leads to the formation of hypochlorous acid (HOCl) and its dissociation product, the hypochlorite ion (OCl–) (Wallis and Chidgey 2022). At the pH of seawater (~8.1), HOCl is 80% dissociated to hypochlorite (pKa = 7.54). The term ‘free chlorine’ refers to the mixture of these three components in equilibrium.

Both chlorine and the hypochlorite ion are powerful oxidants. In particular, bromide ion, present in seawater at approximately 65 mg/L, is rapidly oxidised by hypochlorite to form hypobromous acid (pKa = 8.6), which is only some 20% dissociated to hypobromite ion at the pH of seawater. This reaction is 99% complete in 10 seconds (Jenner et al. 1997). Hypobromous acid is an effective oxidant, although weaker than hypochlorite. The antifouling and oxidative capacity of electrolysed seawater is, therefore, largely due to hypobromite rather than hypochlorite.

The term ‘total residual chlorine’ (TRC) used for freshwater is the equivalent of the term CPO for seawater. CPO are mostly bromine-based in seawater and include hypobromous acid and (in the presence of ammonia) chloramine/bromamine compounds referred to as combined chlorine. By definition, CPO largely refers to the oxidative products hypobromous acid (HOBr), hypobromous ion (OBr-), and bromamines as discussed in detail by Kinani et al. (2022), but does not include chloroform or bromoform.

In water where ammonia is present at elevated concentrations, the formation of chloramines (NH2Cl) (and bromamines) is also possible. Sugam and Helz (1977) estimated that for these to be significant, ammonia concentrations would need to exceed 10 µg/L for chlorination at 1 mg/L, but values of this order are uncommon in seawater. Because the majority of hypochlorous and hypobromous acids are consumed by reaction with organic compounds, the main products are a diverse range of halogenated organics, particularly trihalomethanes. Jenner et al. (1997) found that bromoform was the major product in cooling water discharge from a coastal power station using seawater for cooling purposes. Bromoform was measured at 16 µg/L following a mean chlorine dosage of 0.5–1.5 mg/L as Cl2. The high volatility of such compounds means they are rapidly lost. The half-life of bromoform varies from 16.9 h at 1 m depth to 85 h at 5 m (Abarnou and Miossec 1992), considerably longer than the half-life of chloroform of approximately 30 min (Dilling et al. 1975). Measured CPO includes free chlorine and combined chlorine (as chloramines).

In assessing the ecological impacts of chlorine discharges, the rates at which chlorine and hypochlorite species initially react to form hypobromite species, and further react with other receiving water constituents such as ammonia or natural dissolved organic matter (DOM), are critical. Few studies have examined this in detail. Zeng et al. (2009) showed that at 15°C, an initial chlorine concentration of 2.35 mg/L reduced to approximately 0.8 mg/L in less than 1 min. This reduction resulted from the oxidation of bromide to hypobromous acid, which was too fast to measure. This was followed by a slower first-order decomposition over 15 min to 0.5 mg/L and almost to completion in 30–40 min. The higher the water temperature, the faster the reactions and the reduction in chlorine concentration. They noted that in summer the CPO had fully decayed before discharge, whereas in winter the CPO decomposition was slower and could be incomplete at discharge.

Using CPO decomposition data and models from the literature (Wang et al. 2008; Saeed et al. 2015), a CPO concentration of 100 µg/L is predicted to decay to 50 µg/L within 2 h (~50%), and 25 µg/L within 24 h (~75%) in a 5–15°C receiving seawater environment. The CPO decomposition is slower at salinity <35‰. The rate of reaction with DOM is slower than the reaction with bromide and increases with increasing DOM concentrations (Wang et al. 2008). Similar findings were obtained by Saeed et al. (2015).

The above findings are relevant to how the toxicity testing data might be interpreted and applied to derive DGVs to protect the aquatic receiving environment. In flow-through tests with continuous hypochlorite addition, reaction with bromide would be presumed to have occurred (available bromide reacts rapidly), whereas in static tests, depending on the duration, further oxidative reactions might have progressed (slower reactions with DOM). Applying toxicity data derived in this way must account for the time of exposure required to elicit either acute or chronic toxicity to determine the nature of an impact.

## Default guideline value derivation

### Toxicity data used in derivation

Because of the high reactivity of chlorine, and the short residence time of the reaction products (i.e. several hours maximum) (Wallis and Chidgey 2022), it was more appropriate to derive DGVs that are protective against short-term (acute) effects rather than long-term (chronic) effects. Data from acute toxicity tests are relevant for deriving guideline values for contaminants that are short-lived and non-persistent due to dispersion, volatilisation or degradation—as is the case with chlorine in marine water. Thus, the literature search focused on data from acute exposures.

Because of the short half-lives of chlorine and its reaction products in marine water, flow-through toxicity tests are usually conducted, resulting in the continuous renewal of test water and maintenance of a near-constant chlorine (hypochlorite) exposure to the test organisms. CPO concentrations must be measured frequently to demonstrate that substantial reduction in concentration is not occurring. Static-renewal tests where hypochlorite-containing seawater was replaced regularly (usually daily) have been used in some studies. In static laboratory tests, organisms are exposed to rapidly decaying hypochlorite concentrations, and the LC50 values from such tests are generally higher (i.e. toxicity was lower) than those for flow-through tests. Flow-through tests best simulate continuous rather than intermittent discharges, and they provide a controlled environment that results in less-variable decay rates compared to static and static renewal tests. This has implications for selecting data for DGV derivation. Toxicity tests that use flow-through systems to attempt to maintain a constant chlorine concentration and/or prolong the exposure period will result in higher exposure concentrations than tests undertaken using static/static-renewal conditions. Therefore, the DGVs derived using such flow-through data will be conservative. For static-renewal tests, it is the frequency of renewal (if any) in the context of reaction rate that is important. Hence, 1–15 min static exposures for early life stages (e.g. eggs, sperm, embryos) may be appropriate for use in DGV derivation, whereas static tests where renewal of CPO is ≥24 h should not be used.

The data in Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values are composed of the available acute toxicity data from Chariton and Stauber (2008), CCME (1999), USEPA (1985) and additional recent literature data, all of which were assessed for quality according to the process specified by Warne et al. (2018). Warne et al. (2018) recommends using toxicity data for studies with salinities ≥25‰ for marine guideline values (Table A 1) and not using data from studies where the test salinity <25‰. However, for chlorine, there were a number of tests conducted at salinities just outside the ≥25‰ criterion (i.e. 15–<25‰) as shown in Table A 2. An assessment was undertaken to determine whether data from appropriate tests conducted at 15–<25‰ salinity could be added to the dataset based on tests conducted at ≥25‰. The assessment concluded that the two datasets could be combined (Appendix B: Assessment of datasets for DGV derivation).

