

# An Evaluation of Metal Binding Constants to Cell Surface Receptors in Freshwater Organisms, and their Application in Biotic Ligand Models to Predict Metal Toxicity

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## Supplementary Information

### S1. Zinc

#### S1.1. *Oncorhynchus mykiss*

There have been thirteen studies on the toxicity of zinc to *O. mykiss*, with log  $K_{ZnBL}$  values ranging between 5.60 and 6.23 (average  $\pm$  95 % confidence interval of  $5.94 \pm 0.09$ ). The lowest value is from the uptake study of Alsop and Wood (2000). De Schamphalaere and Janssen (2004a, 2004b) reported a log  $K_{ZnBL}$  value of 5.5, consistent with that of Alsop and Wood (2000), and in their latter study (De Schamphalaere and Janssen, 2004b) also reported a  $(f_c)_{Zn^{50\%}}$  value of 0.246. The remaining ten values have an average binding constant of 6.00 and if the  $(f_c)_{Zn^{50\%}}$  value of De Schamphalaere and Janssen (2004b) is used together with this average value, log  $K_{ZnBL}$  reduces to 5.51, consistent with the three other values (Alsop and Wood, 2000; De Schamphalaere and Janssen, 2004a, 2004b). However, the average of all thirteen values (5.94) is in excellent agreement with the overall average log  $K_{ZnBL}$  ( $5.94 \pm 0.05$ ; Table S1) and, as such, the values derived in the present study are not corrected further for  $(f_c)_{Zn^{50\%}}$ .

De Schamphalaere and Janssen (2004a) studied the toxicity of zinc to juvenile rainbow trout with varying pH (5.68 to 7.87), and concentrations of  $Ca^{2+}$  (8.4 to 159 mg/L) and  $Mg^{2+}$  (1.4 to 76 mg/L), using a 96 h mortality test. The study derived a value for log  $K_{ZnBL}$  of 5.5. The toxicity data obtained in the study were re-evaluated in the present study, where a slightly larger log  $K_{ZnBL}$  of 5.93 was determined. This stability constant is listed in Table S1.

De Schamphalaere and Janssen (2004b) conducted another study on the zinc toxicity to rainbow trout using a chronic endpoint (30 d mortality). Tests were undertaken with variable calcium concentrations (9.1 to 156 mg/L). Analysis of these data and correction for the synthetic water concentrations of  $H^+$ ,  $Na^+$  and  $Mg^{2+}$  led to a value for log  $K_{ZnBL}$  of 5.76 (Table S1). The study also examined the toxicity of zinc to *O. mykiss* in five European natural waters.

Zinc uptake into the gills of juvenile rainbow trout was studied by Alsop and Wood (2000). The study determined values for log  $K_{ZnBL}$  of 5.6 and 5.3 in control and Zn-acclimated fish, respectively. The water quality used in the study had major ion concentrations ( $Ca^{2+}$ : 40 mg/L,  $Mg^{2+}$ : 4.9 mg/L and  $Na^+$ : 13.8 mg/L) that would result in some ameliorative effects on toxicity. The concentrations of the ameliorative cations do not affect the derived log  $K_{ZnBL}$  in an uptake study and, as such, the value of 5.60 is retained (Table S1) from the control; it is expected that Zn-acclimated fish would yield a reduced binding constant.

In a fourth study on zinc toxicity to *O. mykiss*, Cusimano et al. (1986) used 96 h mortality tests at a variable pH (4.7 to 7.0). The  $LC_{50}$  values determined were linearly dependent on the  $H^+$  concentration, with the solution speciation being dominated by  $Zn^{2+}$  at all three pH values (96-97 % of total zinc). Based on the toxicity results obtained, a log  $K_{ZnBL}$  value of 6.06 was determined. This value is listed in Table S1.

Naddy et al. (2015) also studied the toxicity of zinc to rainbow trout. The effect of varying hardness on the toxic response was also considered. The average toxicity in soft water was utilised together with that in hard water. The hard water also had elevated alkalinity and pH compared to the soft water. The  $\text{Zn}^{2+}$  ion dominated zinc speciation in the soft water, but was a much smaller fraction (0.34) of the hard water. When accounting for the speciation and the effects of the ameliorative cations, an average log  $K_{\text{ZnBL}}$  of 6.23 was derived (Table S1).

The toxicity of zinc to rainbow trout was studied by Calfee et al. (2014). The test solution had a pH of 7.9, a hardness of 104 mg/L as  $\text{CaCO}_3$  and an alkalinity of 94 mg/L as  $\text{CaCO}_3$ . The  $\text{Zn}^{2+}$  ion was the dominant zinc species in the test solution and accounting for speciation and the ameliorative cations led to a log  $K_{\text{ZnBL}}$  value of 5.92 (Table S1).

Besser et al. (2007) studied the toxicity of zinc to rainbow trout from populations in the Minnesota and Missouri Rivers. The acute swim-up life stage test results were used in the present analysis. The speciation of the test solutions was dominated by the free zinc ion and together with consideration of the effects of the ameliorative cations led to a log  $K_{\text{ZnBL}}$  value of 6.06 (Table S1).

The study of Chapman (1978) on the toxicity of zinc to the swim-up stage of rainbow trout also led to a log  $K_{\text{ZnBL}}$  value of 6.06 (Table S1) using 96 h  $\text{LC}_{50}$  concentrations. For zinc, the 96 h  $\text{LC}_{50}$  was identical to the 200 h  $\text{LC}_{50}$ . Water in the tests was supplied from wells on the banks of the Willamette River and the derived log  $K_{\text{ZnBL}}$  was adjusted from the inverse of the  $\text{LC}_{50}$  using the ameliorative cation concentrations and the fraction of  $\text{Zn}^{2+}$  in the test water (the free ion dominated zinc speciation).

