An Australian Government Initiative

Australian & New Zealand GUIDELINES FOR FRESH & MARINE WATER QUALITY

Toxicant default guideline values for aquatic ecosystem protection

Dioxins in freshwater

Technical brief

April 2021

Water Quality Guidelines is a joint initiative of the Australian and New Zealand governments, in partnership with the Australian states and territories.

Cataloguing data
This publication (and any material sourced from it) should be attributed as: ANZG 2020, *Toxicant default guideline values for aquatic ecosystem protection: Dioxins in freshwater.* 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.

Contact
Australian Government Department of Agriculture, Water and the Environment
GPO Box 858 Canberra ACT 2601
Switchboard +61 2 6272 3933 or 1800 900 090
Email waterquality@agriculture.gov.au

Acknowledgements
These default guideline values (DGVs) were derived by Dr John C Chapman. Thanks are due to RM Sunderam (Honorary Scientific Fellow, Office of Environment and Heritage NSW) for assistance with quality checking of references and spreadsheets and formatting of graphs, and Olivia King (DSITI Qld) for assistance with modality check. Thanks also to Dr Graeme Batley (CSIRO), who reviewed the draft before submission and provided useful comments. The DGVs were peer reviewed by two anonymous reviewers and by two contracted technical advisors, Dr Rick van Dam and Alicia Hogan. The DGVs were also reviewed and approved by jurisdictional technical and policy oversight groups and a National Water Reform committee prior to being published.

Contents

Figures

Tables

Appendix tables

Appendix figures

Summary

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

Polychlorinated dibenzo-*p*-dioxins (PCDDs or 'dioxins'), dioxin-like coplanar polychlorinated biphenyls (dl-PCBs) and polychlorinated dibenzofurans (PCDFs or 'furans') are highly toxic organic compounds that have very low water solubility and bind readily to organic matter. They are resistant to biological and chemical breakdown and tend to bioaccumulate in organisms. They are highly potent toxicants at very low concentrations and can disrupt the development of the endocrine, reproductive, immune and nervous systems of the offspring of fish, birds and mammals when exposed from conception through post-natal or post-hatching stages (Gatehouse 2004). The most toxic of these is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD; CAS no. 1746–01–6) (Grimwood & Dobbs 1995).

The PCDDs, dl-PCBs and PCDFs are among a group of 12 persistent organic pollutants that were identified in the Stockholm Convention Treaty (2001) on Persistent Organic Pollutants as priority substances for elimination or restriction of release worldwide, which entered into force on 17 May 2004 (Gatehouse 2004). However, due to their persistence and formation as unintentional by-products, dioxins are still present in considerable amounts in the environment (Kanan & Samara 2018).

PCDDs, PCDFs and dl-PCBs are formed as by-products of the synthesis or combustion of chlorine-based compounds that include some of the most toxic chemical substrates. Their formation is mostly due to anthropogenic activities, including the burning of fossil fuels, wood and garbage (Kanan & Samara 2018).

Although there is extensive ecotoxicological literature on 2,3,7,8-TCDD, there were limited, albeit sufficient, data that were suitable for derivation of default guideline values (DGVs). Many of the more recent data are related to toxic effects from egg injection, dietary intake or from sediments, with the major focus being on mechanisms, biomarkers, deformities and histopathological effects. There were no toxicity data on local species. A significant characteristic of dioxin toxicity is the delayed response to short-term exposure; numerous fish studies have reported delayed effects over weeks and months following acute exposures of a few minutes (Elonen et al. 1998) to 4 days. Fish are typically highly sensitive to dioxins, whereas invertebrates, plants and amphibians are relatively insensitive to dioxins.

Based on an acceptable quality dataset of 2,3,7,8-TCDD toxicity to 12 freshwater species from five taxonomic groups (eight fish, insect, mollusc, oligochaete and frog), 2,3,7,8-TCDD was significantly more toxic to fish than to non-fish (invertebrates and amphibians), with a parametric two-sample *t* test result indicating the dataset had a bimodal distribution. Therefore, the DGVs were derived using a mix of chronic and acute toxicity data for only the eight fish species. Rainbow trout, *Oncorhynchus mykiss*, was the most sensitive species to 2,3,7,8-TCDD, with a chronic LOEC of 0.038 ng/L (0.000038 µg/L) (Mehrle et al. 1988), although carp and pike had acute LOECs of 0.1 ng/L.

Based on the number and type of data, and a good fit of the species sensitivity distribution model to the data, moderate reliability DGVs for 2,3,7,8-TCDD in freshwater were derived. The DGVs for 99, 95, 90 and 80% species protection are 0.0002 ng/L, 0.005 ng/L, 0.02 ng/L and 0.08 ng/L, respectively. Due to the bioaccumulative nature of dioxins, the 99% species protection level (0.0002 ng/L or 0.2 pg/L) would be recommended for slightly-to-moderately disturbed ecosystems. However, this concentration would be unmeasurable, so any exceedence of the 95% DGV should lead to examination of sediment and tissue residues, as well as other lines of evidence, in a weight of evidence assessment. As the DGVs were derived using only data from fish, which are known to be more sensitive to dioxins than other taxonomic groups, the DGVs may be over-protective in freshwater ecosystems where fish are not present. Due to the strong hydrophobicity of these compounds, guideline values based on tissue residues and sediment quality, although not yet developed for Australia and New Zealand, would ideally be used alongside water quality DGVs in a weight of evidence approach.

Based on existing, albeit limited, dioxin toxicity data for marine species, the freshwater DGVs appear to be conservatively protective of marine species.

Although insufficient data were available to derive separate DGVs for the other PCDDs, PCDFs or dl-PCBs, guidance is given in Section 5 on the use of accepted toxicity equivalence factors (TEFs). The TEFs will assist in understanding additive toxicity of dioxin mixtures and accounting for the relative potency of different dioxins when applying the DGVs.

1 Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs or 'dioxins'), dioxin-like coplanar polychlorinated biphenyls (dl-PCBs) and polychlorinated dibenzofurans (PCDFs or 'furans') are persistent, bioaccumulative and toxic compounds that are produced as by-products of the synthesis or combustion of certain chlorinated organic chemicals, such as some commercial herbicides and (ortho) chlorophenols, and the burning of fossil fuels, wood and garbage (Grimwood & Dobbs 1995, Wenning et al. 2011, Kanan & Samara 2018). The PCDDs, dl-PCBs and PCDFs are among a group of 12 persistent organic pollutants that were identified in the Stockholm Convention Treaty (2001) on Persistent Organic Pollutants as priority substances for elimination or restriction of release worldwide, which entered into force on 17 May 2004 (Gatehouse 2004). However, notwithstanding the Treaty, their persistence and unintentional formation means that dioxins are still present in considerable amounts in the environment (Kanan & Samara 2018).

The PCDDs are a group of chemicals composed of 75 chemically related compounds; in addition, there are 135 PCDFs and 209 dl-PCBs (USEPA 1984, 1993, Rice et al. 2003). However, only seven PCDDs, 10 PCDFs and 12 dl-PCBs are accepted as having dioxin-like structure and toxicity (USEPA 2008). These molecules are planar, chloro-substituted polycyclic aromatic hydrocarbons. The most toxic dioxin is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD; generally referred to herein as TCDD), a symmetrical, almost planar, molecule with formula $C_{12}H_4Cl_4O_2$ and molecular weight of 321.9. Its water solubility is between 0.2 ng/L and 480 ng/L (Fletcher & McKay 1993) and its log K_{ow} is approximately 6.2 (USEPA 1984). The chemical structures of PCDD, TCDD, PCDF, and dl-PCB are shown in **Figure 1**, **Figure 2**, **Figure 3** and **Figure 4**, respectively.

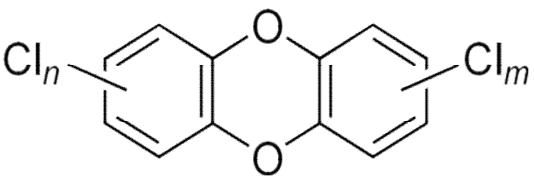


Figure 1 Chemical structure of PCDD

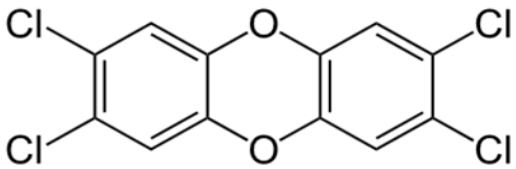


Figure 2 Chemical structure of 2,3,7,8-TCDD

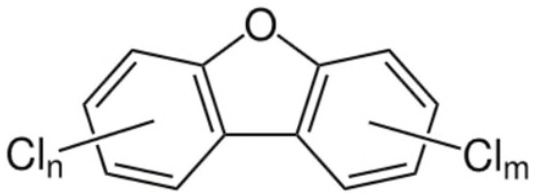


Figure 3 Chemical structure of PCDF

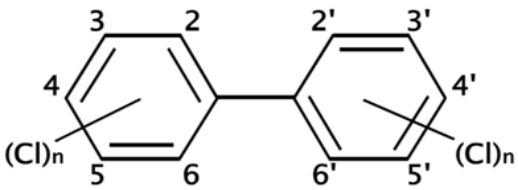


Figure 4 Chemical structure of dl-PCB

Dioxins are highly toxic. The most toxic dioxin and dioxin-like compounds are those 12 compounds that are chlorinated in lateral positions (2, 3, 7 and 8) with four to six 6 chlorine atoms; dioxins can have up to eight chlorine atoms attached. Dioxins are extremely hydrophobic and have very low water solubility (0.0001–1 µg/L) and high log K_{ow} (5.0–10.3). As they are highly hydrophobic compounds, measurement of their concentrations in water is very difficult (USEPA 2008). They partition strongly to organic matter or sediments in aquatic environments, and it is probably more useful to present dioxin guideline values as sediment quality or tissue residue guideline values than

water quality guideline values (CCME 2001a, 2001b). Dioxins are resistant to biodegradation, are persistent in the environment and bioaccumulate in organisms (Grimwood & Dobbs 1995), and are found commonly in sediments and tissues of fish, birds and mammals, even at remote locations.

Concentrations in fauna reflect an animal's position in the food chain, with high trophic level aquatic and terrestrial organisms having the highest levels of dioxins in their bodies relative to other organisms in the same environment (Gatehouse 2004). Concentrations of dioxins and related furans have been found at cleaner sites at between 1 pg/L and 5 pg/L (10^{-12} g/L) and at up to 100 pg/L (0.1 ng/L) at more contaminated sites (Sijm & Opperhuizen 1996).

Dioxins are more readily associated with sediment, and TCDD is usually less than 15% of total dioxins. An Australia-wide survey of dioxins in sediments (Müller et al. 2004) found that the highest concentrations were in estuaries, with a median concentration of 2.3 ng toxic equivalency (TEQ)/kg dry weight (dw), generally higher than those in New Zealand. In contrast, the median concentration in freshwater sediments was an order of magnitude lower, at 0.2 ng TEQ/kg dw, which is similar to levels in marine sediments. The survey noted that the higher median value and most of the high value outliers from estuarine locations were reflective of the placement of most urban/industrial land use in Australia adjacent to estuarine environments. As an example of this, Birch et al. (2007) cited an unpublished consultant document from 1998 that reported a maximum concentration of TCDD on the eastern shore of Homebush Bay, NSW, near historical industrial contamination, of 16.3 µg/kg, while a 2002 document reported a maximum total dioxins plus furans concentration in the estuarine bay sediment of 6 290 µg/kg dw, which is the highest recorded in Australia (Birch et al. 2007). Such high levels resulted in the imposition of a fishing ban in the bay and advisories across Sydney Harbour. PCDD concentrations throughout Port Jackson were strongly correlated with the proximity of sites to Homebush Bay (Roach et al. 2009), and elevated dioxin concentrations in fish in Port Jackson were traced to this source (Manning et al. 2007).