Nearly all reported marine toxicity tests for chlorine were classified as acute, as defined by Warne et al. (2018). The only chronic data reported (as defined by Warne et al. 2018) were for 72-h algal tests (Lopez-Galindo et al. 2010), a 1-h sea urchin fertilisation test (Wallis and Chidgey 2022) and an 8-d fish test (Alderson 1974). For acute effects, usually only LC50 or EC50 data are recorded; however, given that such estimates represent a 50% effect on species survival or some sub-lethal effect, it is more reasonable to use acute negligible effect concentrations such as LC5/EC5 or LC10/EC10 values to derive a DGV that is intended to protect aquatic ecosystems, as these estimates represent a point of incipient toxicity, not 50% lethality/effect (Warne et al. 2018). An exception to this may be where regulations have stipulated that an acute LC50/EC50-based guideline value not be exceeded in a mixing zone. Acute negligible effect values were not available for most species, and it was not possible to estimate these values based on concentration–response data or curves for chlorine toxicity because these data or curves were not published for most of the studies. However, Morgan and Prince (1977) reported both LC5 and LC50 values for flow-through tests on eggs and larvae of five estuarine fish species. Ratios of LC5/LC50 were 0.55, 0.50, 0.66, 0.53 and 0.76 (mean = 0.6). In static tests on the rotifer *Brachionus plicatilis,* Lopez-Galindo et al. (2010) found an LC10/LC50 ratio of 0.75. Given the uncertainties in measurement of LC5 and LC10 values, and in the effects of salinity and temperature, and in flow-through versus static tests, the differences between these acute ‘median effect to negligible effect’ ratios are likely not significant. Therefore, adopting an alternative and more conservative default ratio of 0.2, as used to convert chronic EC50 values to EC10 values (Warne et al. 2018) cannot be justified. Thus, for chlorine, the species sensitivity distribution (SSD) was based on an LC50/EC50 dataset, with a ‘median effect to negligible effect’ conversion factor of 0.6 applied to the resulting protective concentrations (PCs) from the SSD to derive the DGVs. Notably, the DGVs derived by applying the conversion factor to the PCs from the SSD were effectively the same as applying the conversion factor to each EC50/LC50 value prior to constructing the SSD. Also, the adopted approach allows for jurisdictions to use the unconverted EC50/LC50 PCs for other purposes (e.g. assessing the likelihood of significant short-term toxic effects).

The majority of the data used in the derivation were from studies in the 1970s, 1980s and 1990s and, while their quality was acceptable, additional data that looked more closely at the effects of exposure time, salinity and temperature as well as reporting LC10 and LC50 values and concentration–response curves would improve the quality of the dataset.

No chlorine toxicity data for algae or plants were used in the DGV derivation. The European Chemicals Bureau (ECB 2002) recommended using the 72-h (or longer) algal EC50 values as an equivalent a short-term result, with EC10 values being the long-term result. However, most of the available algal toxicity values for chlorine were from chronic static tests lasting 72–96 h, and were not included in the derivation because of the decay in concentration that would occur over this test duration. The single study that assessed chlorine toxicity to two algal species using flow-through exposures (Vannoni et al. 2018) used fluorescence as the endpoint, which is not considered an ecologically relevant endpoint; therefore, the data from this study were not used. As algae are not among the most sensitive species to chlorine (Section 2.2), their exclusion from the derivation does not have a marked influence on the DGVs.

For the oyster *C. virginica*, Capuzzo (1979) found an LC50 of 80 μg/L after a 30‐min exposure in water of 28‰ salinity. Roberts et al. 1975 obtained a 96‐h LC50 of 23 μg/L at 20‰ for the same species. Both studies used a flow‐through test. Although the shorter exposure was possibly more appropriate for a chlorine discharge, for consistency with other data, the 96‐h value was used in the final SSD. For the copepod *Acartia tonsa*, an LC50 of 820 μg/L after 30-min in 28‰ water (Capuzzo 1979) was compared with an LC50 of 29 μg/L after 96-h in 20‰ water (Roberts and Gleeson 1978). The reasons for this difference were unclear. Again, the lower value was used in the final SSD.

As noted in Section 2.2, Wallis and Chidgey (2022) questioned the reliability of the chlorine toxicity data reported by Dinnel et al. (1981) for the two echinoderm species *S.* *droebachiensis* and *D. excentricus*, and suggested that they should not be included in any dataset used to derive guideline values for chlorine in marine water. The arguments presented by Wallis and Chidgey (2022) were scrutinised (Appendix C: Assessment of reliability of echinoderm toxicity data from Dinnel et al. (1981)), and it was concluded that, on the basis of the reaction times used by Dinnel et al. (1981), the data for *S.* *droebachiensis* should be excluded from the DGV derivation and the data for *D. excentricus* should be included in the derivation. For *D. excentricus*, six tests were run using reaction times of 1 min (three tests), 5 min, 15 min and 60 min. Although toxicity across these reaction times did not differ, the geometric mean of the three EC50s from the 1 min reaction time tests (6.4 µg/L) was selected over the other tests. This value was effectively the same as the EC50s from the other reaction times (i.e. 7 µg/L for 5 min, 6 µg/L for 15 min and 8 µg/L for 60 min) (Dinnel et al. 1981).

Based on the key decisions described above, the final dataset used to derive the DGVs for chlorine (as CPO) in marine water comprised toxicity data from 29 species from five different taxonomic groups across a salinity range of 15–35‰ (Table 1). All of the data used in the DGV derivation were based on CPO measurements using either amperometry or iodometric titration. An assessment of the modality of the dataset concluded that the dataset was unimodal and that the data could be used for the DGV derivation (Appendix D: Modality assessment for chlorine).

Further details on the data that passed the quality assessment, and were subsequently used to derive the DGVs, are presented in Table A 1 and Table A 2. Data that passed the quality assessment but were excluded from the DGV derivation are in Table A 3. Details of the data quality assessment and the data that passed the quality assessment are provided as supporting information.

Table 1 Summary, single acute toxicity values, all species used to derive default guideline values for chlorine (CPO) in marine water

| Taxonomic group | Species | Life stage | Exposure duration | Test type | Toxicity measure | Test medium | Concentration (µg/L) |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Echinoderm | *Dendraster excentricus* | Sperm | 5 min | Static | Fertilisation (EC50) | Seawater (28‰) | 6.4 |
| Rotifer | *Brachionus plicatilis* | – | 30 min | Flow-through | Mortality (LC50) | Seawater (28‰) | 90 |
| Mollusc(bivalve) | *Crassostrea virginica* | Larva | 96 h | Flow-through | Mortality (LC50) | Seawater (20‰) | 23 |
| Crustacean(copepod) | *Acartia tonsa* | – | 96 h | Flow-through | Mortality (LC50) | Seawater (20‰) | 29 |
| Crustacean(amphipod) | *Pontogeneia*sp. | Adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 687 |
| *Anonyx*sp. | Adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 145 |
| Crustacean(lobster) | *Homarus americanus* | Larva | 1 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 2 890 |
| Crustacean(mysid) | *Neomysis*sp. | Adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 162 |
| *Mysidopsis bahia* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (20–20.5‰) | 68 |
| Crustacean(shrimp) | *Pandalus danae* | Juvenile and adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 178 |
| *Pandalus goniurus* | Adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 90 |
| *Crangon nigricauda* | Adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 134 |
| *Palaemonetes pugio* | Adult | 96 h | Flow-through | Mortality (LC50) | Seawater (20‰) | 220 |
| Crustacean(crab) | *Hemigrapsus nudus* and *H. oregonensis* | Juvenile and adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 1 420 |
| Fish | *Menidia peninsulae* | Fry | 96 h | Flow-through | Mortality (LC50) | Seawater (22–27‰) | 54 |
| *Pleuronectes* *platessa* | Larva | 96 h | Flow-through | Mortality (LC50) | Seawater (35‰) | 24 |
| *Oncorhynchus kisutch* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 32 |
| *Clupea harengus pailasi* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 65 |
| *Gasterosteus aculeatus* | Juvenile and adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 167 |
| *Cymatogaster aggregata* | Juvenile and adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 71 |
| *Ammodytes hexapterus* | Juvenile and adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 82 |
| *Parophrys vetulus* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 73 |
| *Menidia menidia* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (20‰) | 37 |
| *Menidia beryllina* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (20–20.5‰) | 135 |
| *Synnathus focus* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (20‰) | 270 |
| *Gobiosoma bosci* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (20‰) | 80 |
| *Morone americana* | Egg | 76 h | Flow-through | Mortality (LC50) | Seawater (15‰) | 270 |
| *Morone saxatilis* | Egg | 48 h | Flow-through | Mortality (LC50) | Seawater (15‰) | 200 |
| *Alosa aestivalis* | Egg | 48 h | Flow-through | Mortality (LC50) | Seawater (15‰) | 240 |

### Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the acute marine toxicity data for chlorine (CPO) in Table 1 is presented in Figure 1. The SSD was plotted using the Burrlioz 2.0 software and was based on acute EC50/LC50 data. The model provides a good fit to the data (Figure 1).



Note: dotted line shows the concentration of CPO (17 µg/L) at which 5% of species are potentially affected.

Figure 1 Species sensitivity distribution, chlorine (CPO) in marine water

### Default guideline values

It is important that the DGVs and associated information in this technical brief are used in accordance with the detailed guidance provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality [website](https://www.waterquality.gov.au/anz-guidelines) (ANZG 2018). The chlorine DGVs are expressed as the concentration of CPO.

As the SSD in Figure 1 is based on EC50/LC50 data, PCs derived from the SSD do not represent DGVs but rather the concentrations predicted to protect x% of species from effects greater than 50%. These PCs for the 1st, 5th, 10th and 20th percentiles of the SSD were 7.1 µg/L, 17 µg/L, 27 µg/L and 43 µg/L (as CPO), respectively. The DGVs were calculated by multiplying these PCs by the ‘median to negligible effect’ conversion factor of 0.6 (Section 4.1). The resulting DGVs for 99%, 95%, 90% and 80% species protection are shown in Table 2. The 95% species protection DGV for CPO in marine waters is recommended for application to slightly-to-moderately disturbed ecosystems.

Table 2 Default guideline values, chlorine (CPO) in marine water, very high reliability

| Level of species protection (%) | DGV for chlorine (CPO) in marine water (g/L) **a** |
| --- | --- |
| 99 | 4.3 |
| 95 | 10 |
| 90 | 16 |
| 80 | 26 |

**a** The DGVs were derived using the Burrlioz 2.0 software. They have been rounded to two significant figures.

These are the first marine DGVs for chlorine to be derived using SSDs; all other formal international guideline values are based on using assessment factors applied to data for the most sensitive species. Due to the variation in bioassay durations, but limited overall toxicity data, it was not feasible to develop DGVs for specific exposure durations.

Although marine algae were not used to derive the DGVs, they do not appear to be among the most sensitive of taxonomic groups to chlorine, and the available toxicity data were well above the DGVs. Therefore, the DGVs should be protective of marine algae. Although there are no data for marine plants, they are unlikely to be more sensitive than algae and, therefore, are likely to be protected by the DGVs.

Although short-term DGVs are the most appropriate way to manage the impacts of chlorine in marine water, consideration of the longer-term impacts on biota is also relevant. In terms of defining a chronic exposure guideline value, one option is to apply an acute-to-chronic ratio (ACR) to the PCs based on the LC50 values (Table B 1). Fisher et al. (1994) reported ACRs for flow-through exposures of 3.7 for the mysid *Mysidopsis bahia* and 1.5 for the silverside *Menidia beryllina*. Using the geometric mean of these values (2.4), chronic PC99 and PC95 values of 1.5 µg/L and 5.0 µg/L (as CPO) were obtained. However, these are also conservative, as the most toxic CPO are depleted within 1–2 d, leaving products that are less toxic by at least an order of magnitude. The implication is that compliance with the conservative short-term DGVs is likely to also be protective against long-term effects.

The current DGVs should apply to all field situations, whether the discharges are continuous or intermittent, as there is unlikely to be any significant build-up of CPO beyond a defined mixing zone due to the decay processes and tidal mixing.

### Reliability classification

The chlorine (CPO) in marine water DGVs have a very high reliability classification (Warne et al. 2018) based on the outcomes for the following three criteria:

* sample size—29 (preferred)
* type of toxicity data—acute LC50 converted to acute LC10 and acute EC50 converted to EC10
* SSD model fit—good (Burr Type III).

## Glossary

| Term | Definition |
| --- | --- |
| acute toxicity | A lethal or adverse sub-lethal effect that occurs as the result of a short exposure period to a chemical relative to the organism’s life span. |
| acute-to-chronic ratio (ACR) | The species mean acute value (LC/EC50) divided by the chronic value (e.g. NOEC or EC10) for the same species. |
| assessment factor | A unitless number applied to the lowest toxicity figure for a chemical to derive a concentration that should not cause adverse environmental effects. The size of the assessment factor varies with the type of data. Also called ‘application factor’ or ‘safety factor’. |
| chlorine produced oxidants (CPO) | The sum of free and combined chlorine and bromine, largely involving oxidative products [hypobromous acid (HOBr), hypobromous ion (OBr-), and bromamines, but not chloroform or bromoform] in saltwater. It is generally analogous to total residual chlorine (TRC). |
| chronic toxicity | A lethal or sublethal adverse effect that occurs after exposure to a chemical for a period of time that is a substantial portion of the organism’s life span or an adverse effect on a sensitive early life stage. |
| default guideline value (DGV) | A guideline value recommended for generic application in the absence of a more specific guideline value (e.g. site-specific), in the *Australian and New Zealand Guidelines for Fresh and Marine Water Quality*. |
| DOM | Dissolved organic matter.  |
| EC50 (median effective concentration) | The concentration of a substance in water or sediment that is estimated to produce a 50% change in the response being measured or a certain effect in 50% of the test organisms relative to the control response, under specified conditions. |
| ECx | The concentration of a substance in water or sediment that is estimated to produce an x% change in the response being measured or a certain effect in x% of the test organisms, under specified conditions. |
| endpoint | The specific response of an organism that is measured in a toxicity test (e.g. mortality, growth, a particular biomarker). |
| guideline value  | A measurable quantity (e.g. concentration) or condition of an indicator for a specific community value below which (or above which, in the case of stressors such as pH, dissolved oxygen and many biodiversity responses) there is considered to be a low risk of unacceptable effects occurring to that community value. Guideline values for more than one indicator should be used simultaneously in a multiple lines of evidence approach. |
| LC50 (median lethal concentration) | The concentration of a substance in water or sediment that is estimated to be lethal to 50% of a group of test organisms, relative to the control response, under specified conditions. |
| LCx | The concentration of a substance in water or sediment that is estimated to be lethal to x% of a group of test organisms under specified conditions. |
| PC  | Protective concentration. |
| species (biological) | A group of organisms that resemble each other to a greater degree than members of other groups and that form a reproductively isolated group that will not produce viable offspring if bred with members of another group. |
| species sensitivity distribution (SSD)  | A method that plots the cumulative frequency of species’ sensitivities to a toxicant and fits a statistical distribution to the data. From the distribution, the concentration that should theoretically protect a selected percentage of species can be determined. |
| total residual chlorine (TRC) | The total amount of chlorine present in a sample, equal to the sum of the free chlorine residual and the combined available chlorine residual. The term is mostly used for freshwater and is generally analogous to chlorine produced oxidants (CPO). |
| toxicity | The inherent potential or capacity of a material to cause adverse effects in a living organism. |
| toxicity test | The means by which the toxicity of a chemical or other test material is determined. A toxicity test is used to measure the degree of response produced by exposure to a specific level of stimulus (or concentration of chemical) for a specified test period.  |