Mebane et al. (2021) studied the toxicity of zinc to rainbow trout of varying exposure periods. The toxicity was almost invariant over exposure periods of 48 h to 96 h. The 96 h  $\text{LC}_{50}$  was used in the present study. This value, together with the fraction of zinc as the free ion (the dominant species in the test water) and the effect of the ameliorative cations, were used to derive a log  $K_{\text{ZnBL}}$  value of 5.97, as listed in Table S1. In an earlier study, Mebane et al. (2008) studied the toxicity of three metals to *O. mykiss*, including zinc, using a 96 h mortality assay. The derived  $\text{LC}_{50}$  was 123 mg/L and the  $\text{Zn}^{2+}$  ion was the dominant (90.9 %) form of zinc in the test water at the  $\text{LC}_{50}$ . Using the  $\text{LC}_{50}$  and speciation together with the ameliorative cation concentrations led to a log  $K_{\text{ZnBL}}$  value of 5.90 (Table S1).

The study of Hansen et al. (2002) also examined the toxicity of zinc to rainbow trout using waters of varying hardness and pH. Analysis of the 120 h  $\text{LC}_{50}$  data provided for the various conditions, when adjusted for zinc speciation and the concentrations of the ameliorative cations, led to a log  $K_{\text{ZnBL}}$  value of 5.93. This value is listed in Table S1.

Hogstrand et al. (1998) studied the uptake of zinc into *O. mykiss* using 28 d accumulation assays. A relationship between zinc and calcium influx was observed, from which an intercept (i.e., no Ca) of 1.61 mmol/L was determined. In another test in the absence of calcium, an affinity constant ( $K_m$ ) of 0.682 mmol/L was determined. From these values, an average log  $K_{\text{ZnBL}}$  of 5.98 was calculated. This value is listed in Table S1.

The final study on the toxicity of zinc to rainbow trout was conducted by Bradley and Sprague (1985). The study examined the effect of pH, alkalinity and hardness on the toxicity of zinc to this fish species. In the test waters studied, zinc was predominantly in the form of the free ion and accounting for the concentrations of the ameliorative cations led to a log  $K_{\text{ZnBL}}$  value of 5.85 (Table S1).

### **S1.2. Other *Oncorhynchus* sp.**

There are two studies that have examined the toxicity of zinc to other *Oncorhynchus* species. The log  $K_{\text{ZnBL}}$  values derived for the fish species studied are consistent with those derived for *O. mykiss*.

Chapman (1978) also studied the toxicity of zinc to the swim-up stage of chinook salmon (*O. tshawytscha*) using the same methodology and test water applied to *O. mykiss*. Again, using the 96 h  $\text{LC}_{50}$  concentrations, adjusted for the ameliorative cation concentrations and the fraction of  $\text{Zn}^{2+}$  in the test water (the free ion dominated zinc speciation), led to a log  $K_{\text{ZnBL}}$  value of 6.07 (Table S1).

The toxicity of zinc to green cutthroat trout (*O. clarkii*) was studied by Brinkman and Johnston (2012). Toxicity data reported were for 96 h mortality under varying conditions of pH, hardness and alkalinity; however, the free zinc ion was the dominant species in all test conditions studied. Analysis of all conditions, when adjusted for speciation and ameliorative cation concentrations, led to consistent binding constants and the average value was used ( $\log K_{ZnBL} = 5.77$ ; Table S1).

The average binding constant for the two studies on other *Oncorhynchus* species is  $\log K_{ZnBL} = 5.92$ . This value is in excellent agreement with that determined for *O. mykiss* of  $5.94 \pm 0.09$ . The range of the two values is also consistent with the range of values found for *O. mykiss*.

### S1.3. Other Fish Species

Eleven other studies are cited that have examined the toxicity of zinc to species of fish. These studies relate to seven species of fish. Only two species (*Pimephales promelas* and *Cottus bairdi*) have more than one study, with both having three cited studies.

A study on zinc toxicity to the mottled sculpin (*C. bairdi*) in water of high hardness was conducted by Brinkman and Woodling (2005). Mortality assays were used over varying durations between 4 and 30 days. Only the 4 d  $LC_{50}$  concentration (439 mg/L) differed from all other test duration  $LC_{50}$  concentrations (266 to 302 mg/L; average 277 mg/L). The 7 d value of 278 mg/L (almost identical to the average) was used in the present study to derive the  $\log K_{ZnBL}$  value of 5.97. This value is listed in Table S1. Due to the high hardness used in the study, the amelioration by the  $Ca^{2+}$  and  $Mg^{2+}$  ions was relatively high;  $Zn^{2+}$  was the dominant (84.8 %) Zn species in the test solutions.

Brinkman and Johnston (2012) also studied the toxicity of zinc to mottled sculpin (*C. bairdi*). Toxicity data reported were for 96 h mortality under varying conditions of pH, hardness and alkalinity; however, the free zinc ion was the dominant species in the test condition studied. Analysis of the toxicity data, when adjusted for speciation and ameliorative cation concentrations, led to a binding constant of 5.78 ( $\log K_{ZnBL}$ ), as listed in Table S1.

Besser et al. (2007) also studied the toxicity of zinc to *C. bairdi* from populations in the Minnesota and Missouri Rivers. The acute swim-up life stage test results were used in the present analysis. The speciation of the test solutions was dominated by the free zinc ion (60.4 %) and, together with consideration of the effects of the ameliorative cations, led to a  $\log K_{ZnBL}$  value of 6.45 (Table S1). This binding constant is somewhat high because the  $LC_{50}$  value used in its calculation is relatively low when compared with the  $LC_{50}$  values for other life stages, but, however, is still retained due to consistency with use of other data from the study.