The bioconcentration factor (BCF) for TCDD in whole fish ranged between 7 760 and 28 700 (Mehrle et al. 1988, Fletcher & McKay 1993). As dietary uptake is potentially more significant than uptake through gill membranes, emphasis shifted to measurement of bioaccumulation factors (BAFs), which estimate the net uptake and retention of a chemical through all routes of exposure, uptake, and elimination (USEPA 2008). Fletcher and McKay (1993) reported a BAF of 0.47 for TCDD. USEPA (1984) did not derive a guideline value but considered that water concentrations >0.01 ng/L TCDD could lead to excessive levels of dioxin in fish and shellfish for human consumption, assuming a BCF of >5 000. Biota-sediment accumulation factors (BSAFs) for TCDD were generally below 3 (Niimi 1996). BAFs and BSAFs are obtained from direct measurements in the environment or modelling uptake and elimination rates of each chemical as a result of all routes of exposure (USEPA 2008).

2 Aquatic toxicology

2.1 Overview of dioxin toxicity

There have been a number of reviews on TCDD that have provided useful background information on transport, fate and effects in the environment (USEPA 1984, 1993, Fletcher & McKay 1993, Grimwood & Dobbs 1995, Niimi 1996, Rice et al. 2003, Gatehouse 2004, Müller et al. 2004, USEPA 2008, Wenning et al. 2011). USEPA (2008) provided a summary (their Table 3) of the effects of dioxin-

like chemicals on fish, mammals and birds. Of the 117 supporting references to this table, 22 provided information on effects in fish, and a further three references provided information on aquatic mammals.

The most-studied chemical in the group of PCDDs, PCDFs and co-planar dl-PCBs is TCDD; hence, most of the aquatic toxicity data are for this compound. Demonstrated toxic effects of TCDD in fish, birds and mammals include adverse effects on reproduction, development, and endocrine functions, wasting syndrome, deformities, immunotoxicity, hepatotoxicity, carcinogenicity and mortality at very low concentrations (USEPA 2008). Several PCDDs, PCDFs, and dl-PCBs have been shown to cause toxic responses similar to TCDD, in both laboratory and field situations. They exert such effects through binding to the aryl hydrocarbon receptor (AhR), and they cause a wide spectrum of adverse toxic effects in many vertebrate species (Van den Berg et al. 1998, 2006, Mandal 2005). Although AhR homologs exist in invertebrates, they do not bind with dioxins and dioxin-like compounds; as such, invertebrates are typically less sensitive to dioxin and dioxin-like compounds than vertebrates (Hahn 2002, Hahn et al. 2017)

Fish are generally the most sensitive of purely aquatic species to TCDD; salmonids are the most sensitive, and zebrafish are generally the least sensitive fish species (Walker & Peterson 1994, Henry et al. 1997, Elonen et al. 1998, Tanguay et al. 2003). Acute effects for freshwater fish species exposed to TCDD in water occur at concentrations greater than 1.0 µg/L, but concentrations that cause delayed or chronic toxicity to early life stages of fish following acute exposures are often less than 10 ng/L (USEPA 1984). The more sensitive fish show TCDD effects on mortality, growth and behaviour at between 0.038 ng/L and 40 ng/L (Grimwood & Dobbs 1995), the lowest values being for rainbow trout (*O. mykiss*) larvae (Mehrle et al. 1988). The most toxic furan was TCDF, with effects on *O. mykiss* larvae at 1.79 ng/L (Mehrle et al. 1988). Frogs and toads are considerably less sensitive to TCDD than fish (Jung & Walker 1997, USEPA 2008), while plants and invertebrates have very low sensitivity to TCDD (USEPA 1993, West et al. 1997, Barber et al. 1998, USEPA 2008).

Most of the aquatic toxicity data using waterborne exposure pre-dates the mid-1990s. Since then, the emphasis has shifted to exposure by intraperitoneal injection of eggs (Wannemacher et al. 1992), dietary exposure (West et al. 1997, Tietge et al. 1998, Giesy et al. 2002) or maternal transfer (Johnson et al. 1998, Nichols et al. 1998, Tietge et al. 1998, Heiden et al. 2005, Arnoldsson et al. 2012), usually reporting data as concentrations in eggs regardless of the exposure route (Toomey et al. 2001, Teraoka et al. 2002, 2009). Between 39% and 50% of the TCDD body burden in the female adult fish is maternally transferred to eggs (Wannemacher et al. 1992).

USEPA (2008) suggested that the relative sensitivity of species is better measured on the basis of concentrations of PCDDs, PCDFs, and dl-PCBs in tissues of the species than on a water concentration or administered dose. Jarvinen and Ankley (1999) listed data on tissue residues and associated effects of TCDD for 12 freshwater species and one marine species. Much of the more recent literature on TCDD toxicity reports toxic effects of lethal doses in eggs (pg/g wet weight) or in sublethal biomarker effects or gene expression (Prasch et al. 2003, Bello et al. 2004, Carney et al. 2006, Jönsson et al. 2007). In a few cases, it was possible to interpolate back to water concentrations suitable for the current DGV derivation, but in most cases such studies could not be used for the derivation. Based on concentrations in eggs, Walker et al. (1991, 1992) found toxicity of TCDD to be only slightly higher for exposure by injection than for waterborne exposure.

2.2 Characteristics of dioxin toxicity

Vertebrate embryos are particularly sensitive to TCDD (Grimwood & Dobbs 1995). Dioxins cause developmental toxicity, so exposure to fish embryos, even for very short periods (minutes) (Elonen et al. 1998) causes toxicity to sac fry weeks to months after transfer of the embryos to clean water. The toxic responses following exposure to dioxins, furans and dl-PCBs are similar irrespective of the exposure duration or route, although early life stage fish are significantly more sensitive to the toxic effects of dioxins than adult or juvenile fish (Gatehouse 2004). The toxic effects to early life stages include delayed mortality, decreased food consumption and body weight and histological lesions, yolk-sac oedema, head and spinal deformities, heart deformities, reduced blood flow, haemorrhages and apoptosis (cell death) (Helder & Seinen 1985, Sijm & Opperhuizen 1996, Dong et al. 2002, Teraoka et al. 2002, Antkiewicz et al. 2005). Responses in older fish include lesions, fin necrosis, histopathological changes and death (USEPA 1993). Increased expression of the enzyme cytochrome P450A1 (CYP1A) and immune suppression appear to occur in fish at tissue concentrations that ultimately cause lethal effects (Teraoka et al. 2003). TCDD induction of CYP1A is often used as a biomarker for exposure to dioxin-like compounds (USEPA 1993, Cantrell et al. 1998, Toomey et al. 2001). Much of the recent literature has focused on toxic mechanisms and linking these various effects (Teraoka et al. 2009, Parhizgari & Li 2014).

Higher doses, as well as longer exposure times with lower doses, resulted in increased severity of effects, including mortality (Gatehouse 2004). TCDD displayed steep dose–response curves, where there is only a small difference between the levels causing no effects and those causing lethal effects. Longer-term exposure to TCDD resulted in higher mortalities at lower concentrations. For example, exposure of the swim-up fry of rainbow trout to 0.176 ng/L for 28 days resulted in 50% mortality (and 95% after a further 28-d depuration) (Mehrle et al, 1988), compared to lower mortality and reduced growth when sac fry were exposed to 1 ng/L for 96 h (USEPA 1993).

The observed effects from exposure to TCDD (and other dioxins that bioaccumulate) occur as a function of body burden, rather than the concentration or dose administered. Dioxins accumulate in tissue until a threshold concentration for a given effect is reached, which explains, in part, the delayed effects. The increased severity of effects at higher doses or longer exposure periods occurs because residues in organisms reach a threshold level more rapidly with increasing exposure concentration and time (USEPA 1993). Mortality to older fish would be expected when the fish accumulate between 1 000 pg/g and 15 000 pg/g (USEPA 1993). Early life stage mortality from TCDD has been found at residue levels between 65 pg/g and 400 pg/g for eggs of two species of freshwater trout (Sijm & Opperhuizen 1996). The lowest LOEC for inhibition of growth from eggs of *O. mykiss* was less than 0.1 ng/L, corresponding to a body burden of 0.3 pg/g of egg.

2.3 Delayed toxicity

Fish exposed to TCDD can appear healthy during the exposure period, but may begin to show characteristic toxicity effects after significant depuration periods in clean water, with delayed mortality occurring from weeks to several months after cessation of initial exposure (Gatehouse 2004). For example, in one study, rainbow trout fingerlings exposed to 107 ng TCDD/L for 6 h and then placed in clean water initially appeared healthy, but exhibited delayed mortality up to 139 days

after exposure (Branson 1985). Similar effects are seen when fertilised fish eggs are exposed to TCDD for short periods (USEPA 1993, Elonen et al. 1998).

The toxic effects often manifest in the developing fish soon after hatching. For example, Helder (1981) exposed trout eggs to concentrations of 0.10 ng TCDD/L for 96 h but, after hatching, the fry displayed delayed development and reduced growth for up to 160 days after exposure ceased. Many other species showed similar effects after short-term exposure (Norris & Miller 1974, Miller et al. 1979, Adams et al. 1986, Walker & Peterson 1994, Spitzbergen et al. 1991, Walker et al. 1991, Olivieri & Cooper 1997). Even exposures less than 1 h caused significant mortality and other effects in fish at 32 days after exposure (Elonen et al. 1998). For the purposes of the current DGV derivation, the delayed effects associated with such short-term exposures were considered to represent 'acute' toxicity because the exposures were acute.

2.4 Field exposure and effects

The predominant route of dioxin exposure to pelagic fish in the wild is expected to be through the diet, with direct waterborne exposure through the gills likely to be a minor route of uptake owing to the insolubility of dioxins in water. Dietary exposure data indicate that the toxic responses from exposure to TCDD in food are similar to those observed for aquatic exposure.

Residues of TCDD and related chemicals in fish have not been reported to approach lethal levels in adults, but USEPA (1993) has suggested that population declines through adult mortality may have occurred through undocumented exposures in small localised waterways directly contaminated by nearby sources of TCDD and/or mixtures of related chemicals. The strongest evidence for a link between TCDD contamination and declines in fish population comes from parts of the Great Lakes of North America (USEPA 1993, 2002, Cook et al. 2003). Early life stage lethality, as described above, is more likely to cause fish population declines (Walker et al. 1991).

In an Australian field study, European carp (*Cyprinus carpio*) exposed to highly treated pulp mill effluent in Lake Coleman, Victoria, showed significantly elevated hepatic biomarker levels relative to fish in nearby unexposed water bodies, and this was correlated to low levels of PCDDs/PCDFs measured in carp muscle, which decreased with increasing distance from effluent point sources (Ahokas et al. 1994). Sezmis et al. (2014) and Roach et al. (2007) found that lipid content, range of species, diet and distance from the most contaminated area at Homebush Bay all affected the concentrations of PCDDs, PCDFs and dl-PCBs in fish in Sydney Harbour. Gatehouse (2004) estimated a 'low risk to fish from exposure to ambient dioxin levels found in the Australian aquatic environment', and this is also likely to be the case in New Zealand (Müller et al. 2004).

2.5 Toxicity to freshwater biota

2.5.1 Fish

Given the extreme sensitivity of fish, there is a vast literature on toxicity of TCDD and related compounds to this taxonomic group. However, only a relatively small dataset is suitable for water quality guideline value derivation, mostly dating from the early 1970s to mid-1990s (see Section 4.1). This is because publications have focused on exposure routes through diet, egg injection or sediment since then, and even when waterborne exposures have been used, the toxicity has often been expressed in terms of TCDD concentration in eggs or fry (e.g. as pg/g) rather than the waterborne

concentration. There has also been significant research effort into important issues such as understanding mechanisms of PCDD toxicity (often using model species such as zebrafish (Hill et al. 2005)), developing biomarkers and genetic tools, understanding the significance of tissue body burdens, bioaccumulation (West et al. 1997, Heiden et al. 2005) and food web models (Loonen et al. 1996, Wintermeyer & Cooper 2007), field exposures (Roach et al. 2009, Segstro et al. 1995), and determining toxic equivalency (Hornung et al. 1996, Safe 1998, Bhavsar et al. 2008). Thus, many of the data were generated for purposes other than developing a concentration–response relationship in water.