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

This appendix documents all toxicity data that passed the screening and quality assessment processes. Table A 1 contains the ≥25‰ salinity data that were used in the DGV derivation. Table A 2 contains the ≥15–<25‰ salinity data that were used in the DGV derivation. Table A 3 lists the ≥25‰ salinity and ≥15–<25‰ salinity data that were excluded from the DGV derivation.

Table A 1 Summary, acute toxicity data that passed screening and quality assessment processes, chlorine (CPO) in marine water, salinity ≥25‰ – used in DGV derivation

| Taxonomic group | Species | Life stage | Exposure duration | Test type | Toxicity measure | Test medium | Temp. (°C) | Concentration (µg/L) | Reference |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Rotifer | *Brachionus plicatilis* | – | 30 min | Flow-through | Mortality (LC50) | Seawater (28‰) | 25 | **90** | Capuzzo 1979**Value used in SSD** |
| Crustacean (amphipod) | *Pontogeneia*sp. | Adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 15 | **687** | Thatcher 1978**Value used in SSD** |
| *Anonyx*sp. | Adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 15 | **145** | Thatcher 1978**Value used in SSD** |
| Crustacean(shrimp) | *Pandalus danae* | Juvenile and adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 15 | **178** | Gibson et al. 1975; Thatcher 1978**Value used in SSD** |
| *Pandalus goniurus* | Adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 15 | **90** | Thatcher 1978**Value used in SSD** |
| *Crangon nigricauda* | Adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 15 | **134** | Thatcher 1978**Value used in SSD** |
| Crustacean(lobster) | *Homarus americanus* | Larva | 1 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 20–22 | **2 890** | Capuzzo et al. 1976**Value used in SSD** |
| Crustacean(mysid) | *Neomysis*sp. | Adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 15 | **162** | Thatcher 1978**Value used in SSD** |
| Crustacean(crab) | *Hemigrapsus nudus* and *H. oregonensis* | Juvenile and adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 15 | **1 420** | Thatcher 1978**Value used in SSD** |
| Echinoderm | *Dendraster excentricus* | Sperm | 5 min | Static | Fertilisation (EC50) | Seawater (28‰) | 14 | **6.4** | Dinnel et al. 1981**Value used in SSD***Geometric mean of 3 values* |
| Fish | *Menidia peninsulae* | Fry | 96 h | Flow-through | Mortality (LC50) | Seawater (22–27‰) | 25 | **54** | Goodman et al. 1983**Value used in SSD** |
| *Pleuronectes platessa* | Larva | 96 h | Flow-through | Mortality (LC50) | Seawater (35‰) | 8 | **24** | Alderson 1972**Value used in SSD** |
| *Oncorhynchus kisutch* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 15 | **32** | Thatcher 1978**Value used in SSD** |
| *Clupea harengus pailasi* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 15 | **65** | Thatcher 1978**Value used in SSD** |
| *Gasterosteus aculeatus* | Juvenile and adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 15 | **167** | Thatcher 1978**Value used in SSD** |
| *Cymatogaster aggregata* | Juvenile and adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 15 | **71** | Thatcher 1978**Value used in SSD** |
| *Ammodytes hexapterus* | Juvenile and adult | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 15 | **82** | Thatcher 1978**Value used in SSD** |
| *Parophrys vetulus* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (28‰) | 15 | **73** | Thatcher 1978**Value used in SSD** |

–: No data available/not stated.

Table A 2 Summary, flow-through acute toxicity data that passed screening and quality assessment processes, chlorine (CPO) in marine water, salinity ≥15‰–<25‰ – used in DGV derivation

| Taxonomic group | Species | Life stage | Exposure duration | Test type | Toxicity measure | Test medium | Temp. (°C) | Concentration (µg/L) | Reference |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Mollusc(bivalve) | *Crassostrea virginica* | Larva | 96 h | Flow-through | Mortality (LC50) | Seawater (20‰) | 19–28 | **23** | Roberts et al. 1975**Value used in SSD** |
| Crustacean(copepod) | *Acartia tonsa* | – | 96 h | Flow-through | Mortality (LC50) | Seawater (20‰) | 20 | **29** | Roberts and Gleeson 1978**Value used in SSD** |
| Crustacean(shrimp) | *Palaemonetes pugio* | Adult | 96 h | Flow-through | Mortality (LC50) | Seawater (20‰) | 19–28 | **220** | Roberts and Gleeson 1978**Value used in SSD** |
| Crustacean(mysid) | *Mysidopsis bahia* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (20.5‰) | 20 | 73 | Fisher et al. 1994 |
| Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (20‰) | 20 | 62 | Fisher et al. 1999 |
| – | **68** | **Value used in SSD***Geometric mean* |
| Fish | *Menidia menidia* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (20‰) | 19–28 | **37** | Roberts and Gleeson 1978**Value used in SSD** |
| *Menidia beryllina* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (20.5‰) | 20 | 128 | Fisher et al. 1994 |
| Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (20‰) | 20 | 143 | Fisher et al. 1999 |
| – | **135** | **Value used in SSD***Geometric mean* |
| *Synnathus focus* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (20‰) | 17–28 | **270** | Roberts et al. 1975**Value used in SSD** |
| *Gobiosoma bosci* | Juvenile | 96 h | Flow-through | Mortality (LC50) | Seawater (20‰) | 17–28 | **80** | Roberts et al. 1975**Value used in SSD** |
| *Morone americana* | Egg | 76 h | Flow-through | Mortality (LC50) | Seawater (15‰) | 8–12 | **270** | Morgan and Prince 1977**Value used in SSD** |
| *Morone saxatilis* | Egg | 48 h | Flow-through | Mortality (LC50) | Seawater (15‰) | 8–12 | **200** | Morgan and Prince 1977**Value used in SSD** |
| *Alosa aestivalis* | Egg | 48 h | Flow-through | Mortality (LC50) | Seawater (15‰) | 8–12 | **240** | Morgan and Prince 1977**Value used in SSD** |

–: No data available/not stated.