The average value for  $\log K_{ZnBL}$  for the three studies on *C. bairdi* is 6.07. Even considering the relatively high value from Besser et al. (2007), this average value is still within the uncertainty range of the *O. mykiss* data.

Bringolf et al. (2006) studied the toxicity of zinc to fathead minnow (*P. promelas*) with varying dissolved organic carbon concentrations (DOC) using a 96 h mortality assay. Calculation of the toxicity concentration with zero DOC, and correcting for the concentrations of the ameliorative cations, led to a  $\log K_{ZnBL}$  value of 5.54 (Table S1). The zinc toxicity to *P. promelas* was also studied in five North American natural waters as well as two reference waters.

Schubauer-Berigan et al. (1993) measured the toxicity of zinc to four freshwater species including *P. promelas* as a function of pH. For *P. promelas*, the toxicity was found to depend on pH and the  $\log K_{ZnBL}$  value (5.56) was determined from the relationship of toxicity with respect to the  $H^+$  concentration. The  $\log K_{ZnBL}$  value is listed in Table S1.

Diamond et al. (1997) also studied the toxicity of zinc to *P. promelas* in both laboratory water and Lehigh River water sampled at different times of year. The geometric average of the  $LC_{50}$  data for the laboratory water was used in the present analysis, which gave a concentration of 251 mg/L. Use of this toxicity concentration, together with the zinc speciation ( $Zn^{2+}$  is 86.4 % of total zinc) and the ameliorative cation concentrations resulted in a  $\log K_{ZnBL}$  value of 5.86 (Table S1).

The average log  $K_{ZnBL}$  value for *P. promelas* is 5.65, which is lower than the values found for other species, in particular, that for *O. mykiss*. However, the range of values determined for *P. promelas* is still consistent with the average and the 95 % confidence interval derived for *O. mykiss*.

Trenfield et al. (2023) studied the toxicity of zinc to several species, including *Mogurnda mogurnda* (7 d growth assay) in waters of low hardness. Speciation calculations indicated that the free zinc ion was the dominant zinc species, being 67.5 % of total zinc. The  $EC_{50}/LC_{50}$  concentrations determined for all species studied ranged between 52 and 74 mg/L only, a relatively narrow range, with *M. mogurnda* having an  $EC_{50}$  concentration of 72 mg/L. Combining this zinc toxicity concentration with the fraction of zinc as the free ion leads to a log  $K_{ZnBL}$  value of 6.13 (Table S1).

Vardy et al. (2014) studied the toxicity of zinc to white sturgeon (*Acipenser transmontanus*) in both laboratory and Columbia River water. The data for the youngest life-stage (8 days post-hatch) and laboratory water were used in the present analysis, together with the zinc speciation and ameliorative cation concentrations, to determine a log  $K_{ZnBL}$  value of 6.11. This value is included in Table S1.

Chen et al. (2016) conducted a comprehensive study on the toxicity of zinc to grass carp (*Ctenopharyngodon idellus*) using both acute and chronic endpoints. Using the longest endpoints for both (96 h and 28 d),  $LC_{50}/EC_{50}$  concentrations of 475 and 149 mg/L, respectively, were determined. For these endpoints, Chen et al. (2016) determined separate  $(f_c)_{Zn^{50\%}}$  values of 0.435 and 0.209, respectively. Using both the toxicity concentrations at the respective endpoints and the associated  $(f_c)_{Zn^{50\%}}$  values, together with the ameliorative cation concentrations, leads to almost identical log  $K_{ZnBL}$  values. As such, the average value is used in the present study, namely 5.69 (Table S1).

Another fish species studied by Brinkman and Johnston (2012) for the toxicity of zinc was the mountain whitefish (*Prosopium williamsoni*). Toxicity data reported were for 96 h mortality under varying conditions of pH, hardness and alkalinity. In all conditions studied, the free zinc ion was dominant. Analysis of the toxicity data, when adjusted for speciation and ameliorative cation concentrations, led to an average binding constant of 5.46 (log  $K_{ZnBL}$ ), as listed in Table S1.

The study of Hansen et al. (2002) also examined the toxicity of zinc to bull trout (*Salvelinus confluentus*), using waters of varying hardness and pH. Analysis of the 120 h  $LC_{50}$  data provided for the various conditions, when adjusted for zinc speciation and the concentrations of the ameliorative cations, led to a log  $K_{ZnBL}$  value of 5.74. This value is listed in Table S1.

Overall, the eleven studies for fish species that are not *Oncorhynchus* sp. have an average log  $K_{ZnBL}$  value of  $5.84 \pm 0.20$ . Again, this average value and its associated 95 % uncertainty interval is fully consistent with the average value and 95 % uncertainty interval found for *O. mykiss*. This indicates that the log  $K_{ZnBL}$  values derived for fish species are fully internally consistent.

#### S1.4. *Daphnia magna*

There have been nine studies on the toxicity of zinc to *Daphnia magna*, with log  $K_{ZnBL}$  values ranging between 5.31 and 6.24 (average of  $5.83 \pm 0.26$ ). The lowest values are from the studies of Heijerick et al. (2005a) and Van Regenmortel et al. (2017), who selected the same value. Heijerick et al. (2005a) reported a  $(f_c)_{Zn^{50\%}}$  value of 0.42 whereas Van Regenmortel et al. (2017) used a lower value of 0.12. From the earlier study of Heijerick et al. (2002a), a  $(f_c)_{Zn^{50\%}}$  value of 0.45 was derived. The log  $K_{ZnBL}$  value of Van Regenmortel et al. (2017) would be substantially larger if either of the larger  $(f_c)_{Zn^{50\%}}$  values were used. Nevertheless, the average log  $K_{ZnBL}$  value determined for *D. magna* is consistent with that derived for *O. mykiss*.