A peculiarity of dioxin toxicity is the delayed toxicity observed in fish (see Section 2.2 and Section 2.3). In studies assessing this, toxicity frequently occurred shortly after fish hatched from previously exposed eggs (Grimwood & Dobbs 1995, Spitzbergen et al. 1991), and yolk-sac fry were often the most sensitive life stage. Many of these data are reported as acute NOECs or LOECs, and sometimes with only one or no more than three concentrations. Juvenile coho salmon *Oncorhynchus kisutch* were very sensitive to TCDD when exposed for 4 days and observed in clean water for 60 to 114 days (Miller et al. 1973, Miller et al. 1979); a 12% mortality was reported at 0.056 ng/L after 60 days, and other mortality NOECs were reported at 0.56 ng/L and 1.05 ng/L (growth NOECs were slightly higher). Northern pike *Esox lucius* sac fry were also very sensitive when embryos were exposed to TCDD for 4 days and observed for up to 21 days (Helder 1980); various adverse effects occurred between 0.1 ng/L and 1 ng/L, with 100% mortality at 10 ng/L. Zebrafish and medaka were among the less sensitive fish (Wisk & Cooper 1990, Henry et al. 1997).

Elonen et al. (1998) undertook some direct species comparisons for seven freshwater fish species based on TCDD concentration in eggs that caused significant decreases in survival or growth of juveniles. NOECs were between 175 pg/g and 1 190 pg/g, and LOECs were between 270 pg/g and 2 000 pg/g. Lake herring *Coregonus artedii* and fathead minnow *Pimephales promelas* were most sensitive (the zebrafish *Danio rerio* was the least), but even these were at least eight times less sensitive than lake trout *Salvelinus namaycush* (40 ng/kg) (Spitzbergen et al. 1991). Although it was possible to deduce equivalent nominal water concentrations for NOECs and LOECs, the data could not be used for DGV derivation because, although they reported graded concentrations of TCDD in eggs, they achieved this by varying the time of exposure, rather than water concentrations, which were adjusted for different species according to egg size.

Chronic toxicity data that linked water exposure to toxic effects were available for four fish species: carp *C. carpio*, rainbow trout *O. mykiss*, lake trout *S. namaycush*, and medaka *Oryzias latipes*. The most sensitive fish was rainbow trout *O. mykiss* swim-up fry, with chronic LOEC for growth inhibition of 0.038 ng/L after 28-d exposure and mortality after a further 28-d depuration period (Mehrle et al. 1988).

2.5.2 Other biota

Freshwater plants (algae and macrophytes) and invertebrates appear to be relatively insensitive to dioxins compared to fish. Several papers describing model ecosystem effects, although failing quality checks for the current DGV derivation, provided useful information on the lack of TCDD toxicity to non-fish species in contrast to fish toxicity. The alga *Oedogonium cardiacum*, the duckweed *Lemna minor* and the snail *Physa* sp. were not affected by 30-d exposures to between 710 ng/L and 1 300 ng/L (Isensee 1978, Isensee & Jones 1978). A model ecosystem exposed to 2.4 to 4.2 ng/L

TCDD resulted in 100% mortality of mosquitofish and channel catfish within 20 days (Yockim et al. 1978), but showed no effects on *O. cardiacum* or the snail *Helosoma* sp., despite high accumulation of TCDD. Although these data were not used for the current DGV derivation, they provide the only available information on the lack of aquatic plant toxicity.

Acceptable quality chronic toxicity data for invertebrates were available for three species from different taxonomic groups (Miller et al. 1973). Two of these, the mosquito larva *Aedes aegypti* and the snail *Physa* sp., showed no significant mortality at the only concentration tested (200 ng/L), while *Physa* sp. and the oligochaete *Paranais* sp. showed some effect on reproduction at this concentration. Acute exposure of *Daphnia magna* up to 1 030 ng/L TCDD for 2 days followed by a 7-d depuration period did not elicit any toxic effects on three different life stages (Adams et al. 1986).

Amphibians are generally less sensitive than fish, based on data from four species (McKinney et al. 1985, Jung & Walker 1997), although effects on *Rana pipiens* were noted at 3 ng/L after 1-d exposure and 28-d depuration (Jung & Walker 1997). Jung and Walker (1997) reported no effects on eggs of a frog species and toad species at the highest concentrations tested (100 ng/L and 30 ng/L, respectively (acute NOEC; 1-d exposure and 48-d to 67-d depuration)). However, Jung and Walker (1997) found a significant 35% increase in mortality to *Rana pipiens* at 3 µg/L (a single test concentration) after 1-d exposure and 28-d depuration (acute LOEC), relative to controls. McKinney et al. (1985) reported significant growth inhibition to *Xenopus laevis* exposed for 30 days to the lowest concentration tested (100 ng/L TCDD) followed by 21-d depuration (chronic LOEC).

2.6 Toxicity to marine biota

Although the current DGVs relate to freshwater toxicity, a brief summary of toxicity to marine biota is provided here. There are limited data on TCDD toxicity to marine biota, but they seem to be generally less sensitive to the toxic effects of TCDD than most freshwater species (Prince & Cooper 1995, Gatehouse 2004, Yamauchi et al. 2006, Wintermeyer & Cooper 2007, Cooper & Wintermeyer 2009, Anselmo et al. 2011). Marine fish that have been previously exposed to TCDD are less sensitive to TCDD exposure than unadapted fish (Prince & Cooper 1995). Additionally, and in line with results in freshwater, marine invertebrates were significantly less sensitive than fish (Barber et al. 1998). The available data for marine water would suggest that a freshwater DGV would be conservatively protective of marine and estuarine species, a conclusion also reached by Gatehouse (2004).

3 Factors affecting toxicity

It is expected that chemicals such as PCDDs with log K_{ow} (octanol-water partition coefficient) ≥6 would be strongly bound to organic and suspended matter and may be mostly unavailable, except by dietary consumption (Fletcher & McKay 1993). They would also strongly bind to sediment but, given their high toxicity, even very low concentrations in equilibrium with contaminated sediment could be toxic in some locations. Tissue residue guideline values (CCME 2001a) or sediment quality guideline values (CCME 2001b) may, in time, be more appropriate for dioxins and related compounds.

4 Default guideline value derivation

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

4.1 Toxicity data used in derivation

A search of publicly available data yielded over 110 papers on the toxic effects of TCDD in the aquatic environment. However, as noted in Section 2.5, many of the data from these were unsuitable for water quality DGV derivation because they dealt with other issues (e.g. mechanisms of toxicity, biomarkers and genetic tools, bioaccumulation, food web models, field exposures, determining toxic equivalency), exposure routes were through diet, egg injection or sediment, and even when waterborne exposures had been used, the toxicity was often expressed in terms of TCDD concentration in eggs or fry (pg/g) and could not readily be converted to a waterborne concentration.

The toxicity data used to derive the DGVs included chronic data as well as acute data converted to chronic-equivalent data. Many of the fish tests were short-term exposures (sometimes as short as 15 minutes (Elonen et al. 1998)) but had long observation periods in clean water after exposure, to assess delayed toxicity (USEPA 1993). Given that toxic effects increase with increasing exposure time, as well as increasing concentration (Gatehouse 2004), these studies were considered as 'acute' exposures for the purpose of DGV derivation. A peculiarity of the dioxin literature was the frequency of acute NOEC and LOEC data, due largely to the often observed delayed toxic effects following short-term exposure and the fact that experiments were often not designed for the primary purpose of obtaining an aquatic concentration–response relationship. However, Warne et al. (2018) does not permit the use of acute NOEC or LOEC data for guideline value derivation; hence, such data were not used for the current derivation. Instead, acute LC50 values, divided by the default factor of 10 to derive a chronic 'no or low effect' equivalent, were selected from acute toxicity studies, where possible. The permissible dataset comprised toxicity data for 12 species from 5 taxonomic groups.

There were few acceptable studies on invertebrates. Miller et al. (1973) assessed the chronic toxicity of a single concentration (200 ng/L) of TCDD on three invertebrate species (A. *aegypti*, *Physa* sp., *Paranais* sp.). These were deemed to represent NOECs or LOECs depending on whether or not a significant effect at this concentration was observed. The invertebrate and plant data from microcosms (Isensee 1978, Isensee & Jones 1978, Yockim et al. 1978) could not be used.

Although there were data on four amphibian species (McKinney et al. 1985, Jung & Walker 1997), three comprised acute NOEC and LOEC data that could not be used for DGV derivation. However, McKinney et al. (1985) reported a significant chronic growth inhibition LOEC of 100 ng/L TCDD to *Xenopus laevis* exposed for 30 days followed by 21-d depuration.

Most of the fish toxicity data were on embryos. The comparative toxicity data for seven fish species by Elonen et al. (1998) could not be used (see Section 2.5), which eliminated three species, *Catastomus commersoni*, *Coregonus artedi*, *Ictalurus punctatus*, and reduced the amount of data available for four others.

Cook et al. (1991) provided the only waterborne exposure data on carp *C. carpio* (adults) in a 71-d chronic exposure to a single concentration of 0.06 ng/L TCDD in an experiment designed to determine bioconcentration. The authors reported that 11 of 90 (i.e. 12%) exposed fish died just before or during the depuration period compared to none in the control group, but that the number of deaths would likely have been greater had fish not been sub-sampled for residue analysis throughout the experiment. Additionally, a wide range of lesions and deformities were observed in surviving fish that had been exposed to TCDD. Hence, the single exposure concentration was considered equivalent to a LOEC. Despite the experimental limitations, the toxicity value was included in the final dataset, largely because it demonstrated chronic lethal and sub-lethal effects on a fish species at the more sensitive end of the fish species sensitivity distribution.

Although the zebrafish is used as a model organism for determining mechanisms of TCDD toxicity, the only data on this species suitable for DGV derivation were by Henry et al. (1997), who exposed embryos for 1 hour and observed effects for 10 days; they found no effect at the lowest concentration (acute NOEC) of 35 ng/L (longer exposures may have given lower values) and, therefore, the data were not used.

Helder (1980) exposed *Esox lucius* embryos to TCDD for 4 days and observed effects for up to 21 days. Various adverse effects occurred between 0.1 ng/L and 1 ng/L, with 49% mortality at 1 ng/L and 100% mortality at 10 ng/L after 21 days. The 49% mortality rate at 1 ng/L was deemed to be sufficiently representative of an LC50 (given typical errors in LC50 estimation) and, thus, was used for the derivation after being converted to a chronic 'no or low effect' equivalent of 0.1 ng/L by applying the default acute-to-chronic ratio (ACR) of 10.

Miller et al. (1973, 1979) exposed juvenile *O. kisutch* to TCDD for 4 days and observed effects for 60 to 114 days in three separate experiments. A mortality rate of 12% was reported at 0.056 ng/L after 60 days post-exposure (Miller et al. 1973). However, mortality at 0.56 ng/L for the same period was very similar (Miller et al. 1973), indicating the lack of a concentration–response relationship in this concentration range. The next highest concentration of 5.6 ng/L resulted in 55% mortality after 60 days post-exposure. This value was deemed to be sufficiently representative of an LC50 (given typical errors in LC50 estimation) and, thus, was used for the derivation after being converted to a chronic 'no or low effect' equivalent of 0.56 ng/L by applying the default ACR of 10.

Five papers on TCDD toxicity to eggs, yolk-sac fry and larvae of *O. mykiss* provided mortality and growth data (Helder 1981, Helder & Seinen 1985, Mehrle et al. 1988, Bol et al. 1989, Walker et al. 1992). Most were on 2-d to 4-d exposures, followed by depuration periods of between 16 days and 160 days. Mehrle et al. (1988) reported a chronic growth inhibition LOEC to swim-up fry at the lowest concentration tested, 0.038 ng/L, after 28-d exposure and a mortality LOEC also at 0.038 ng/L after a further 28-d depuration period. This value was used after being converted to a chronic 'no or low effect' equivalent of 0.015 ng/L by applying the default conversion factor of 2.5.

Wisk and Cooper (1990) exposed embryos of *O. latipes* to TCDD for 11 days and determined an LC50 of 9 to 13 ng/L; other effects, such as life-threatening lesions and prevention of hatching, had EC50 values of 14 to 17 ng/L. Kim and Cooper (1999) determined a NOEC and LOEC of 2.5 ng/L and 5.6 ng/L, respectively, for mortality to embryos of this species, with the NOEC being used for the DGV derivation.