Table A 3 Summary, toxicity data that passed screening and quality assessment processes, chlorine (CPO) in marine water, salinity ≥15–<25‰ and ≥25‰ – not used in DGV derivation

| Taxonomic group | Species | Life stage | Exposure duration | Acute / chronic | Test type | Toxicity measure | Test medium | Temp. (°C) | Concen-tration (µg/L) | Reference | Comments |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Salinity ≥25‰ |
| Green microalga | *Isochrysis galbana* | – | 96 h | Chronic | Static | Growth (IC15) | Seawater (salinity not reported) | 20 | 172 | Lopez-Galindo et al. 2010 | CPO measured every 30 min. IC50 1 390 µg/L. |
| *Dunaliella salina* | – | 96 h | Chronic | Static | Growth (IC15) | Seawater (salinity not reported) | 20 | 481 | Lopez-Galindo et al. 2010 | Daily biomass (optical density) measurements. IC50 824 µg/L. |
| Dinoflagellate | *Prorocentrum minimum* | – | 6 h | Acute | Static | Growth (IC10) | – | 20 | 914 | Ebenezer and Ki 2013 | Decaying CPO by 75%. No IC50 calculable. |
| Rotifer | *Brachionus plicatilis* | 30 min old | 24 h | Acute | Static | Mortality (LC50) | Seawater (salinity not reported) | 20 | 586 (LC50), 438 (LC10) | Lopez-Galindo et al. 2010 | Measured concentrations in 0.3 mL well plates. |
| Crustacean(amphipod) | *Hyale barbicornis* | Juvenile | 96 h | Acute | 24-h renewal | Mortality (LC50) | Seawater (34‰) | 20 | 1 050 | Anasco et al. 2008 | Measured concentration decayed rapidly. Nominal concentration used for 24-h exposure, measured concentrations for other exposure times. |
| Crustacean(copepod) | *Acartia tonsa* | – | 30 min | Acute | Flow-through | Mortality (LC50) | Seawater (28‰) | 20 | 820 | Capuzzo 1979 | Acceptable quality. |
| Echinoderm | *Strongylocentrtus droebachiensis* | Sperm | 5 h | Acute | Static | Fertilisation (EC50) | Seawater (28‰) | 14 | <5 | Dinnel et al. 1981 | See Appendix C: Assessment of reliability of echinoderm toxicity data from Dinnel et al. (1981). Reaction time too long. |
| *Heliocidaris tuberculata* | Sperm | 1 h | Chronic | Static | Fertilisation (EC50) | Seawater (salinity not reported)  | 16 | 133 | Wallis and Chidgey (2022) | Deemed a chronic endpoint for exposures ≥1 h according to Warne et al. (2018). |
| Mollusc(bivalve) | *Crassostrea virginica* | Larva | 30 min | Acute | Flow-through | Mortality (LC50) | Seawater (28‰) | 25 | 80 | Capuzzo 1979 | Acceptable quality. |
| Fish | *Oryzias javanicus* | Larva | 96 h | Acute | 24-h renewal | Mortality (LC50) | Seawater (34‰) | 26 | 91 | Anasco et al. 2008 | Concentration decayed rapidly. Nominal for 24-h, measured for others. |
| Larva | 24 h | Acute | 24-h renewal | Mortality (LC50) | Seawater (34‰) | 26 | 152 | Anasco et al. 2008 | – |
| *Pleuronectes* *platessa* | Egg | 8 d | Chronic | Flow-through | Mortality (LC50) | Seawater (35‰) | 8 | 120 | Alderson 1972, 1974 | Low temperature. |
| Salinity ≥15–<25‰ |
| Crustacean | *Amphiporeia virginiana* | Juvenile | 48 h | Acute | Static | Mortality (LC50) | Seawater (21‰) | 10 | 626 | Wan et al. 2000 | Acceptable quality. |
| *Eohaustorius washingtonianus* | Juvenile | 48 h | Acute | Static | Mortality (LC50 | Seawater (21‰) | 15 | 567 | Wan et al. 2000 | Acceptable quality. |
| Mollusc(bivalve) | *Crassostrea virginica* | Larva | 48 h | Acute | Flow-through | Mortality (LC50) | Seawater (20‰) | 19–28 | 26 | Roberts and Gleeson 1978 | Acceptable quality. |
| Fish | *Menidia menidia* | Egg | 48 h | Acute | Flow-through | Mortality (LC50) | Seawater (15‰) | 8–12 | 300 | Morgan and Prince 1977 | Acceptable quality. |

–: No data available/not stated.

## Appendix B: Assessment of datasets for DGV derivation

There were three possible datasets that were assessed for use in the derivation of DGVs for chlorine (CPO) in marine water:

* Dataset 1: based on short-term tests conducted at ≥25‰ salinity, and comprising toxicity values for 18 species from five taxonomic groups
* Dataset 2: based on dataset 1 minus toxicity values from static exposure tests
* Dataset 3: based on short-term tests conducted at ≥15‰ salinity, and comprising toxicity values for 29 species from five taxonomic groups.

An assessment of the most appropriate dataset to use for the derivation of the DGVs is presented below. The acute toxicity protective concentrations (PCs) reported below have not been converted to chronic-equivalent PCs via the application of the ACR of 0.6 described in Section 4.1.

Using only the ≥25‰ acute toxicity data from flow-through or very short-term static tests (i.e. <15 min) from Table A 1, an SSD was plotted (Figure B 1) and used to derive PCs. The PCs for 99%, 95%, 90% and 80% species protection were 6.1 µg/L, 15 µg/L, 24 µg/L and 40 µg/L (as CPO), respectively (Table B 1).

If data from static tests were omitted, the PCs for 99% and 95% species protection increased to 20 µg/L and 30 µg/L (as CPO), respectively. This increase was largely due to the removal of the most sensitive endpoint, which was from a static test using a sand dollar species. Therefore, this species was included in the dataset used for the DGV derivation (see Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values).

Given there were considerable additional chlorine toxicity data for salinity between 15‰ and 25‰, the differences in the toxicity of chlorine between ≥15–<25‰ and ≥25‰ salinity were assessed, with a view to potentially combine the datasets (Table A 1 and Table A 2), assuming that the lowered salinity did not result in different toxicity. This required a comparison between the ≥25‰ and 15–<25‰ salinity datasets. The SSD of the 11 values in Table A 2 for salinity 15–<25‰ (Figure B 2) gave PCs of 3.2 µg/L, 15 µg/L, 29 µg/L and 57 µg/L (as CPO) for 99%, 95%, 90% and 80% protection, respectively (Table B 1), which was similar to the PCs based on the ≥25‰ salinity dataset (Table B 1), despite the 15–<25‰ salinity dataset not including the sensitive sand dollar species. Combining the data from Table A 1 and Table A 2 resulted in a dataset comprising 29 species from five  taxonomic groups and resulted in the SSD shown in Figure B 3 (and in Figure 1), and PCs of 7.1 µg/L, 17 µg/L, 27 µg/L and 43 µg/L (as CPO) for 99%, 95%, 90% and 80% protection, respectively (Table B 1). The 95% confidence limits of all of the data derived from the ≥25‰ and 15–<25‰ salinity datasets overlapped, and given there were only minor differences between the PCs from the different datasets (Table B 1), it was deemed appropriate to use the combined dataset (i.e. for ≥15‰ salinity) for deriving the DGVs.