Heijerick et al. (2002a) studied the toxicity of zinc to *D. magna* at varying pH levels (6.0 to 8.0) and concentrations of  $Na^+$  (1.8 to 345 mg/L),  $K^+$  (3.0 to 78 mg/L),  $Ca^{2+}$  (5.0 to 320 mg/L) and  $Mg^{2+}$  (5.1 to 194 mg/L) in synthetic water using acute 48 h immobilisation assays. The study determined values for both log  $K_{ZnBL}$  and  $(f_c)_{Zn^{50\%}}$  as 5.31 and 0.42, respectively. The latter value is very similar to that found for the toxicity of nickel to *D. magna* (0.45) from the studies of both

Deleebeeck et al. (2008) and Mano and Shinobara (2020). If a value for  $(f_c)_{Zn^{50\%}}$  of 0.45 were used for the toxicity data of Heijerick et al. (2002a), the log  $K_{ZnBL}$  would reduce marginally to 5.25. However, the values obtained by Heijerick et al. (2002a) have been used in the present study and are listed in Table S1.

In a later study, Heijerick et al. (2005a) examined the toxicity of zinc to *D. magna* using chronic 21 d reproduction assays. The study used synthetic water with varying pH levels (5.5 to 8.0) and concentrations of  $Na^+$  (48 to 290 mg/L),  $Ca^{2+}$  (10 to 120 mg/L) and  $Mg^{2+}$  (6.0 to 97 mg/L). The study found the same log  $K_{ZnBL}$  value (5.31), but a lower  $(f_c)_{Zn^{50\%}}$  value (0.13) than in their earlier study. The toxicity data were reanalysed in the present study, with values for log  $K_{ZnBL}$  and  $(f_c)_{Zn^{50\%}}$  of 6.14 and 0.45 determined (Table S1). Although the reanalysed data appear quite different to the values obtained by Heijerick et al. (2005a), the two values from each study when used together lead to very similar values for the  $EC_{50}$ , as would be expected. It should be noted that the value determined for  $(f_c)_{Zn^{50\%}}$  in the present study (0.45) is identical to that found for *D. magna* with respect to nickel toxicity (from the studies of Deleebeeck et al. (2008) and Mano and Shinobara (2020)).

Okamoto et al. (2015) also studied the toxic effects of zinc to *D. magna* at a water hardness of 72 mg/L (as  $CaCO_3$ ) using a 48 h acute immobilisation test. Zinc toxicity values from other studies (Biesinger and Christensen, 1972; Khangarot and Ray, 1989; Rathore, 2001) at hardness values of 45 and 240 mg/L (as  $CaCO_3$ ) were cited in the study. The data from these studies were used to determine an average zinc toxicity at the average hardness, as there was not a reasonable relationship between hardness and toxicity from the studies. From the average values, log  $K_{ZnBL}$  was determined by correcting for the concentrations of  $H^+$ ,  $Na^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$ . The obtained value (5.88) is listed in Table S1. The value is in reasonable agreement with the other three zinc toxicity values given for *D. magna*, even though the analysis methodology used on, and, therefore, for the log  $K_{ZnBL}$  value obtained from the data cited by Okamoto et al. (2015), is more uncertain.

Another study on the toxicity of zinc to *D. magna* was conducted by Van Regenmortel et al. (2017) using a 21 d reproduction assay. The study examined toxicity in two synthetic waters and four European natural waters. A log  $K_{ZnBL}$  value of 5.31 was obtained in the study, the same value as obtained by Heijerick et al. (2002a) for the same species, but the  $(f_c)_{Zn^{50\%}}$  value of 0.12 was considerably lower than that found by Heijerick et al. (2002a). The values determined by Van Regenmortel et al. (2017) are retained in the present study (Table S1). This value is considered different to that obtained by Heijerick et al. (2002a), even though it was adopted from that study, because of the very different  $(f_c)_{Zn^{50\%}}$  value determined and used in the study. If 0.45 were used for the  $(f_c)_{Zn^{50\%}}$  value, then the log  $K_{ZnBL}$  value would increase to 6.10.

Muyssen and Janssen (2005) studied the toxicity of zinc to *D. magna* and found that the 48 h immobilisation ranged between 608 and 713 mg/L for acute tests conducted over six generations of organisms, with a central  $EC_{50}$  of 661 mg/L. The free zinc ion was found to dominate zinc speciation in the test water used and after considering the effect of the ameliorative cation concentrations, a log  $K_{ZnBL}$  value of 5.67 was determined.

The toxicity of zinc to *D. magna* was studied by Paulauskis and Winner (1988) at variable hardness and humic acid concentrations. As expected, the toxicity of zinc to the organisms decreased as both hardness and the humic acid concentration increased. Taking account of zinc speciation in the test waters and the ameliorative cation concentrations led to a log  $K_{ZnBL}$  value of 6.09. This value is listed in Table S1.

Paylar et al. (2022) studied the toxicity of zinc to *D. magna* in waters of variable hardness. Toxicity was found to decrease as the water hardness increased. The study determined zinc speciation and found that the free ion was the dominant zinc species in all test waters. Accounting for the ameliorative cation concentrations led to a log  $K_{ZnBL}$  value of 5.88, as listed in Table S1.

Zinc toxicity to *D. magna* was studied by Mount and Norberg (1984) at pH 7.3 and test waters of relatively low hardness. The free zinc ion accounted for the vast majority (94.8 %) of total zinc. For *D. magna*, a toxicity of 68 mg/L was determined, which led to a log  $K_{ZnBL}$  value of 6.24 (Table S1) when the ameliorative cation concentrations were considered.

### S1.5. Other *Daphnia* sp.

There are four other studies that have examined the toxicity of zinc to other *Daphnia* species. The log  $K_{ZnBL}$  values derived for these cladoceran species are consistent with those derived for *D. magna*.