Cook et al. (1991) exposed fathead minnow *P. promelas* juveniles for 71 days, followed by 61-d depuration to assess bioconcentration, but there was insufficient information on mortality and other endpoints to derive a toxicity value for use; the authors noted just 'a variety of toxic signs' at 0.049 to 0.067 ng/L. Adams et al. (1986) exposed *P. promelas* to TCDD for 4-d (acute), followed by 60-d depuration, and noted significant mortality at 7.1 ng/L but not at 0.7 ng/L (NOEC). After a 28-d continuous exposure (chronic), there was 53% mortality at 1.7 ng/L. Olivieri and Cooper (1997) reported a chronic LC50 of approximately 7.1 ng/L (based on conversion from a tissue dose) for *P. promelas* embryos exposed for 7 days. Zero mortality was observed up to and including a concentration of 0.59 ng/L, with 16% mortality reported at 1.2 ng/L. These could be reliably assumed to represent a chronic NOEC and LOEC, respectively. When 1-month-old larvae were exposed for 1 day, followed by 64-d depuration, the LOEC for growth was 40 ng/L and the NOEC was 3.8 ng/L (Olivieri & Cooper 1997). Overall, the chronic mortality NOEC of 0.59 ng/L for embryos was used, although the other data support this value.

Only acute data were available for *Poecilia reticulata*, with 1 to 5-d exposures followed by a 30 to 42-d depuration. The fin necrosis endpoint (Miller et al. 1979) was expressed as fish body weight (NOEC of 0.08 ng/g) and could not be converted to a waterborne concentration, while other references (Miller et al. 1973, Norris & Miller 1974) showed effects at much higher concentrations (100 ng/L), which were the lowest concentrations tested. Hence, no data on this species could be used.

Walker and Peterson (1994) exposed embryos of brook trout *Salvelinus fontinalis* to TCDD for 2 days followed by an 80-d depuration period, reporting an acute LC50 of 200 pg/g egg, which translated to a waterborne concentration of 9 ng/L (as estimated using waterborne concentration versus egg concentration data). This value was used after being converted to a chronic 'no or low effect' equivalent of 0.9 ng/L by applying the default acute-to-chronic ratio (ACR) of 10.

For lake trout, *S. namaycush*, there were two key studies that produced toxicity data from water exposures of eggs to TCDD (Walker et al. 1991, 1996). Walker et al. (1991) reported an acute LD50 of 65 pg/g (47 pg/g for injection) for sac fry following a 2-d waterborne exposure of eggs and a subsequent 119-d monitoring period, which equated to a waterborne concentration of approximately 21 ng/L (as estimated using waterborne concentration versus egg concentration data). Walker et al. (1996) reported an approximate 50% effect on sac fry survival following a 2-d waterborne exposure of eggs to 7.5 ng/L TCDD and a subsequent 90-d monitoring period, which could be reliably assumed to represent an acute LC50. The two acute LC50 values from Walker et al. (1991, 1996) were used for the derivation after conversion to chronic NOECs, and with a resulting geometric mean of 1.25 ng/L.

It is generally accepted that vertebrates are more sensitive to dioxin and dioxin-like compounds than invertebrates, and that this is likely due to differing mechanisms of toxicity between the two groups (Hahn 2002, Van den Berg et al. 2006, Hahn et al. 2017) (see Section 2.1). To assess this, a modality assessment was undertaken on the dioxin dataset (i.e. 12 species from five taxonomic groups) as per the process described by Warne et al. (2018) (see Appendix B: Modality assessment for dioxin). The assessment supported the existing knowledge on the relative toxicity of dioxin; therefore, as recommended by Warne et al. (2018), only the toxicity data for the most sensitive group of organisms, in this case fish, were used to calculate the DGVs. In such cases, the minimum data requirement for the number of species applies (i.e. five species), but the usual criterion of requiring

data for organisms that belong to at least four taxonomic groups can be relaxed. This can only be done if all the data of the same type as those used to derive the DGVs (in this case, chronic and chronic estimated using converted acute data) meet both requirements (Warne et al. 2018). A summary of the toxicity data and conversions for the eight species of fish used to derive the DGVs is provided in Table 1. Further details on all the data suitable for DGV derivation and test conditions are given in Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values. Details of the data quality assessment and the data that passed the quality assessment are provided as supporting information.

Table 1 Summary of single toxicity values, all species used to derive default guideline values for 2,3,7,8-TCDD in freshwater

Taxonomic group	Species	Life stage	Duration (d)	Type (acute/ chronic)	Toxicity measure (test endpoint) [a]	Final toxicity value (ng/L) [b]
Chordata	*Cyprinus carpio*	Adult	71 + 61	Chronic	LOEC (mortality)	0.024 [c]
	Esox lucius	Egg	4 + 11	Acute	LC49 (mortality)	0.1 [d]
	Oncorhynchus kisutch	Juvenile	4 + 60	Acute	LC55 (mortality)	0.56 [d]
	Oncorhynchus mykiss	Fry	28 + 28	Chronic	LOEC (growth)	0.015 [c]
	Oryzias latipes	Embryo	11	Chronic	NOEC (mortality)	2.5
	Pimephales promelas	Embryo	7	Chronic	NOEC (mortality)	0.59
	Salvelinus fontinalis	Egg	2 + 80	Acute	LC50 (mortality)	0.9 [d]
	Salvelinus namaycush	Egg	2 + 90–119	Acute	LC50 (mortality)	1.25 [d, e]

a The measure of toxicity being estimated/determined: LCx—concentration causing x% lethality (where x = 49, 50 or 55%); LOEC—lowest observed effect concentration; NOEC—no observed effect concentration.
b This is the final value after conversion to a chronic zero or negligible effect concentration (calculating geometric mean where appropriate). Note that values are in ng/L (nanograms/litre)—to convert to µg/L, divide by 1 000 (i.e. 1 ng/L = 0.001 µg/L).
c Toxicity value after conversion from chronic LOEC by application of factor of 2.5.
d Toxicity value after conversion from acute LC50 by application of factor of 10 (default ACR).
e The geometric mean of two acute LC50 values (21 ng/L and 7.5 ng/L after application of ACR).

4.2 Species sensitivity distribution

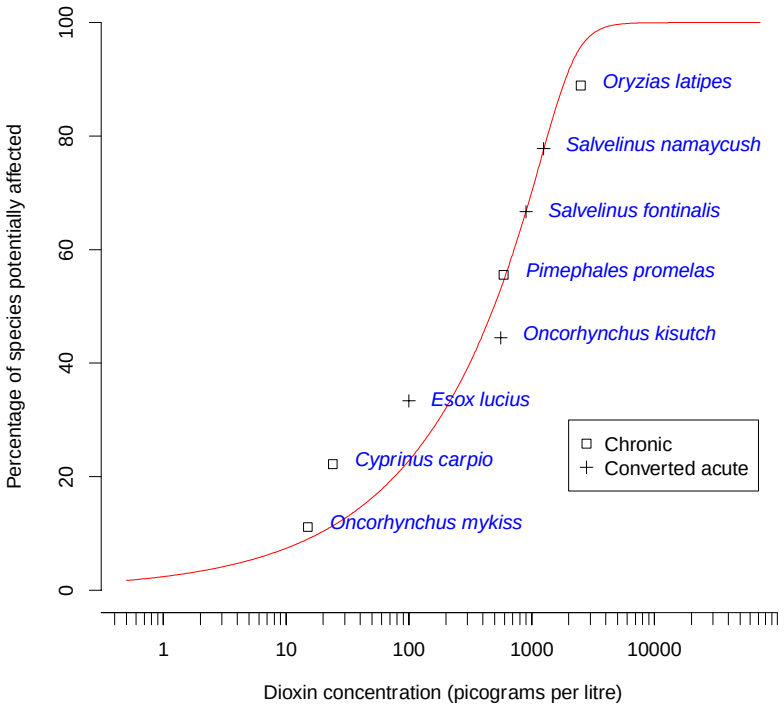
The cumulative frequency (species sensitivity) distribution (SSD) of the eight freshwater chronic and acute (converted to chronic) toxicity data for 2,3,7,8-TCDD reported in Table 1 is shown in Figure 5. The SSD was plotted using the Burrlioz 2.0 software. The model was judged to provide a good fit to the data.

4.3 Default guideline values

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

The DGVs for 99, 95, 90 and 80% species protection are shown in Table 2. Because dioxins bioaccumulate, the 99% species protection DGV would normally be recommended for slightly-to-moderately disturbed systems, but this value is below current analytical detection limits. ANZG

(2018; see Accounting for local conditions) provides guidance on what to do in the event guideline values are below analytical detection limits. It is important to highlight that, due to the DGVs being derived using only data from fish, which are known to be highly sensitive to dioxins relative to most other freshwater biota, the DGVs may be over-protective in freshwater ecosystems where fish are not present. Assessment of the risks of dioxins to aquatic ecosystems should always adopt a weight of evidence approach that, wherever possible, also considers sediment and tissue concentrations.



Note: dioxin concentrations are in picograms per litre (pg/L).

Figure 5 Species sensitivity distribution, 2,3,7,8-TCDD in freshwater

In the absence of marine DGVs for dioxins, the limited available toxicity data for marine and estuarine species would suggest that the freshwater DGVs presented here would be conservatively protective of marine and estuarine species.

Although insufficient data were available to derive separate DGVs for the other PCDDs, PCDFs or dl-PCBs, guidance is provide in Section 5 on the use of accepted toxicity equivalence factors (TEFs) that will assist in determining their toxicity, understanding additive toxicity of mixtures of these compounds and accounting for the relative potency of different dioxins when applying the DGVs.

Table 2 Toxicant default guideline values, 2,3,7,8-TCDD in freshwater, moderate reliability

Level of species protection (%)	DGV for 2,3,7,8-TCDD in freshwater (ng/L) [a]
99	0.0002
95	0.005
90	0.02
80	0.08

a The DGVs were derived using the Burrlioz 2.0 software and are reported to two significant figures.

4.4 Reliability classification

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

- Sample size—8 (good)
- Type of toxicity data—chronic data and acute data converted to chronic equivalent data
- SSD model fit—good (Burr Type III model).

5 Toxicity equivalence factors

PCDDs, PCDFs, and dl-PCBs in the environment are generally found as complex mixtures, necessitating a means of quantifying their combined effects to determine ecological risk (USEPA 2008). In human toxicology, the toxic equivalency (TEQ) method has been used for equating the toxicities of chemicals of similar nature and mode of action when these chemicals are together in a mixture. For instance, the potency of each of the relevant PCDDs, PCDFs, and dl-PCBs is compared to the potency of TCDD, which is the most toxic dioxin. The potency of TCDD is allotted a TEQ of 1. Each of the other relevant compounds is then allocated a fractional value for the ratio of their potency compared to the potency of TCDD. The concentration of the compounds can then be measured, and using the TEQ, a concentration equivalent to TCDD can be calculated.

Toxicity equivalence factors (TEFs) are more established for human toxicology than for fish (Van den Berg et al. 1998, Van den Berg et al. 2006). Given the insensitivity of invertebrates, the TEQ methodology is generally not applicable to invertebrates. However, invertebrates may be vulnerable to some of these chemicals via other toxicological effects. For instance, dl-PCBs measured as aroclors have been shown to be chronically toxic to cladocerans at low µg/L levels (USEPA 2008). USEPA (2008) defines TEF as 'An estimate of the potency, relative to TCDD, of an individual PCDD, PCDF or polychlorinated biphenyl congener, determined using careful scientific judgment after considering all available relative potency data'. This is distinguished from the Relative Potency Factor (RPF), which is an 'estimate based on one or more studies of the potency, relative to TCDD, of an individual chemical to cause AhR mediated toxicity or biological effects, determined using careful scientific judgment after considering all available relative potency data' including multiple endpoints, species, and/or *in vitro* or *in vivo* studies (USEPA 2008).