Data for two species were common to the datasets, namely for the oyster *Crassostrea virginica* and the copepod *Acartia tonsa*. For the oyster, Capuzzo (1979) observed an LC50 of 80 µg/L following a 30-min exposure in seawater at 28‰ salinity, while Roberts et al (1975) reported a 96-h LC50 of 23 µg/L in 20‰ salinity (these were both flow-through exposures). While the shorter exposure was possibly more appropriate for a chlorine discharge, the lower of the two values was used in the SSD. For the copepod, an LC50 of 820 µg/L after 30 min in 28‰ salinity seawater (Capuzzo 1979) was compared to a 96-h LC50 of 29 µg/L in 20‰ salinity seawater (Roberts and Gleeson 1978). The more sensitive 96-h value was used in the SSD.



Note: dotted line shows the concentration of CPO (15 µg/L) at which 5% of species are potentially affected.

Figure B 1 SSD for chlorine (CPO) based on flow-through LC50 data, static EC50 data and water salinity ≥25‰



Note: dotted line shows the concentration of CPO (15 µg/L) at which 5% of species are potentially affected.

Figure B 2 SSD for chlorine (CPO) based on flow-through LC50 data and water salinity 15–<25‰



Note: dotted line shows the concentration of CPO (17 µg/L) at which 5% of species are potentially affected.

Figure B 3 Combined dataset SSD for chlorine (CPO) – based on flow-through LC50 data, static EC50 data and water salinity ≥15‰

Table B 1 Summary, short-term protective concentrations for chlorine (CPO) in marine water – three datasets assessed

|  |  |
| --- | --- |
| Level of species protection (%) | CPO protective concentration values (µg/L) **a** |
| ≥25‰ salinity, flow-through data, static data (n = 18) | 15–<25‰ salinity, flow-through data (n = 11) | ≥15‰ salinity, flow-through data, static data (n = 29) |
| 99 | 6.1  | 3.2 | 7.1 |
| 95 | 15  | 15 | 17 |
| 90 | 24 | 29 | 27 |
| 80 | 40 | 57 | 43 |

**a** Conversion to DGVs requires multiplication by an ACR of 0.6.

## Appendix C: Assessment of reliability of echinoderm toxicity data from Dinnel et al. (1981)

Wallis and Chidgey et al. (2022) questioned the reliability of the chlorine toxicity data reported by Dinnel et al. (1981) for the two echinoderm species *S.* *droebachiensis* and *D. excentricus*, and suggested that they should not be included in datasets used to derive guideline values for chlorine in marine water. An assessment of their arguments follows.

The details of the echinoderm toxicity tests and associated results reported by Dinnel et al. (1981) are described in Section 2.2. A summary of the experimental design for both species is shown in Table C 1. In assessing the quality of the Dinnel et al. (1981) study for inclusion in current DGV derivation, the data for both echinoderm species received a score of approximately 60% (see quality assessment supporting information). While this is considered acceptable for deriving DGVs (i.e. a score of ≥50% is required for a toxicity value to be deemed acceptable), it is possible for acceptable quality studies to be excluded based on critical flaws that might not have been identified or did not sufficiently penalise the study during the formal quality assessment process. Consequently, the concerns raised by Wallis and Chidgey (2022) were individually addressed below.

Table C 1 Bioassay details for assessing chlorine toxicity in unfiltered seawater to two sea urchins, sourced from Dinnel et al. (1981)

| Species | Endpoint | Reaction time **a** | Sperm exposure duration | Sperm-egg incubation duration |
| --- | --- | --- | --- | --- |
| *Dendraster excentricus* | Sperm viability | 1–60 min | 5 min | 15 min |
| *Strongylocentrotus droebachiensis*  | Sperm viability | 24–48 h | 5 min | 15 min |

**a** Reaction time refers to the time allowed for chlorine or bromine in the test solution to react with the dilution seawater *before* adding the sperm.

##### Canadian guideline values for chlorine in marine water did not use the Dinnel et al. (1981) data

CCME (1999) stated that there were reservations about the analytical methods and testing protocols reported by Dinnel at al. (1981). However, no further details of these reservations were provided in the CCME (1999) guideline document, making it difficult to assess the justification for this decision. Nevertheless, the data met the quality requirements for derivation of ANZG DGVs (as per Warne et al. 2018). Moreover, the methods were sufficiently clear in terms of the exposure regimes used (Table C 1). In terms of the analytical methods, Dinnel et al. (1981) clearly stated that they measured chlorine as total residual oxidant (TRO; analogous to CPO) and cited the APHA iodometric method by which this was done. They also reported the limit of detection, which was below the reported EC50 values.

##### Information lacking on the time gap between the toxicity tests and chlorine analyses

The study did not state when and how often during the experiment the samples for analysis were collected. While this is a limitation, it does not automatically exclude the data from the derivation.

##### Long reaction times (24–48 h) would reduce chlorine in the test water to very low concentrations

Theoretically, long reaction times reduce chlorine and CPO concentrations and, therefore, reduce toxicity. Wallis and Chidgey (2022) showed that, at CPO concentrations of approximately 600 µg/L, most of the CPO had reacted within 1 h. Both echinoderms exhibited very high sensitivity to chlorine. Dinnel et al. (1981) acknowledged the unexpected high toxicity of chlorine to *S. droebachiensis* after the longer reaction times of 24–48 h. In discussing chlorine fate, Dinnel et al. (1981) referred to an initial phase of rapid loss followed by a second phase of slower loss that is not measurable as TRO, but which represents a potential source of toxicity until shown otherwise. They also noted that measurable TRO may not be a completely adequate measure of toxicity, and that ‘lost chlorine’ may produce chlorinated by-products not measurable as TRO, which are persistent in the marine environment.

Notwithstanding the discussion by Dinnel et al. (1981), the uncertainty introduced by the relatively long reaction times of 24–48 h for *S. droebachiensis* tests may provide sufficient justification for excluding these data from the DGV derivation.

##### Using data for unfiltered water confounded the chlorine toxicity results

Wallis and Chidgey (2022) claimed that the data for the *D. excentricus* in unfiltered seawater showed higher toxicity, which suggests that there was an additional stressor. However, there was no evidence for this. Moreover, no direct comparison can be made between the filtered and unfiltered seawater used by Dinnel et al. (1981) because the reaction time for the filtered sample was longer (1–2 h) than for the unfiltered sample (1 min to 1 h). Thus, the change in toxicity could also be attributed to the reaction time length. Also, if there was an additional stressor in the unfiltered water causing toxicity, this would presumably have been seen across all the chlorine treatments because there was no dilution series of the seawater, just straight seawater with different chlorine concentrations. However, Dinnel et al. (1981) stated that fertilisation in seawater control was ≥90% and that any tests for which control fertilisation was <90% were discarded. This provides evidence that: the seawater was not having an adverse effect on the sperm; and where control performance was below acceptability, the test data were not used for the analyses. These factors were not acknowledged by Wallis and Chidgey (2022).