Cooper et al. (2009) studied the toxicity of zinc to *D. carinata* using 48 h mortality assays. The  $LC_{50}$  concentration determined for *D. carinata* was 339.8 mg/L. The  $Zn^{2+}$  ion was the dominant species of zinc present in the test solutions. The derived log  $K_{ZnBL}$  value determined from the study was 5.70 when the concentrations of the ameliorative cations were considered. The derived binding constant is listed in Table S1.

Mount and Norberg (1984) also studied zinc toxicity to *D. pulex* at pH 7.3 and test waters of relatively low hardness. Again, the free zinc ion accounted for the vast majority (93.4 %) of total zinc. For this *Daphnia* species, a larger toxicity of 107 mg/L (compared to their value for *D. magna*) was determined, which led to a log  $K_{ZnBL}$  value of 6.05 (Table S1) when the ameliorative cation concentrations were considered.

Clifford and McGeer (2009) also studied the toxicity of zinc to *D. pulex* in soft waters. However, the toxicity was also examined at varying pH levels (6.32 to 8.01) and concentrations of  $Ca^{2+}$  (1.6 to 60 mg/L),  $Mg^{2+}$  (0.24 to 15 mg/L),  $Na^{+}$  (2.3 to 27 mg/L) and  $K^{+}$  (0.39 to 54 mg/L). The study found a value for log  $K_{ZnBL}$  of 5.6. The reanalysis of the data in the present study led to a value for log  $K_{ZnBL}$  of 5.70 (Table S1), in good agreement with the value found in the study.

Stauber et al. (2023) studied the toxicity of zinc to *D. thomsoni* in New Zealand river waters of differing conditions. Although the free zinc ion was the dominant zinc species in all the test waters studied, it varied from 55.0 to 76.9 % of total zinc. The hardness and pH of the river waters was also variable, but when speciation and ameliorative cation concentrations were considered, reasonably consistent binding constants were derived, with an average value of log  $K_{ZnBL}$  of 6.27.

The average binding constant for the four studies on other *Daphnia* species is log  $K_{ZnBL}$  = 5.93. This value is in excellent agreement with that determined for *D. magna* of  $5.83 \pm 0.26$ . Moreover, it is also in very good agreement with all the data derived for fish species.

### S1.6. *Ceriodaphnia dubia*

Like *D. magna*, there have also been eight studies on the toxicity of zinc to *Ceriodaphnia dubia*, with log  $K_{ZnBL}$  values ranging between 5.55 and 6.28 (average of  $5.90 \pm 0.20$ ). The lowest value is from the study of Mebane et al. (2021) and the largest from Naddy et al. (2015). The average log  $K_{ZnBL}$  value of 5.90 is consistent with those found for both *D. magna* and *O. mykiss* as well as more broadly for other freshwater species. Only two values were used for  $(f_c)_{M^{50\%}}$ , either the relatively low value derived from the study of Keithly et al. (2004) or what might be regarded as a more standard value of 0.5. The lower value was used for the data from only two of the eight studies on *C. dubia*.

Cooper et al. (2009) also studied the toxicity of zinc to *C. dubia* using 48 h mortality assays. The  $LC_{50}$  concentration determined was 21.8 mg/L. This toxicity concentration endpoint was considerably different to the concentration they found for *D. carinata*; however, if the  $(f_c)_{M^{50\%}}$  from Keithly et al. (2004) is used for the response of *C. dubia* to the toxicity of zinc, the log  $K_{ZnBL}$  value determined for the species is 5.63, which is in very good agreement with the value derived for *D. carinata* from the study. This binding constant is also given in Table S1.

Nys et al. (2016b) also studied the toxicity of zinc to *C. dubia* and found an average  $EC_{50}$  zinc concentration of 890 nmol/L. They also studied the toxicity of nickel and lead to *C. dubia*, where for both the latter metals it was necessary to combine the measured  $EC_{50}$  together with the  $(f_c)_{Ni^{50\%}}$  value derived from the study of Keithly et al. (2004) to determine the values of log  $K_{MBL}$ . However, it would appear that in the study of Nys et al. (2016b) that *C. dubia* is less sensitive to zinc exposures, whereas it was substantially more sensitive to nickel and lead exposures. As such, the obtained log  $K_{ZnBL}$  value (corrected for pH and the concentrations of  $Na^{+}$ ,  $Ca^{2+}$  and  $Mg^{2+}$ ) is 6.10 (Table S1). It should be noted that *C. dubia*, when exposed to zinc in the study of Cooper et al. (2009), was much more sensitive than the same species in the study

of Nys et al. (2016b). There do not appear to be differences in the water quality of the test solutions in the two studies that could explain the substantial difference in the sensitivity of *C. dubia* in the two studies.

A third study examining the toxicity of zinc to *C. dubia* was conducted by Hyne et al. (2005) where a 48 h immobilisation assay was utilised. The effect of pH (5.5 to 7.5) was studied and, when corrected for pH and the concentrations of the major ions, the  $EC_{50}$  was found to range between 65.6 (pH 7.5) and 128 (pH 6.5) mg/L, with an average of 90.4 mg/L. This latter value leads to a  $\log K_{ZnBL}$  of 5.86 (Table S1), also assuming the  $(f_c)_{Zn^{50\%}}$  value is 0.50.

Stauber et al. (2023) studied the toxicity of zinc to *C. dubia* in Australian river/creek waters of differing conditions. In the present study, the 7 d reproduction endpoint data were used (Woronora River, Jingellic Creek). Again, the free zinc ion was the dominant zinc species in the test waters studied, varying from 74.9 to 78.5 % of total zinc. The hardness and pH of the river waters was also variable, but when speciation and ameliorative cation concentrations were considered, very consistent binding constants were derived, with an average value of  $\log K_{ZnBL}$  of 5.97, without invoking a lower  $(f_c)_{Zn^{50\%}}$  value than 0.5.