The TEQ method offers a means to derive a single estimate of exposure concentration of various concentrations of multiple chemicals found in environmental mixtures, which can then be compared with the relevant DGV. This approach has several advantages compared with alternative methods for estimating risks of mixtures of these chemicals. There is a growing body of evidence that the use of congener-specific analyses decreases the overall uncertainty associated with assessing the risks posed by mixtures of PCDDs, PCDFs, and dl-PCBs (USEPA 2008). The TEF approach relies on the constituent chemicals having the same mode of action and that their toxicity is dose-additive. PCDDs, PCDFs and dl-PCBs are considered to have the same mode of action (they bind to the AhR as an initial step in toxic effects), and their toxicity seems to be additive (USEPA 1993, Van den Berg et al. 1998, 2006, USEPA 2008).

The TEQ method takes into account the possible effects of the suite of dioxin-like chemicals found in complex environmental mixtures; hence, it is less likely to underestimate risk than methods based on TCDD (USEPA 2008), entails less uncertainty, and is less likely to underestimate risks than methods based on single chemicals. It provides a means for accounting for the variable potency of the different dioxin-like chemicals. USEPA (1993) considered that the uncertainties associated with using the method were not higher than other sources of uncertainty within the ecological risk assessment process.

International TEFs have been established for mammals, birds, and fish by WHO working groups (Van den Berg et al. 1998, 2006), and they represent reasonable values for estimating the TEQ. Even with the inherent uncertainties, the TEQ method provides a reasonable, scientifically justifiable, and widely accepted method to estimate the relative potency of dioxin-like chemicals (USEPA 2008). Nonetheless, the method should only be applied in a manner consistent with its underlying assumptions; that is, it should only be used for appropriate chemicals with similar modes of action. Other researchers have developed TEFs for PCDDs, PCDFs and dl-PCBs for specific species or situations (Walker & Peterson 1991, Newstead et al. 1995, Parrott et al. 1995, Zabel et al. 1995, Berntssen et al. 2007).

The WHO-recommended fish TEFs from 1998 (Van den Berg et al. 1998) (Table 3) were adopted by USEPA (2008), and they still represent the most accepted available TEFs for fish. The TEFs for fish are preferentially based on the work of Walker and Peterson (1991) and Zabel et al. (1995), who developed TEFs based on dioxin tissue concentrations and mortality data (i.e. an ecologically relevant endpoint) for early life stage fish or, where mortality-related TEF data were not available, based on other endpoints, such as enzyme induction or quantitative structure activity relationships. They are usually consistent with relative toxicity from either exposure by injection or water (Walker et al. 1992) and, hence, are considered to give a reasonable estimate of mixture toxicity in water. TEFs based on measurements or estimates of PCDD, PCDF, and dl-PCB concentrations in tissues are considered to be the most accurate for assessment of effects in fish and birds.

When there is a mixture of dioxin-like chemicals, the total TEQ to TCDD (TEQ_{total}) can be calculated by summing the multiplication of the concentration of each compound with its respective TEF, as in the equation (Bhavsar et al. 2008):

$$TEQ_{total} = \sum(C\mathit{dioxin}_{d} * TEF\mathit{dioxin}_{d}) + \sum(C\mathit{furan}_{f} * TEQ\mathit{furan}_{f} + \sum(C\mathit{dl\text{-}PCB}_{p} * TEQ\mathit{dl\text{-}PCB}_{p})$$

where C = concentration, and d, f and p = congeners of dioxin, furan and dl-PCBs respectively.

If the TEQ*total* is >DGV for TCDD, the DGV is exceeded and the normal decision scheme can be followed, although weight of evidence from sediment and organism tissue concentrations would be an important component of any further work.

Van den Berg et al. (1998) and USEPA (2008; section 3.4.3) have summarised uncertainties inherent to the TEQ method and the uncertainties associated with applications in ecological risk assessment. It is important to note that risk assessment based on the TEQ method for these very hydrophobic and bioaccumulative compounds in water is the very first step, if indeed it is relevant at all. Measurement of residues in sediment or in fish against a sediment guideline or tissue residue guideline may be more useful. While the TEQ method can be used for evaluating the risk from concentrations of dioxin-like chemicals in fish tissue, there are a number of critical steps to undertake before a risk estimate can be made using tissue residues. These include application of species-specific BAFs or

BSAFs. USEPA (2008; section 3.3) details the approach for evaluating risk using the TEQ approach for residues in fish tissues or eggs (available from the National Service Center for Environmental Publications).

It is possible that TEFs can also be developed for polybrominated diphenyl ethers and other brominated aromatics that may act through the AhR. Hornung et al. (1996) have proposed TEFs for such compounds, which could be used where mixtures of these compounds occur with PCDDs, PCDFs and/or dl-PCBs.

Table 3 Toxicity equivalence factors used to extrapolate toxicity of 2,3,7,8-TCDD to other dioxins, furans and dl-PCBs

CAS no.	Compound	Toxicity equivalence factor [a]
Polychlorinated dibenzo-*p*-dioxins (PCDDs)		
01746–01–6	2,3,7,8-TCDD	1
40321–76–4	1,2,3,7,8-PeCDD	1
39227–28–6	1,2,3,4,7,8-HxCDD	0.5
57653–85–7	1,2,3,6,7,8-HxCDD	0.01
19408–74–3	1,2,3,7,8,9-HxCDD	0.01
35822–46–9	1,2,3,4,6,7,8-HpCDD	0.001
03268–87–9	OCDD	<0.0001
Polychlorinated dibenzofurans (PCDFs)		
51207–31–9	2,3,7,8-TCDF	0.05
57117–41–6	1,2,3,7,8-PeCDF	0.05
57117–31–4	2,3,4,7,8-PeCDF	0.5
70648–26–9	1,2,3,4,7,8-HxCDF	0.1
57117–44–9	1,2,3,6,7,8-HxCDF	0.1
72918–21–9	1,2,3,7,8,9-HxCDF	0.1
60851–34–5	2,3,4,6,7,8-HxCDF	0.1
67562–39–4	1,2,3,4,6,7,8-HpCDF	0.01
55673–89–7	1,2,3,4,7,8,9-HpCDF	0.01
39001–02–0	OCDF	<0.0001
Polychlorinated biphenyls (dl-PCBs)		
32598-13-3	3,3’,4,4’-TCB (77)	0.0001
70362-50-4	3,4,4’,5-TCB (81)	0.0005
57465-28-8	3,3’,4,4’,5-PeCB (126)	0.005
32774-16-6	3,3’,4,4’,5,5’-HxCB (169)	0.00005
32598-14-4	2,3,3’,4,4’-PeCB (105)	<0.000005
74472-37-0	2,3,4,4’,5-PeCB (114)	<0.000005
31508-00-6	2,3’,4,4’,5-PeCB (118)	<0.000005
65510-44-3	2’,3,4,4’,5-PeCB (123)	<0.000005
38380-08-4	2,3,3’,4,4’,5-HxCB (156)	<0.000005

CAS no.	Compound	Toxicity equivalence factor [a]
69782-90-7	2,3,3',4,4',5'-HxCB (157)	<0.000005
52663-72-6	2,3',4,4',5,5'-HxCB (167)	<0.000005
39635-31-9	2,3,3',4,4',5,5'-HpCB (189)	<0.000005

a USEPA (2008)—TEF levels specifically for fish adopted from WHO fish TEFs derived by Van den Berg et al. (1998).
Terms used in table: HpCB: Heptachlorinated biphenyl; HpCDD: Heptachlorinated dibenzo-*p*-dioxin; HpCDF: Heptachlorinated dibenzofuran; HxCB: Hexachlorinated biphenyl; HxCDD: Hexachlorinated dibenzo-*p*-dioxin; HxCDF: Hexachlorinated dibenzofuran; PeCB: Pentachlorinated biphenyl; PeCDD: Pentachlorinated dibenzo-*p*-dioxin; PeCDF: Pentachlorinated dibenzofuran; TCB: Tetrachlorinated biphenyl; TCDF: Tetrachlorinated dibenzofuran; 2,3,7,8-TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; 2,3,7,8-TCDF: 2,3,7,8-tetrachlorinated dibenzofuran.

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.
AhR	Aryl hydrocarbon receptor.
ANZECC	Australian and New Zealand Environment and Conservation Council.
ARMCANZ	Agricultural and Resource Management Council of Australia and New Zealand.
bioaccumulation factor (BAF)	The ratio of the concentration of a contaminant in an organism to its concentration in the ambient environment at a steady state, where the organism can take in the contaminant through ingestion with its food as well as through direct contact. It can be expressed on a wet weight, dry weight or lipid weight basis.
bioconcentration factor (BCF)	The ratio of the concentration of a contaminant in an organism to its concentration in the ambient water (or sediment) at a steady state. It can be expressed on a wet weight, dry weight or lipid weight basis.
BSAF	Biota-sediment accumulation factor.
CAS no. (Chemical Abstracts Service number)	Each chemical has a unique identifying number that is allocated to it by the American Chemical Society.
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 guideline value) in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Formerly known as 'trigger values'.
depuration	A process in which an aquatic organism is placed into a uncontaminated environment for a period of time following a period of being exposed to a contaminant or contaminants.
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.

Term	Definition
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. (Also refer to default guideline value and site-specific guideline value.)
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, relative to the control response, under specified conditions.
lowest observed effect concentration (LOEC)	The lowest concentration of a material used in a toxicity test that has a statistically significant adverse effect on the exposed population of test organisms as compared with the controls.
Log K_{ow}	Logarithm of the octanol-water coefficient, the ratio of the compound's concentration in a known volume of n-octanol to its concentration in a known volume of water after the octanol and water have reached equilibrium.
maximum acceptable toxicant concentration (MATC)	The geometric mean of the lowest exposure concentration that causes a statistically significant adverse effect (LOEC) and the highest exposure concentration where no statistically significant effect is observed (NOEC).
ng/L	Nanograms/litre (i.e. 10^{-9} g/L); 1 ng/L = 0.001 µg/L.
no observed effect concentration (NOEC)	The highest concentration of a material used in a toxicity test that has no statistically significant adverse effect on the exposed population of test organisms as compared with the controls.
OCDD	Octachlorinated dibenzo-*p*-dioxin.
OCDF	Octachlorinated dibenzofuran.
PCBs (polychlorinated biphenyls)	Highly toxic and persistent compounds derived from the replacement by Cl radicals of numerous H radicals on biphenyl, which consists of two benzene rings joined by a covalent bond, with the elimination of two H radicals ($C_{12}H_{10}$).
PCDDs	Polychlorinated dibenzo-*p*-dioxins.
PCDFs	Polychlorinated dibenzofurans.
pg/g	Picograms/gram (i.e. 10^{-12} g/g); 1 pg/g = 0.001 ng/g or 0.000001 µg/g.
pg/L	Picograms/litre (i.e. 10^{-12} g/L); 1 pg/L = 0.001 ng/L or 0.000001 µg/L.
sac fry	Fish fry (larvae) that still have their yolk sac present.
site-specific guideline value	A guideline value that is relevant to the specific location or conditions that are the focus of a given assessment or issue.
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.
swim-up fry	Fish fry (larvae) that have absorbed their yolk sac, are ready to start feeding and rise to the surface to gulp air into the swim bladder.

Term	Definition
TCDD	Tetrachlorinated dibenzo-*p*-dioxin; used in this document interchangeably as 2,3,7,8-TCDD.
TCDF	Tetrachlorinated dibenzofuran.
2,3,7,8-TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin.
TEF	Toxicity equivalence factor. An estimate of the potency, relative to TCDD, of an individual PCDD, PCDF or polychlorinated biphenyl congener, determined using careful scientific judgment after considering all available relative potency data.
TEQ	Toxic equivalency—referring to the approach using toxicity equivalence factors.
toxicity	The inherent potential or capacity of a material to cause adverse effects in a living organism.
toxicity test	The means by which the toxicity of a chemical or other test material is determined. A toxicity test is used to measure the degree of response produced by exposure to a specific level of stimulus (or concentration of chemical) for a specified test period.