##### Replacement of Dinnel et al. (1981) echinoderm toxicity data with new toxicity data for another echinoderm species

In deriving marine guideline values for chlorine, Wallis and Chidgey (2022) suggested replacing the Dinnel et al. (1981) data for *S.* *droebachiensis* and *D. excentricus* with new toxicity data for the echinoderm *Heliocidaris tuberculata*. However, the DGV derivation method (Warne et al. 2018) does not permit the replacement of data for one or more species from a taxonomic group with data for another species from the same taxonomic group. In such cases, if all data are acceptable, all species would be included.

##### Conclusion

Although the evidence is not strong, the relatively long reaction times (24–48 h) used for *S.* *droebachiensis* provide sufficient justification to exclude the data from the derivation. However, there is insufficient justification to exclude the data for *D. excentricus*. Thus, the final dataset for the DGV derivation included data for *D. excentricus* but excluded data for *S. droebachiensis*.

## Appendix D: Modality assessment for chlorine

A modality assessment was undertaken for the chlorine in marine water toxicity dataset according to the four questions stipulated in Warne et al. (2018). These questions and their answers are as follows.

##### Is there a specific mode of action that could result in taxa-specific sensitivity?

Although the specific mode of action of chlorine toxicity is unclear, it is understood to not be specific to any taxa-specific biological processes and, as such, its toxicity is not expected to result in taxa-specific sensitivity.

##### Does the dataset suggest bimodality?

A histogram of the ln-transformed toxicity data (Figure D 1) indicates that the dataset has a unimodal, albeit slightly right skewed, distribution. Calculation of the bimodality coefficient (BC) on the ln-transformed data yielded a value of 0.26, which is below the BC threshold (0.55) that indicates bimodality, suggesting the dataset is not bimodal.



Figure D 1 Histogram, ln-transformed chlorine marine acute toxicity data

##### Do data show taxa-specific sensitivity (i.e. through distinct groupings of different taxa types)?

The sample sizes for most taxonomic groups are very small, making it difficult to draw conclusions about taxa-specific sensitivity, except that fish appear to be slightly more sensitive to chlorine than crustaceans (Figure D 2). Based on the data of Dinnel et al. (1981), echinoderms appear to be highly sensitive to chlorine; however, the data of Wallis and Chidgey (2022) suggest the high sensitivity does not exist for all echinoderms.



Note: the line in the box represents the median; ‘x’ represents the mean; unfilled circles represent suspected outliers; filled circles represent known outliers; error bars represent minimum and maximum values.

Figure D 2 Box plot, sensitivity of taxonomic groups to chlorine in marine water

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

Based on the outcomes of the above questions, the dataset is not bimodal and, on this basis, was used for the DGV derivation.

## References

Abarnou A and Miossec L (1992) ‘Chlorinated waters discharged to the marine environment chemistry and environmental impact: an overview’, *Science of the Total Environment*, 126:173–197.

Alderson R (1972) ‘Effects of low concentrations of free chlorine on eggs and larvae of plaice (*Pleuronectes platessa L.*)’, in Riuvo M (ed) *Marine pollution and sea life*, Fishing News (Books) Ltd, Surrey, England.

Alderson R (1974) ‘Sea-water chlorination and the survival and growth of the early development stages of plaice, *Pleuronectes platessa* (L) and Dover sole, *Solea solea* (L)’, *Aquaculture*, 4:41–53.

Anasco N, Koyama J, Imai S and Nakamura K (2008) ‘Toxicity of residual chlorines from hypochlorite treated seawater to marine amphipod *Hyale barbicornis* and estuarine fish *Oryzias javanicus*’, *Water Air and Soil Pollution*, 195:129–136.

ANZECC/ARMCANZ (2000) *Australian and New Zealand guidelines for fresh and marine water quality*, Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand.

ANZG (2018) [*Australian and New Zealand Guidelines for Fresh & Marine Water Quality*](https://www.waterquality.gov.au/anz-guidelines) [website], Australian and New Zealand governments and Australian state and territory governments.

APHA (2017) *Standard methods for the examination of water and wastewater*, 23rd edn, American Public Health Association, Washington, DC.

ATSDR (2010) *Toxicological profile for chlorine*, Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services.

Batley GE and Simpson SL (2020) ‘A short-term guideline value for chlorine in marine waters’, *Environmental Toxicology and Chemistry*, 39:754–764.

Batley GE, van Dam RA, Warne MStJ, Chapman JC, Fox DR, Hickey CW and Stauber JL (2018) *Technical rationale for changes to the method for deriving Australian and New Zealand water quality guideline values for toxicants – update of 2014 version*, Australian and New Zealand governments and Australian state and territory governments.

Capuzzo JM (1979) ‘The effect of temperature on the toxicity of chlorinated cooling waters to marine animals – a preliminary review’, *Marine Pollution Bulletin*, 10:45–47.

Capuzzo JM, Lawrence SA and Davidson JA (1976) ‘Combined toxicity of free chlorine, chloramine and temperature to Stage I larvae of the American lobster *Homarus americanus*’, *Water Research*, 10:1093–1099.

CCME (1999) ‘Canadian water quality guidelines for the protection of aquatic life: reactive chlorine’, in *Canadian Environmental Quality Guidelines*, Canadian Council of Ministers of the Environment.

Chariton AA and Stauber JL (2008) *Toxicity of chlorine and its major by-products in seawater: a literature review*, Report No. 54/08, CSIRO Land and Water Science.

Dilling WL, Tefertiller NB and Kallos, GJ (1975) ‘Evaporation rates and reactivities of methylene chloride, chloroform, 1, 1, 1-trichloroethane, trichloroethylene, tetrachloroethylene, and other chlorinated compounds in dilute aqueous solutions’, *Environmental Science & Technology*, 9:833–838.

Dinnel PA, Stober QJ and DiJulio DH (1981) ‘Sea urchin sperm bioassay for sewage and chlorinated seawater and its relation to fish bioassays’, *Marine Environmental Research*, 5:29–39.

Ebenezer V and Ki J-S (2013) ‘Physiological and biochemical responses of the marine dinoflagellate *Prorocentrum minimum* exposed to the oxidizing biocide chlorine’, *Ecotoxicology and Environmental Safety*, 92:129–134.

ECB (2002) [*Technical guidance document on risk assessment, Part II*](http://publications.jrc.ec.europa.eu/repository/bitstream/JRC23785/EUR%2020418%20EN-2.pdf), European Chemicals Bureau report no. EUR 20418 EN/2, European Commission Joint Research Centre, accessed 17 April 2019.

El-Sherbiny MM, Satheesh S and Ba-Akdah MA (2021) ‘Physiological responses of marine macroalgae to chlorine dioxide treatment’, *Thalassas: An International Journal of Marine Sciences*, 37:291–302.