The study of Diamond et al. (1997) also examined the toxicity of zinc to *C. dubia* in both laboratory water and Lehigh River water sampled at different times of year. The geometric average of the  $LC_{50}$  data for the laboratory water was used in the present analysis and gave a concentration of 189 mg/L. The use of this toxicity concentration, together with the zinc speciation ( $Zn^{2+}$  is 86.3 % of total zinc) and the ameliorative cation concentrations, resulted in a  $\log K_{ZnBL}$  value of 5.98 (Table S1).

One of the four freshwater species studied by Schubauer-Berigan et al. (1993) was *C. dubia*, with toxicity assessed as a function of pH. For *C. dubia*, the toxicity was found to depend on pH and the  $\log K_{ZnBL}$  value (5.84) was determined from the average toxicity with respect to the  $H^+$  concentration. This average  $\log K_{ZnBL}$  value is listed in Table S1.

Naddy et al. (2015) also studied the toxicity of zinc to *C. dubia*. The effect of varying hardness on the toxic response was also considered. The average toxicity in soft water was utilised together with that in hard water. The hard water also had elevated alkalinity and pH compared to the soft water. The  $Zn^{2+}$  ion dominated zinc speciation in the soft water, but was a much smaller fraction (0.32) of the hard water. When accounting for the speciation and the effects of the ameliorative cations, an average  $\log K_{ZnBL}$  of 6.28 was derived (Table S1).

Mebane et al. (2021) also studied the toxicity of zinc to *C. dubia* using varying exposure periods. The toxicity was almost invariant over exposure periods of 36 h to 96 h. The 48 h  $LC_{50}$  was used in the present study. This value, together with the fraction of zinc as the free ion (the dominant species in the test water) and the effect of the ameliorative cations, as well as utilising the  $(f_c)_{M^{50\%}}$  from Keithly et al. (2004), led to a  $\log K_{ZnBL}$  value of 5.55, as listed in Table S1.

### S1.7. Other Ceriodaphnia sp.

There is only a single study that has examined the toxicity of zinc to other *Ceriodaphnia* species. Mount and Norberg (1984), in addition to studying *D. magna* and *D. pulex*, also studied zinc toxicity to *C. reticulata* at a pH of 7.3 and with test waters of relatively low hardness. As with the studies of the two other cladoceran species, the free zinc ion accounted for the vast majority (94.8 %) of total zinc. The toxicity found for *C. reticulata* was similar to that found for the other two species (i.e., 76 mg/L). This toxicity led to a  $\log K_{ZnBL}$  value of 6.19 (Table S1) when the ameliorative cation concentrations were considered. The binding constant derived is consistent with other values determined for the toxicity of zinc to freshwater species.

### S1.8. Other Crustacean Species

There have been five other studies on the toxicity of zinc to freshwater crustacean species. Four of these are on other cladoceran species, with two of those on *Hyaella azteca*. The fifth study is on the prawn, *Macrobrachium rosenbergi*. The average  $\log K_{ZnBL}$  of these five studies is 5.94, in excellent agreement with those of other crustacean species and, more broadly, other freshwater species.

Another of the four freshwater species studied by Schubauer-Berigan et al. (1993) was *H. azteca*, with toxicity assessed as a function of pH. For this species, the toxicity at pH 8-8.5 was used to determine the log  $K_{ZnBL}$  value (5.86; Table S1), accounting for the zinc speciation in the test solution.

Poynton et al. (2019) also examined the toxicity of zinc to the freshwater amphipod *Hyaella azteca* in comparison with that of nanoparticulate ZnO. The  $LC_{50}$  concentration of zinc was found to be 260 mg/L. A reasonably large correction was needed for the ameliorative cations due to a moderate hardness of 110 mg/L (as  $CaCO_3$ ). The log  $K_{ZnBL}$  value determined from the study was 5.83, as given in Table S1, and in very good agreement with the value calculated for the same species from the study of Schubauer-Berigan et al. (1993).

Trenfield et al. (2023) also studied the toxicity of zinc to *Moinodaphnia macleayi* (96 h reproduction assay) in waters of low hardness. Speciation calculations indicated that the free zinc ion was the dominant zinc species, as 55.6 % of total zinc. The  $EC_{50}$  concentration determined for the species was 64 mg/L. Combining the zinc toxicity concentrations with the fraction of zinc as the free ion leads to a log  $K_{ZnBL}$  value of 6.26 (Table S1).

Zou and Bu (1994) studied the toxicity of zinc to *Moina irrasa* in very soft water at pH 8. The toxicity found in the study was 59.24 mg/L using a 48 h mortality endpoint. The magnitude of the adjustment of this value is quite small due to the relatively high pH and very soft water that led to a selected log  $K_{ZnBL}$  value of 6.18 (as listed in Table S1).

The toxicity of zinc to *M. rosenbergi* was studied by Satapornvanit et al. (2009) using a 48 h mortality endpoint. The test waters were at pH 7.2 and used a relatively low hardness and alkalinity and, as such, the zinc ion was the dominant zinc species in the test water. The measured  $LC_{50}$  (329 mg/L), adjusted for speciation and ameliorative cation concentrations, led to a selected log  $K_{ZnBL}$  value of 5.58 (Table S1).

### S1.9. *Chlorella* sp.

There are four studies that examined the toxicity of zinc to *Chlorella* sp. The log  $K_{ZnBL}$  values determined from the four studies cover a relatively narrow range of 5.77 to 5.92, with an average value of 5.83. This average value is in very good agreement with other average values found for log  $K_{ZnBL}$  for other freshwater species and groups of species.