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

Table A 1 Summary, toxicity data that passed the screening and quality assurance processes, dioxin in freshwater

Taxonomic group	Species	Life stage	Exposure duration (d)	Test type	Toxicity measure (test endpoint) [a]	Test medium	Temperature (°C)	pH	Water hardness (mg $CaCO_3$/L)	Concentration (ng/L)	Final concentration (ng/L)	Reference
Chordata	*Cyprinus carpio*	Adult	71 + 61	Chronic	LOEC (Mortality)	Lake Superior – filtered	25	–	–	0.06	0.024 [b, c]	Cook et al. (1991)
Chordata	*Esox lucius*	Egg	4 + 11	Acute	LC49 (Mortality)	Dechlorinated tap water	14 ± 0.5	6.9	94–102	1	0.1 [b, c]	Helder (1980)
Chordata	*Oncorhynchus kisutch*	Juvenile	4 + 60	Acute	LC55 (Mortality)	Well water	12–18	6.9	64	5.6	0.56 [b, c]	Miller et al. (1973)
Chordata	*Oncorhynchus mykiss*	Fry	28 + 28	Chronic	LOEC (Growth – wet weight)	Reconstituted water	9–13	7.5–7.9	50–87	0.038	0.015 [b, c]	Mehrle et al. (1988)
Chordata	*Oryzias latipes*	Embryo	11	Chronic	NOEC (Mortality)	'Rearing solution' [e]	25 ± 2	–	2	2.5	2.5 [b, c]	Kim and Cooper (1999)
Chordata	*Pimephales promelas*	Embryo	7	Chronic	NOEC (Mortality)	Dechlorinated tap water	23 ± 0.5	–	–	0.59	0.59 [b, c]	Olivieri and Cooper (1997)
Chordata	*Salvelinus fontinalis*	Egg	2 + 80	Acute	LC50 (Mortality)	–	7 ± 1	7.5	350	9	0.9 [b, c]	Walker and Peterson (1994)

Taxonomic group	Species	Life stage	Exposure duration (d)	Test type	Toxicity measure (test endpoint) [a]	Test medium	Temperature (°C)	pH	Water hardness (mg $CaCO_3$/L)	Concentration (ng/L)	Final concentration (ng/L)	Reference
Chordata	*Salvelinus namaycush*	Egg	2 + 90	Acute	LC50 (Mortality)	Dechlorinated tap water	7 ± 1	7.5	304	7.5	0.75	Walker et al. (1996)
		Egg	2 + 119	Acute	LC50 (Mortality)	Dechlorinated tap water	8 ± 0.5	7.5	136	21	2.1	Walker et al. (1991)
–											1.25 [c, d]	–

a The measure of toxicity being estimated/determined. LCx: Concentration causing x% lethality to a group of organisms (where x = 49, 50 or 55%). NOEC: No observed effect concentration. LOEC= Lowest observed effect concentration.

b The lowest value for a species.

c Value used in the SSD to derive the DGVs.

d The geometric mean of two values (0.75 and 2.1 ng/L) for *Salvelinus namaycush*.

e Deionised water + salts: NaCl (1 g/L); KCl (0.3 g/L); $CaCl_2$ (0.4 g/L); $MgCl_2$ (1.6 g/L); Hardness calculated from Ca (0.144 g/L) and Mg (0.403 g/L) concentrations.

Appendix B: Modality assessment for dioxin

A modality assessment was undertaken for dioxin according to the four questions in Warne et al. (2018). These questions and their answers are listed as follows.

Is there a specific mode of action that could result in taxa-specific sensitivity?
It is generally accepted that most, if not all, toxic and biological effects of dixon and dioxin-like compounds are mediated through the aryl hydrocarbon receptor (AhR), which is a cytosolic receptor protein present in most vertebrate tissues (Mandal 2005, van den Berg et al. 2006). Although AhR homologs exist in invertebrates, they do not bind with dioxins and dioxin-like compounds, which is thought to explain the relative insensitivity of invertebrates to such compounds (Hahn 2002, Hahn et al. 2017). Consistent with the known mode of action of dioxins, the large amount of literature on dioxin effects on fish indicates that fish appear to be much more sensitive to dioxin than other taxa.

Does the dataset suggest bimodality?
The modality assessment was undertaken on the lowest toxicity value for each species (or the appropriate geometric mean of the lowest values) that passed the screening and quality assessment stipulated in Warne et al. (2018). Table B 1 summarises the data considered for the modality assessment.

The data were subject to visualisation, calculation of the bimodality coefficient (BC), and consideration of the range in the effect concentrations. These factors are recommended lines of evidence in evaluating whether bimodality or multimodality of the dataset is apparent. This is discussed as follows.

- The natural log (ln) transformed histogram suggests bimodality (Figure B 1).
- Data that span large ranges (>4 orders of magnitude) indicate potential for underlying bimodality or multimodality (Warne et al. 2018); the dioxin data span 5 orders of magnitude. The fish data range from 0.024 ng/L to 2.5 ng/L (3 orders of magnitude range) and the data for other species range from 40 ng/L to 200 ng/L (2 orders of magnitude range).
- When the BC is greater than 0.555 it indicates that the dataset does not follow a typical normal distribution and may be bimodal. The BC for the ln transformed data was 0.35, which is less than the 0.555 cutoff stipulated in Warne et al. (2018); therefore, it suggests that the dataset may not be bimodal.

When considered together, the above lines of evidence are equivocal in indicating bimodality, However, two of the three lines of evidence suggest bimodality and the only contradictory line of evidence, the BC, is not a definitive test of bimodality.

Table B 1 Lowest toxicity value, each species that passed the screening and quality assessment stipulated in Warne et al. (2018)

Concentration (ng/L)	Toxicity value	Species	Taxonomic group	Toxicity measure
0.015	LOEC	*Oncorhynchus mykiss*	Fish	Chronic LOEC
0.024	LOEC	*Cyprinus carpio*	Fish	Chronic LOEC
0.1	LC49	*Esox lucius*	Fish	Acute to estimated chronic
0.56	NOEC	*Oncorhynchus kisutch*	Fish	Acute to estimated chronic
0.59	NOEC	*Pimephales promelas*	Fish	Chronic NOEC
0.9	LC50	*Salvelinus fontinalis*	Fish	Acute to estimated chronic
1.25	LC50	*Salvelinus namaycush*	Fish	Acute to estimated chronic
2.5	NOEC	*Oryzias latipes*	Fish	Chronic NOEC
40	LOEC	*Xenopus laevis*	Amphibian	Chronic LOEC
80	LOEC	*Paranais* sp.	Annelid	Chronic LOEC
80	LOEC	*Physa* sp.	Mollusc	Chronic LOEC
200	NOEC	*Aedes aegypti*	Insect	Chronic NOEC

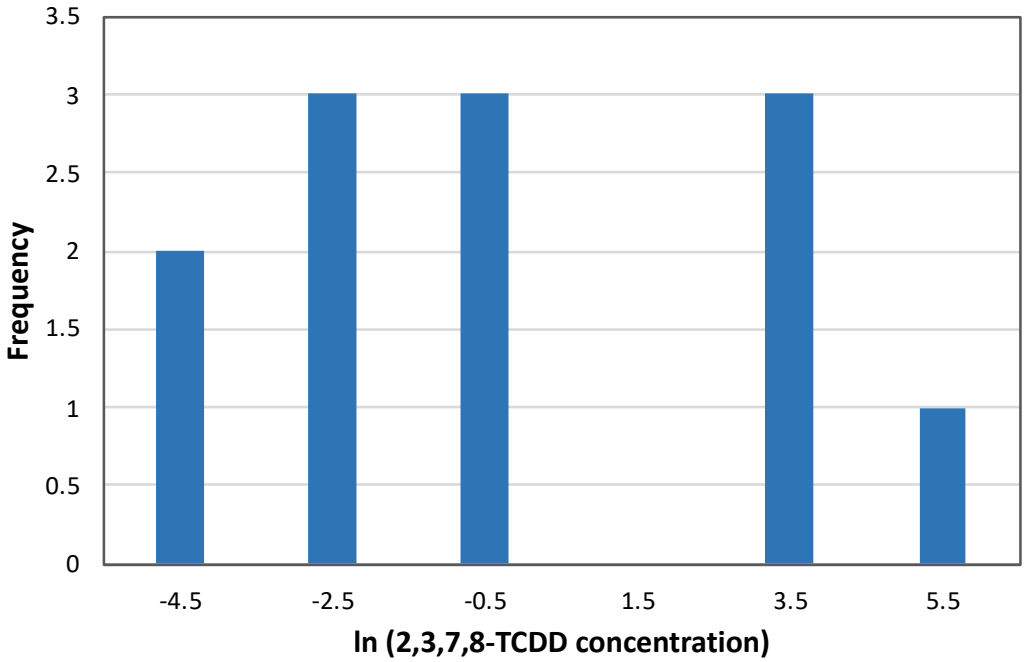


Figure B 1 Histogram, ln transformed toxicity data, 2,3,7,8-TCDD

Do data show taxa-specific sensitivity (i.e. through distinct groupings of different taxa types)?
It is anticipated that dioxin will show taxa-specific sensitivity given its mode of action. Taxa-specific sensitivity is considered likely to account for the bimodality suggested in the data. A box plot of the data for the eight fish, the single amphibian and the three invertebrate species showed large differences in toxicity values, with no overlap, between these groups, with the fish clearly the most sensitive group (Figure B 2).

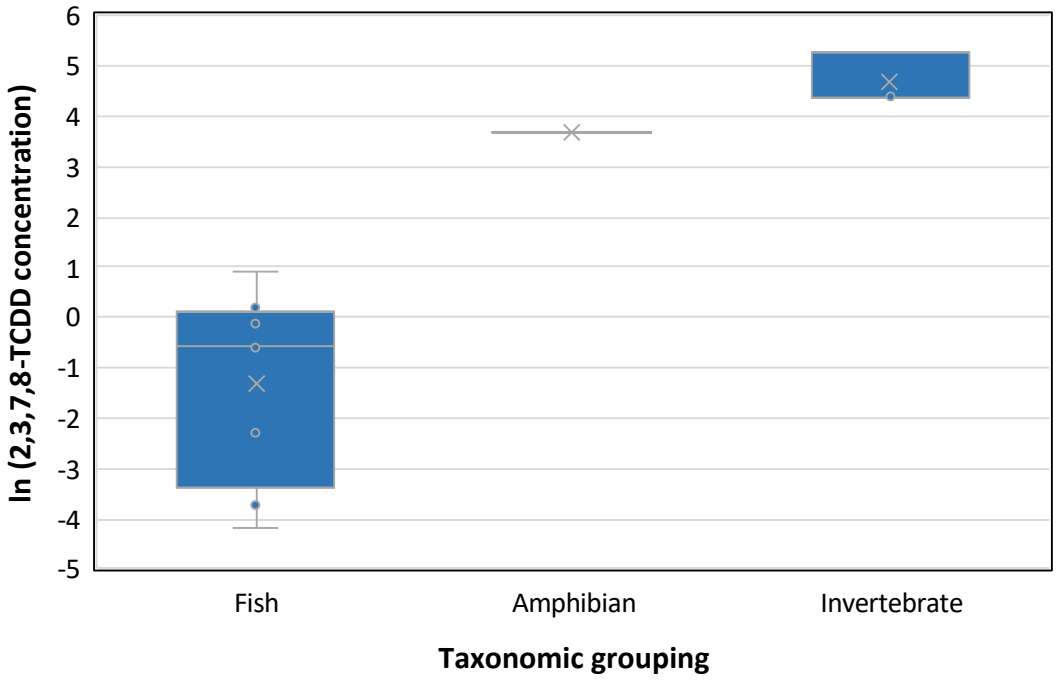


Figure B 2 Histogram, ln transformed toxicity data, 2,3,7,8-TCDD

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?

Notwithstanding the relatively small sample size for non-fish species, review of the data and taxonomic groups used in preparation of the DGVs supports the conclusion that it is unlikely that the modality of the dataset is an artefact of data selection, small sample size, test procedures, or other reasons unrelated to a specific mode of action. Thus, the weight of evidence supported use of the data for only the fish species in deriving the DGVs.

References

Adams, WJ, de Graeve, GM, Sabourin, TD, Cooney, JD & Mosher, GM 1986. Toxicity and bioconcentration of 2,3,7,8-TCDD to fathead minnows (*Pimephales promelas*). *Chemosphere*, 15, 1503–1511.