Eppley RW, Renger EH and Williams PM (1976) ‘Chlorine reactions with sea-water constituents and the inhibition of photosynthesis of natural marine phytoplankton’ *Estuarine Coastal and Marine Science*, 4:147–161.

Fisher DJ, Burton DJ, Yonkos LT, Turley SD and Ziegler GP (1999) ‘The relative acute toxicity of continuous and intermittent exposures of chlorine and bromine to aquatic organisms in the presence and absence of ammonia’, *Water Research*, 33:760–779.

Fisher DJ, Burton DT, Yonkos LT, Turley SD, Turley BS, Ziegler GP and Zillioux EJ (1994) ‘Acute and short-term chronic effects of continuous and intermittent chlorination on *Mysidopsis bahia* and *Menidia beryllina*’, *Environmental Toxicology and Chemistry*, 13:1525–1534.

Gibson CI, Thatcher TO and Apts CW (1975) *Some effects of temperature, chlorine and copper on the survival and growth of the coon stripe shrimp, Pandalus danae*, report no. BNWL-SA-5344, Battelle Northwest.

Goodman L, Douglas P, Middaugh DJ, Hansen PJ, Higdon PK and Cripe GM (1983) ‘Early life-stage toxicity test with tidewater silversides (*Menidia peninsulae*) and chlorine-produced oxidants’, *Environmental Toxicology and Chemistry*, 2:337–342.

Harp DL (2002) *Current technology of chlorine analysis for water and wastewater*, technical information series booklet no.17, Hach Company.

Jenner HA, Taylor CJL, van Donk M and Khalanski M (1997) ‘Chlorination by-products in chlorinated cooling water of some European coastal power stations’, *Marine Environmental Research*, 43:279–293.

Kinani S, Roumiguières A and Bouchonnet S (2022) ‘A critical review on chemical speciation of chlorine-produced oxidants (CPOs) in seawater. Part 1: chlorine chemistry in seawater and its consequences in terms of biocidal effectiveness and environmental impact’, *Critical Reviews in Analytical Chemistry*, Nov 3:1–14, doi: 10.1080/10408347.2022.2139590.

Lewis S, Cartwright NG, Jerman E, Tynan P, Sims IR and Wellstein N (1994) *Proposed environmental quality standards for chlorine in fresh and marine waters*, Water Research Centre, Medmenham, UK.

Lopez-Galindo C, Garrido MC, Casanueva JF and Nebot E (2010) ‘Degradation models and ecotoxicity in marine waters of two antifouling compounds: sodium hypochlorite and an alkylamine surfactant’, *Science of the Total Environment*, 408:1779–1785.

Morgan RP and Prince RD (1977) ‘Chlorine toxicity to eggs and larvae of five Chesapeake Bay fishes’, *Transactions of the American Fisheries Society*, 106:380–385.

Nguyen T, Roddick FA and Fan L (2012) ‘Biofouling of water treatment membranes: a review of the underlying causes, monitoring techniques and control measures’, *Membranes*, 2:804–840.

Patrick R and McLean R (1971) *Entrainment simulation studies on some estuarine organisms for the Potomac Electric Power Company*, Academy of Natural Sciences, Department of Limnology, Philadelphia.

Rajagopal S (2011) ‘Chlorination and biofouling control in industrial cooling water systems’, in Rajagopal S, Jenner HA and Venugopalan VP (eds) *Operational and Environmental Consequences of Large Industrial Cooling Water Systems*, Springer, New York.

Roberts MH and Gleeson RA (1978) ‘Acute toxicity of bromochlorinated seawater to selected estuarine species with a comparison to chlorinated seawater toxicity’, *Marine Environmental Research*, 1:19–30.

Roberts MH, Diaz RJ, Bender ME and Huggett RJ (1975) ‘Acute toxicity of chlorine to selected estuarine species’, *Journal of the Fisheries Research Board of Canada*, 32:2525–2528.

Saeed S, Prakash S, Deb N, Campbell R, Kolluru V, Febbo E and Dupont J (2015) ‘Development of a site-specific kinetic model for chlorine decay and the formation of chlorination by-products in seawater’, *Journal of Marine Science and Engineering*, 3:772–792.

Sorokin N, Atkinson C, Aldous E, Rule K and Comber S (2007) [*Proposed EQS for water framework directive annex VIII substances: chlorine (free available)*](https://www.wfduk.org/sites/default/files/Media/chlorine.pdf), Science Report No. SC040038/SR4, Environment Agency, Bristol.

Stewart ME, Blogoslawski WJ, Hsu RY and Helz GR (1979) ‘By-products of oxidative biocides: toxicity to oyster larvae’, *Marine Pollution Bulletin*, 10:166–169.

Sugam R and Helz GR (1977) ‘Speciation of chlorine produced oxidants in marine waters’, *Chesapeake Science*, 18:113–115.

Thatcher TO (1978) ‘The relative sensitivity of Pacific Northwest fishes and invertebrates to chlorinated sea water’, in Jolley RL, Gorchev H and Hamilton DH (eds) *Water chlorination: environmental impact and health effects, Vol. 2*, Ann Arbor Science Publishers, USA.

UK Marine SAC (2019) [Biocides used in cooling water disinfection](http://ukmpa.marinebiodiversity.org/uk_sacs/activities/water-quality/wq8_28.htm), UK Marine Special Areas of Conservation website, accessed 15 April 2019.

USEPA (1985) *Ambient water quality criteria for chlorine – 1984*, report no. EPA 440/5-84-030, United States Environmental Protection Agency, Office of Water, Washington, DC.

Vannoni M, Creach V, Barry J and Sheahan D (2018) ‘Chlorine toxicity to *Navicula pelliculosa* and *Achnanthes* spp. in a flowthrough system: the use of immobilised microalgae and variable chlorophyll fluorescence’, *Aquatic Toxicology*, 202:80–89.

Wallis I and Chidgey S (2022) ‘New guidance values for chlorine in marine waters’, *Water e-Journal*, 8(1), doi: 10.21139/wej.2022.030.

Wan MT, Van Aggelen G, Cheng W and Watts RG (2000) ‘Acute toxicity of chlorine-produced oxidants (CPO) to the marine invertebrates *Amphiporeia virgiiana* and *Eohaustorius washingtonianus*’, *Bulletin of Environmental Contamination and Toxicology*, 64:205–212.

Wang J-T, Chen M-H, Lee H-J, Chang W-B, Chen C-C, Pai S-C and Meng P-J (2008) ‘A model to predict total chlorine residue in the cooling seawater of a power plant using iodine colorimetric method’, *International Journal of Molecular Science*, 9:542–553.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW, and Stauber JL (2018) *Revised method for deriving Australian and New Zealand water quality guideline values for toxicants – update of 2015 version*, Australian and New Zealand governments and Australian state and territory governments.

Wilson RE, Stoianov I and O’Hare D (2019) ‘Continuous chlorine detection in drinking water and a review of new detection methods’, *Johnson Matthey Technology Reviews*, 63:103–118.

Zeng J, Jiang Z, Chen Q, Zheng P and Huang Y (2009) ‘The decay kinetics of residual chlorine in cooling seawater simulation experiments’, *Acta Oceanologica Sinica*, 28:54–59.