Wilde et al. (2006) measured zinc uptake in, and toxicity to, *Chlorella* sp. as a function of pH using a 48 h growth assay. At a pH of 7.5, the study reported a log  $K_{ZnBL}$  value of 5.8, which is consistent with the values for this parameter determined from the data of other studies of zinc toxicity to *Chlorella* sp. At the lower pH of 6.5, however, the study found a lower value for log  $K_{ZnBL}$  of 5.0, a value that is not consistent with those from other studies. The value of 5.8 is retained in the present study (Table S1).

Franklin et al. (2002a) studied the toxicity of zinc to *Chlorella* sp. using 48 h and 72 h growth assays. The pH of the synthetic water used in the study was 7.5. Using the average of the  $EC_{50}$  concentrations determined at the two endpoints, a log  $K_{ZnBL}$  value of 5.92 was calculated (Table S1), in good agreement with the study of Wilde et al. (2006) at the same pH.

Johnson et al. (2007) used a 72 h growth assay to examine the toxicity of zinc to *Chlorella* sp. also using synthetic solutions with a pH of 7.5. Based on the  $IC_{50}$  value found for zinc (110 mg/L) and the fraction of zinc determined to be in the form of the free ion,  $Zn^{2+}$ , a log  $K_{ZnBL}$  value of 5.83 was determined, in good agreement with the previous two studies on this species (Table S1).

In another study examining the toxicity of zinc to *Chlorella* sp., Price et al. (2023) used a 72 h growth assay to determine the  $EC_{50}$  in a buffered, no-dissolved-organic-matter synthetic control water. The log  $K_{ZnBL}$  value determined for this control was 5.77 (Table S1). The pH of the control water was 7.7, similar to the other studies that have assessed the toxicity of zinc to *Chlorella* sp. Given the similarity of the pH, it is not possible to assess further whether the toxicity of zinc to *Chlorella* sp. decreases with a decrease in pH, as was found by Wilde et al. (2006).



#### S1.10. *Raphidocelis subcapitata*

There are nine studies considered that have examined the toxicity of zinc to *Raphidocelis subcapitata*. The log  $K_{ZnBL}$  values determined the range from 5.81 to 6.33, with an average of  $6.09 \pm 0.13$ . Although the average value is somewhat higher than those found for other species, the value is still consistent with the other values within the obtained 95 % confidence intervals.

The variation of major ions ( $Na^+$  (62 to 166 mg/L),  $K^+$  (3.1 to 44 mg/L),  $Ca^{2+}$  (4.8 to 80 mg/L) and  $Mg^{2+}$  (2.9 to 61 mg/L)) and pH (5.6 to 7.8) on the toxicity of zinc to *R. subcapitata* was studied by Heijerick et al. (2002b) using a 72 h growth inhibition assay in synthetic waters. Analysis of the toxicity data from the study led to values for log  $K_{ZnBL}$  and  $(f_c)_{Zn^{50\%}}$  of 6.15 and 0.25, respectively. The log  $K_{ZnBL}$  value is listed in Table S1.

The same zinc toxicity assay endpoint (72 h growth) was used by Franklin et al. (2007) on the same species (*R. subcapitata*). The toxicity ( $IC_{50}$ ) concentrations found in Zn-only experiments was 60 mg/L. Under the conditions studied,  $Zn^{2+}$  was the predominant form (89 %) of zinc in solution which led to a calculated value for log  $K_{ZnBL}$  of 6.09 (Table S1), consistent with that determined from the study of Heijerick et al. (2002b). The pH in the study was maintained at 7.5.

Earlier, Franklin et al. (2001) also studied the toxicity of zinc to *R. subcapitata* using the same assay endpoint. In this study, a lower  $EC_{50}$  value of 38 mg/L was determined at the same pH as the later study and in waters of very low hardness. The relatively low  $EC_{50}$ , however, even considering the low hardness, led to a relatively large log  $K_{ZnBL}$  value of 6.33 (Table S1).

Graff et al. (2003) also studied the toxicity of zinc to *R. subcapitata* using the same 72 h growth assay used in the previous two studies. The study used the International Standards Organisation (ISO) medium to determine an  $EC_{50}$  of 56 mg/L (similar to that found by Franklin et al. (2007)). The free zinc ion was also the dominant species in solution in the water tested, but only accounted for 67 % of total zinc. Based on these data, the log  $K_{ZnBL}$  value determined was 6.17 (Table S1). The pH of the test media used was 8.2.

Van Regenmortel et al. (2017) also studied the toxicity of zinc to *R. subcapitata* using the same 72 h growth inhibition assay. Analysis of the results obtained in the study led to a log  $K_{ZnBL}$  value of 5.91 (Table S1). This value was based on the average  $EC_{50}$  value determined over the range of pH (5.85 to 8.53) studied. However, the study concluded that the zinc  $EC_{50}$  concentration is dependent on pH and this needs to be considered when considering the effect of zinc on *R. subcapitata*. These results differ from the toxicity of zinc to *D. magna* found in the same study.

Filova et al. (2021) studied the toxicity of several metals including zinc to *R. subcapitata* in waters of relatively low hardness. The study determined a 96 h growth endpoint of 200 mg/L where the zinc ion was the dominant form of zinc (93.4 %) of zinc in the test solutions. Using the  $EC_{50}$  concentration and the zinc speciation, together with the ameliorative cation concentrations, led to a log  $K_{ZnBL}$  value of 5.81, as is listed in Table S1.

The toxicity of zinc to *P. subcapitata* was also studied by Alves et al. (2017) in waters of relatively low hardness and at a pH of 7. A 72 h growth endpoint of 91 mg/L was determined in the study. The correction for ameliorative cations was quite small and the zinc ion was the dominant (89.2 %) form of zinc in the test water, leading to a log  $K_{ZnBL}$  value of 6.08, as is listed in Table S1.