Ahokas, J, Holdway, D, Brennan, S, Goudey, R & Bibrowska, H 1994. MFO activity in carp (*Cyprinus carpio*) exposed to treated pulp and paper mill effluent in Lake Coleman, Victoria, Australia, in relation to AOX, EOX, and muscle PCDD/PCDF. *Environmental Toxicology and Chemistry*, 13, 41–50.

Anselmo, HMR, Koerting, L, Devito, S, van den Berg, JHJ, Dubbeldam, M, Kwadijk, C & Murk, AJ 2011. Early life developmental effects of marine persistent organic pollutants on the sea urchin *Psammechinus miliaris*. *Ecotoxicology and Environmental Safety*, 74, 2182–2192.

Antkiewicz, DS, Burns, CG, Carney, SA, Peterson, RE & Heideman, W 2005. Heart malformation is an early response to TCDD in embryonic zebrafish. *Toxicological Sciences*, 84, 368–377.

ANZG 2018. Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Governments and Australian state and territory governments, Canberra, ACT, Australia. Viewed 13/09/2019, https://www.waterquality.gov.au/anz-guidelines.

Arnoldsson, K, Haldén, AN, Norrgren, L & Haglund, P 2012. Retention and maternal transfer of environmentally relevant polybrominated dibenzo-*p*-dioxins and dibenzofurans, and polychlorinated biphenyls in zebrafish (*Danio rerio*) after dietary exposure. *Environmental Toxicology and Chemistry*, 31, 804–812.

Barber, TR, Chappie, DJ, Duda, DJ, Fuchsman, PC & Finley, BL 1998. Using a spiked sediment to establish a no-effect concentration for dioxin exposure to the amphipod *Ampelisca abdita*. *Environmental Toxicology and Chemistry*, 17, 420–424.

Bello, SM, Heideman, W & Peterson, RE 2004. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin inhibits regression of the common cardinal vein in developing zebrafish. *Toxicological Sciences*, 78, 258–266.

Berntssen, MHG, Giskegjerde, TA, Rosenlund, G, Tortensen, BE & Lundebye, A-K 2007. Predicting World Health Organization toxic equivalency factor dioxin and dioxin-like polychlorinated biphenyl levels in farmed Atlantic salmon (*Salmo salar*) based on known levels in feed. *Environmental Toxicology and Chemistry*, 26, 13–23.

Bhavsar, SP, Hayton, A & Jackson, DA 2008. Uncertainty analysis of dioxin-like polychlorinated biphenyls-related toxic equivalents in fish. *Environmental Toxicology and Chemistry*, 27, 997–1005.

Birch, GF, Harrington, C, Symons, RK & Hunt, JW 2007. The source and distribution of polychlorinated dibenzo-*p*-dioxin and polychlorinated dibenzofurans in sediments of Port Jackson, Australia. *Marine Pollution Bulletin*, 54, 295–308.

Bol, J, van den Berg, M & Seinen, W 1989. Interactive effects of PCDD's, PCDF's and PCB's as assessed by the E.L.S. – bioassay. *Chemosphere*, 19, 899–906.

Branson, DR, Takahashi, IT, Parker, WM & Blau, GE 1985. Bioconcentration kinetics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rainbow trout. *Environmental Toxicology and Chemistry*, 4, 779–788.

Cantrell, SM, Joy-Schlezinger, J, Stegeman, JJ, Tillitt, DE & Hannink, M 1998. Correlation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced apoptotic cell death in the embryonic vasculature with embryotoxicity. *Toxicology and Applied Pharmacology*, 148, 24–34.

Carney, SA, Chen, J, Burns, CG, Xiong, KM, Peterson, RE & Heideman, W 2006. Aryl hydrocarbon receptor activation produces heart-specific transcriptional and toxic responses in developing zebrafish. *Molecular Pharmacology*, 70, 549–561.

CCME 2001a. Canadian tissue residue guidelines for the protection of wildlife consumers of aquatic biota: Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs). In: CCME 1999. Canadian environmental quality guidelines. Canadian Council of Ministers of the Environment, Winnipeg, Canada.

CCME 2001b. Canadian sediment quality guidelines for the protection of aquatic life: Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs). In: CCME 1999. Canadian environmental quality guidelines. Canadian Council of Ministers of the Environment, Winnipeg, Canada.

CCREM 1987. Canadian water quality guidelines. Canadian Council of Resource and Environment Ministers, Ontario.

Cook, PM, Kuehl, DW, Walker, MK & Peterson, RE 1991. Bioaccumulation and toxicity of TCDD and related compounds in aquatic ecosystems. In: Gallo, MA, Scheuplein, RJ, van der Heijden, CA (eds.). Biological Basis for Risk Assessment of Dioxins and Related Compounds. Banbury Report 35. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 143–167.

Cook, PM, Robbins, JA, Endicott, DD, Lodge, KB, Guiney, PD, Walker, MK, Zabel, EW & Peterson, RE 2003. Effects of aryl hydrocarbon receptor-mediated early life stage toxicity on lake trout populations in Lake Ontario during the 20th Century. *Environmental Science and Technology*, 37, 3864–3877.

Cooper, KR & Wintermeyer, M 2009. A critical review: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) effects on gonad development in bivalve molluscs. *Journal of Environmental Science and Health*, Part C, 27, 226–245.

Dong, W, Teraoka, H, Yamazaki, K, Tsukiyama, S, Imani, S, Imagawa, T, Stegeman, JJ, Peterson, RE & Hiraga, T 2002. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin toxicity in the zebrafish embryo: Local circulation failure in the dorsal midbrain is associated with increased apoptosis. *Toxicological Sciences*, 69, 191–201.

Elonen, GE, Spehar, RL, Holcombe, GW, Johnson, RD, Fernandez, JD, Erickson, RJ, Tietge, JE & Cook, PM 1998. Comparative toxicity of 2,3,7,8- tetrachlorodibenzo-*p*-dioxin to seven freshwater fish species during early life stage development. *Environmental Toxicology and Chemistry*, 17, 472–483.

Fletcher, CL & McKay, WA 1993. Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in the aquatic environment: A literature review. *Chemosphere*, 26, 1041–1069.

Gatehouse, R 2004. Ecological Risk Assessment of Dioxins in Australia, National Dioxins Program Technical Report No. 11. Australian Government Department of the Environment and Heritage, Canberra.

Giesy, JP, Jones, PD, Kannan, K, Newstead, JL, Tillitt, DE & Williams, LL 2002. Effects of chronic dietary exposure to environmentally relevant concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on survival, growth, reproduction and biochemical responses of female rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology*, 59, 35–53.

Grimwood, MJ & Dobbs, TJ 1995. A review of the aquatic ecotoxicology of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. *Environmental Toxicology and Water Quality*, 10, 67–75.

Hahn, ME 2002. Aryl hydrocarbon receptors: Diversity and evolution. *Chemico-Biological Interactions*, 141, 131–160.

Hahn, ME, Karchner, SI & Merson RR 2017. Diversity as opportunity: Insights from 600 million years of AHR evolution. *Current Opinions in Toxicology*, 2, 57–71.

Heiden, TK, Hutz, RJ & Carvan, MJ 2005. Accumulation, tissue distribution, and maternal transfer of dietary 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin: impacts on reproductive success of zebrafish. *Toxicological Sciences*, 87, 497–507.

Helder, TH & Seinen, W 1985. Standardization and application of an E.L.S.-bioassay for PCDDs and PCDFs. *Chemosphere*, 14, 183–193.

Helder, TH 1980. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on early life stages of the pike (*Esox lucius L.*). *The Science of the Total Environment*, 14, 255–264.

Helder, TH 1981. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on early life stages of rainbow trout (*Salmo gairdneri Richardson*). *Toxicology*, 19, 101–112.

Henry, TR, Spitzbergen, JM, Hornung, MW, Abnet, CC & Peterson, RE 1997. Early life stage toxicity of 2,3,7,8- tetrachlorodibenzo-*p*-dioxin in zebrafish (*Danio rerio*). *Toxicology and Applied Pharmacology*, 142, 56–68.

Hill, AJ, Teraoka, H, Heideman, W & Peterson, RE 2005. Zebrafish as a model vertebrate for investigating chemical toxicity. *Toxicological Sciences*, 86, 6–19.

Hornung, MW, Zabel, EW & Peterson, RE 1996. Toxic equivalency factors of polybrominated dibenzo-*p*-dioxin, dibenzofuran, biphenyl, and polyhalogenated diphenyl ether. *Toxicology and Applied Pharmacology*, 140, 227–234.

Isensee, AR & Jones, GE 1978. Distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in aquatic model ecosystem. *Environmental Science and Technology*, 9, 668–672.

Isensee, AR 1978. Bioaccumulation of 2,3,7,8-tetrachlorodibenzo-para-dioxin. *Ecological Bulletin (Stockholm)*, 27, 255–262.

Jarvinen, AW & Ankley, GT 1999. Linkage of effects to tissue residues: Development of a comprehensive database for aquatic organisms exposed to inorganic and organic chemicals. SETAC Technical Publication Series, SETAC Press, Pensacola, FL.

Johnson, RD, Tietge, JE, Jensen, KM, Fernandez, JD, Linnum, AL, Lothenbach, DB, Holcombe, GW, Cook, PM, Christ, SA, Lattier, DL & Gordon, DA 1998. Toxicity of 2,3,7,8- tetrachlorodibenzo-*p*-dioxin to early life stage brook trout (*Salvelinus fontinalis*) following parental dietary exposure. *Environmental Toxicology and Chemistry*, 17, 2408–2421.

Jönsson, ME, Jenny, MJ, Woodin, BR, Hahn, ME & Stegeman, JJ 2007. Role of AHR2 in the expression of novel cytochrome P450 1 family genes, cell cycle genes, and morphological defects in developing zebra fish exposed to 3,3',4,4',5-pentachlorobiphenyl or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicological Sciences*, 100, 180–193.

Jung, KE & Walker, MK 1997. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on development of anuran amphibians. *Environmental Toxicology and Chemistry*, 16, 230–240.

Kanan, S & Samara, F 2018. Dioxins and furans: A review from chemical and environmental perspectives. *Trends in Environmental Analytical Chemistry*, 17, 1–13.

Kim, Y & Cooper, KR 1999. Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and polychlorinated biphenyls (PCBs) in the embryos and newly hatched larvae of the Japanese medaka (*Oryzias latipes*). *Chemosphere*, 39, 527–538.

Loonen, H, van de Guchte, C, Parsons, JR, de Voogt, P & Govers, HAJ 1996. Ecological hazard assessment of dioxins: hazards to organisms at different levels of aquatic food webs (fish-eating birds and mammals, fish and invertebrates). *Science of the Total Environment*, 182, 93–103.

Mandal, PK 2005. Dioxin: A review of its environmental effects and its aryl hydrocarbon receptor biology. *Journal of Comparative Physiology B*, 175, 221–230.

Manning, T, Roach, A & Ferrell, D 2007. Dioxins in fish and other seafood from Sydney Harbour, Australia. *Organohalogen Compounds*, 69, 343–346.

McKinney, JD, Fawkes, J, Jordan, S, Chae, K, Oatley, S, Coleman, RE & Brinert, W 1985. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) as a potent and persistent thyroxine agonist: A mechanistic model for toxicity based on molecular reactivity. *Environmental Health Perspectives*, 61, 41–53.

Mehrle, PM, Buckler, DR, Little, EE, Smith, LM, Petty, JD, Peterman, PH, Stalling, DL, de Graeve, GM, Coyle, JJ & Adams, WJ 1988. Toxicity and bioconcentration of 2,3,7,8-tetrachlorodibenzodioxin and 2,3,7,8-tetrachlorodibenzofuran in rainbow trout. *Environmental Toxicology and Chemistry*, 7, 47–62.

Miller, RA, Norris, LA & Hawkes, CL 1973. Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in aquatic organisms. *Environmental Health Perspectives*, 5, 177–186.

Miller, RA, Norris, LA & Loper, BA 1979. The response of coho salmon and guppies to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in water. *Transactions of the American Fisheries Society*, 108, 401–407.

Müller, J, Muller, R, Goudkamp, K, Shaw, M, Mortimer, M, Haynes, D, Paxman, C, Hyne, R, McTaggart, A, Burniston, D, Symons, R & Moore, M 2004. Dioxins in Aquatic Environments in Australia, National Dioxins Program Technical Report No. 6. Australian Government Department of the Environment and Heritage, Canberra.

Newstead, JL, Giesy, JP, Ankley, GT, Tillitt, DE, Crawford, RA, Gooch, JW, Jones, PD & Denison, MS 1995. Development of toxic equivalency factors for PCB congeners and the assessment of TCDD and PCB mixtures in rainbow trout. *Environmental Toxicology and Chemistry*, 5, 861–871.

Nichols, JW, Jensen, KM, Tietge, JE & Johnson, RD 1998. Physiologically based toxicokinetic model for maternal transfer of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in brook trout (*Salvelinus fontinalis*). *Environmental Toxicology and Chemistry*, 17, 2422–2438.

Niimi, AJ 1996. Review article: Evaluation of PCBs and PCDD/Fs retention by aquatic organisms. *The Science of the Total Environment*, 192, 123–150.

Norris, LA & Miller, RA 1974. The toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in guppies (*Poecilia reticulatus Peters*). *Bulletin of Environmental Contamination and Toxicology*, 12, 76–80.

Olivieri, CE & Cooper, KR 1997. Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in embryos and larvae of the fathead minnow (*Pimephales promelas*). *Chemosphere*, 34, 1139–1150.

Parhizgari, Z & Li, J 2014. A physiologically-based pharmacokinetic model for disposition of 2,3,7,8-TCDD in fathead minnow and medaka. *Environmental Toxicology and Chemistry*, 33, 1064–1071.

Parrott, JE, Hodson, PV, Servos, MR, Huestis, SL & Dixon, DG 1995. Relative potency of polychlorinated dibenzo-*p*-dioxins and dibenzofurans for inducing mixed-function oxygenase activity in rainbow trout. *Environmental Toxicology and Chemistry*, 14, 1041–1050.

Prasch, AL, Teraoka, H, Carney, SA, Dong, W, Hiraga, T, Stegeman, JJ, Heideman, W & Peterson, RE 2003. Aryl hydrocarbon receptor 2 mediates 2,3,7,8-tetrachlorodibenzo-*p*-dioxin developmental toxicity in zebrafish. *Toxicological Sciences*, 76, 138–150.

Prince, R & Cooper, KR 1995. Comparisons of the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on chemically impacted and nonimpacted subpopulations of *Fundulus heteroclitus*: 1. TCDD toxicity. *Environmental Toxicology and Chemistry*, 14, 579–587.

Rice, CP, O'Keefe, PW & Kubiak, TJ 2003. Sources, pathways and effects of PCBs, dioxins, and dibenzofurans. In: Hoffman, DJ, Rattner, BA, Burton, GA Jr & Cairns, J Jr (eds.). Handbook of Ecotoxicology, 2nd Edition, Lewis Publishers, Boca Raton.

Roach, A, Ferrell, D & Manning, T 2007. The interaction between life history and dioxin levels in fish from Sydney Harbour, Australia. *Organohalogen Compounds*, 69, 1560–1565.

Roach, A, Symons, R & Stevenson, G 2009. Contrasting patterns of spatial autocorrelation of PCDD/Fs, dioxin-like PCBS and PBDES in sediments in Sydney Harbour, Australia. *Organohalogen Compounds*, 71, 366–371.

Safe, SH 1998. Hazard and risk assessment of chemical mixtures using the toxic equivalency factor approach. *Environmental Health Perspectives*, 106, Suppl 4, 1051–1058.

Segstro, MD, Muir, DCG, Servos, MR & Webster, GRB 1995. Long-term fate and bioavailability of sediment-associated polychlorinated dibenzo-*p*-dioxins in aquatic mesocosms. *Environmental Toxicology and Chemistry*, 14, 1799–1807.

Sezmis, AL, Birch, G & Covaci, A 2014. Relationships between dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like biphenyls (dl-PCBs) congener concentrations in aquatic organisms from Sydney Estuary, Australia and physiology, spatial, seasonality, trophodynamic and life history traits. *Science of the Total Environment*, 490, 50–58.

Sijm, DTHM & Opperhuizen, A 1996. Dioxins: An environmental risk to fish? In: Beyer, WN, Heinz, GH & Redmon-Norwood, AW (eds.). Environmental contaminants in wildlife: Interpreting tissue concentrations. SETAC Special Publication Series. CRC Press, Lewis Publishers, Boca Raton, FL, USA, 500–573.

Spitzbergen, JM, Walker, MK, Olson, JR & Peterson, RE 1991. Pathological alterations in the early life stages of lake trout, *Salvelinus namaycush*, exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as fertilized eggs. *Aquatic Toxicology*, 19, 41–72.

Tanguay, RL, Andreason, EA, Walker, MK & Peterson, RE 2003. Dioxin toxicity and aryl hydrocarbon receptor signaling in fish. In: Schecter, A & Gasiewicz, TA (eds.). Dioxin and Health, 2nd Edition. John Wiley and Sons Inc, NY, USA, 603–628.

Teraoka, H, Dong, W, Tsujimoto, Y, Iwasa, H, Endoh, D, Ueno, N, Stegeman, JJ, Peterson, RE & Hiraga, T 2003. Induction of cytochrome P450 1A is required for circulation failure and edema by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in zebrafish. *Biochemical and Biophysical Research Communications*, 304, 223–228.

Teraoka, H, Kubota, A, Dong, W, Kawai, Y, Yamazaki, K, Mori, C, Harada, Y, Peterson, RE & Hiraga, T 2009. Role of the cyclooxygenase 2-thromboxane pathway in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced decrease in mesencephalic vein blood flow in the zebrafish embryo. *Toxicology and Applied Pharmacology*, 234, 33–40.

Teraoka, H, Wu Dong, W, Ogawa, S, Tsukiyama, S, Okuhara, Y, Niiyama, M, Ueno, N, Peterson, RE & Hiraga, T 2002. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin toxicity in the zebrafish embryo: Altered regional blood flow and impaired lower jaw development. *Toxicological Sciences*, 65, 192–199.

Tietge, JE, Johnson, RD, Jensen, KM, Cook, PM, Elonen, GE, Fernandez, JD, Holcombe, GW, Lothenbach, DB & Nichols, JW 1998. Reproductive toxicity and disposition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in adult brook trout (*Salvelinus fontinalis*) following a dietary exposure. *Environmental Toxicology and Chemistry*, 17, 2395–2407.

Toomey, BH, Bello, S, Hahn, ME, Cantrell, S, Wright, P, Tillitt, DE & Di Giulio, RT 2001. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin induces apoptotic cell death and cytochrome P4501A expression in developing *Fundulus heteroclitus* embryos. *Aquatic Toxicology*, 53, 127–138.

USEPA 1984. Ambient water quality criteria for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Report EPA 440/5-84-007. United States Environmental Protection Agency, Office of Water Regulations and Standards, Washington DC, USA.

USEPA 1993. Interim report on data and methods for assessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin risk to aquatic life and associated wildlife. Report EPA/600/R- 93/055. United States Environmental Protection Agency, Washington DC.

USEPA 2002. National recommended water quality criteria: 2002. Report EPA-822-R-02-047. United States Environmental Protection Agency, Office of Water Regulations and Standards, Washington DC, USA.

USEPA 2008. Framework for the application of the toxicity equivalence methodology for polychlorinated dioxins, furans, and biphenyls in ecological risk assessment. Report EPA/100/R-08/004. United States Environmental Protection Agency, Office of the Science Advisor, Washington, DC.

Van den Berg, M, Birnbaum, L, Bosveld, ATC, Brunstrom, B, Cook, P, Feely, M, Giesy, JP, Hanberg, A, Hasegawa, R, Kennedy, SW, Kubiak, T, Larsen, JC, van Leeuwen, FXR, Djien Liem, AK, Nolt, C, Peterson, RE, Poellinger, L, Safe, S, Schrenk, D, Tillitt, D, Tysklind, M, Younes, M, Waern, F & Zacharewski, T 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environmental Health Perspectives*, 106, 775–792.

Van den Berg, M, Birnbaum, LS, Denison, M, de Vito, M, Farland, W, Feely, M, Fiedler, H, Hakansson, H, Hanberg, A, Haws, L, Rose, M, Safe, S, Schrenk, D, Tohyama, C, Tritscher, A, Tuomisto, J, Tysklind, M, Walker, N & Peterson, RE 2006. The 2005 World Health Organization Reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicological Sciences*, 93, 223–241.

Walker, MK & Peterson, RE 1991. Potencies of polychlorinated dibenzo-*p*-dioxin, dibenzofuran, and biphenyl congeners, relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin for producing early life stage mortality in rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology*, 21, 219–238.

Walker, MK & Peterson, RE 1994. Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to brook trout (*Salvelinus fontinalis*) during early development. *Environmental Toxicology and Chemistry*, 13, 817–820.

Walker, MK, Cook, PM, Butterworth, BC, Zabel, EW & Peterson, RE 1996. Potency of a complex mixture of polychlorinated dibenzo-*p*-dioxin, dibenzofuran, and biphenyl congeners compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in causing fish early life stage mortality. *Fundamental and Applied Toxicology*, 30, 178–186.

Walker, MK, Hufnagle, LC, Clayton, MK & Peterson, RE 1992. An egg injection method for assessing early life stage mortality of polychlorinated dibenzo-*p*-dioxin, dibenzofuran, and biphenyls in rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology*, 22, 15–38.

Walker, MK, Spitzbergen, JM, Olson, JR & Peterson, RE 1991. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity during early life stage development of lake trout (*Salvelinus namaycush*). *Canadian Journal of Fisheries and Aquatic Sciences*, 48, 875–883.

Wannemacher, R, Rebstock, A, Kulzer, E, Schrenk, D & Bock, KW 1992. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on reproduction and oogenesis in zebrafish (*Brachydanio rerio*). *Chemosphere*, 24, 1361–1368.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW & Stauber JL 2018. Revised Method for Deriving Australian and New Zealand Water Quality Guideline Values for Toxicants – update of 2015 version. Prepared for the revision of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Governments and Australian state and territory governments, Canberra, 48 pp.

Wenning, RJ, Martello, L & Prusak-Daniel, A 2011. Dioxins, PCBs, and PBDEs in aquatic organisms. In Beyer, WN & Meador, JP (eds.). Environmental Contaminants in Biota: Interpreting tissue concentrations. CRC Press, Boca-Raton, FL, USA, 103–169.

West, CW, Ankley, GT, Nichols, JW, Elonen, GE & Nessa, DE 1997. Toxicity and bioaccumulation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in long term tests with the freshwater benthic invertebrates *Chironomus tentans* and *Lumbriculus variegatus*. *Environmental Toxicology and Chemistry*, 16, 1287–1294.

Wintermeyer, ML & Cooper, KR 2007. The development of an aquatic bivalve model: Evaluating the toxic effects on gametogenesis following 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) exposure in the eastern oyster (*Crassostrea virginica*). *Aquatic Toxicology*, 81, 10–26.

Wisk, JD & Cooper, KR 1990. The stage specific toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in embryos of the Japanese medaka (*Oryzias latipes*). *Environmental Toxicology and Chemistry*, 9, 1159–1169.

Yamauchi, M, Kim, E-Y, Iwata, H, Shima, Y & Tanabe, S 2006. Toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in developing red seabream (*Pagrus major*) embryo: An association of morphological deformities with AHR1, AHR2 and CYP1A expressions. *Aquatic Toxicology*, 80, 166–179.

Yockim, RS, Isensee, AR & Jones, GE 1978. Distribution and toxicity of TCDD and 2,4,5-T in an aquatic model ecosystem. Che*mistry, Physics, Biology and Toxicology as Focused on Environmental Problems*, 7, 215–220.

Zabel, EW, Cook, PM & Peterson, RE 1995. Toxic equivalency factors of polychlorinated dibenzo-*p*-dioxin, dibenzofuran and biphenyl congeners based on early life stage mortality in rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology*, 31, 315–328.