Blaise et al. (1986), in studying the toxicity of zinc to *P. subcapitata*, found similar  $EC_{50}$  concentrations using two differing endpoints (cell count and ATP) of 44.7 and 55.0 mg/L, with an average value of 49.9 mg/L. The pH of the test solutions was 7 and the hardness was relatively low, with the zinc ion (94.8 %) dominating the zinc speciation. These conditions led to a log  $K_{ZnBL}$  value of 6.25 (Table S1).

Stauber et al. (2023) also studied the toxicity of zinc to *P. subcapitata* in both a reference water and several New Zealand river waters. In the reference water (pH 7.6 and relatively low hardness), an  $EC_{50}$  concentration of 99 mg/L was determined, which was similar to the  $EC_{50}$  values found for the river waters, which had varying hardness and DOC concentrations.

Considering speciation and the ameliorative cation concentrations, a log  $K_{ZnBL}$  value of 5.99 was determined (Table S1).

#### S1.11. Mollusc Species

There have been studies into twelve species of molluscs, with each species being studied on a single occasion. Within these studies, three *Hyridella* sp. were examined in the same study (Markich, 2017), two *Velesunio* sp. and also two *Lampsilis* sp. in the same study (Wang et al., 2010b). The log  $K_{ZnBL}$  values determined for these twelve mollusc species ranged between 5.70 and 6.32, with an average value of  $6.02 \pm 0.13$ . This value is again in good agreement with all other values found for the toxicity of zinc to freshwater species.

Trenfield et al. (2023) studied the toxicity of zinc to several species including *Amerianna cumingi* (6 d reproduction assay) and *Velesunio angasi* (24 h mortality assay) in waters of low hardness. Speciation calculations indicated that the free zinc ion was the dominant zinc species, with contents of 64.9 and 67.3 % of total zinc, respectively. The  $EC_{50}/LC_{50}$  concentrations determined for these two mollusc species were 68 and 52 mg/L, respectively. Combining these zinc toxicity concentrations with the fraction of zinc as the free ion leads to log  $K_{ZnBL}$  values of 6.17 and 6.27 (Table S1).

The toxicity of zinc to a New Zealand freshwater mussel, *Echyridella menziesii*, was studied by Clearwater et al. (2014) using three natural waters from the North Island of New Zealand, employing a survival assay. In the waters studied, the free zinc ion ( $Zn^{2+}$ ; 88 %) was the dominant zinc species, with the average  $LC_{50}$  concentration from the three lake waters being used to derive the log  $K_{ZnBL}$  value, which was found to be 5.70 (Table S1).

Wang et al. (2010b) studied the toxicity of metals, including zinc, to the early life stages of freshwater mussels using various mortality assays (1 to 28 days). For juvenile fatmucket, *Lampsilis siliquoides*, the 96 h assay led to reportable  $LC_{50}$  concentrations (151 and 175 mg/L). Free zinc (78.5 %) was the dominant zinc species and correcting for the effect of the ameliorative cations, and using the average  $LC_{50}$ , led to a log  $K_{ZnBL}$  value of 6.27 (Table S1). Similar analyses in the same study on *Lampsilis rafinesqueana* led to a similar log  $K_{ZnBL}$  value of 6.32, as also listed in Table S1.

Mortality (24 to 72 hours) studies of six freshwater mussels (glochidia: *Alathyria profuga*, *Cucumerunio novaehollandiae*, *Hyridella australis*, *Hyridella depressa*, *Hyridella drapeta* and *Velesunio ambiguus*) were undertaken by Markich (2017) to study the sensitivity of the mussel glochidia to exposures of metals, including zinc. The study found an average value for log  $K_{ZnBL}$  of 6.10, with a range of 5.96 to 6.23 (48 h mortality data). The free zinc ion (96.8 %) was the dominant species in solution. Reanalysis of the toxicity data in the present study led to marginally smaller log  $K_{ZnBL}$  values, ranging between 5.84 and 6.04, with an average of 5.94. All six values are listed in Table S1.

Hoang and Tong (2015) studied the toxicity of zinc to the apple snail (*Pomacea papudosa*) under varying conditions of pH, hardness and DOC. The 96 h mortality data were found to be quite variable depending on conditions, but when speciation and ameliorative cation concentrations were considered, the toxicity was quite similar, with an average log  $K_{ZnBL}$  value determined of 5.81 (Table S1).

#### S1.12. Macrophyte Species

There has only been a single study examining the toxicity of zinc to a macrophyte species. Naumann et al. (2007) studied the toxicity of a range of metals to *Lemna minor* in relatively hard water. The zinc ion was found to be only 39 % of total zinc in the test solutions, where the observed toxicity occurred at a concentration of 627 mg/L. Utilising the  $EC_{50}$  concentration and the zinc speciation, together with the ameliorative cation concentrations, led to a log  $K_{ZnBL}$  value of 6.22.

#### S1.13. Cnidaria Species

There has also only been a single study with respect to the toxicity of zinc to cnidaria species. Trenfield et al. (2023) also studied the toxicity of zinc to *Hydra viridissima* (96 h growth

assay) in waters of low hardness. Speciation calculations indicated that the free zinc ion was the dominant zinc species, being 64.3 % of total zinc. The EC<sub>50</sub> concentration determined for the species was 74 mg/L. Combining the zinc toxicity concentrations with the fraction of zinc as the free ion leads to log  $K_{ZnBL}$  values of 6.14 (Table S1).

#### S1.14. Rotifer Species

The toxicity of zinc to five individual rotifer species has been studied. The values determined for log  $K_{ZnBL}$  for the five species range between 5.50 and 6.27, with an average value of 5.96, again in very good agreement with other values found for the toxicity of zinc to freshwater aquatic species.

Sarma et al. (2007) studied the toxicity of zinc to the two freshwater rotifers, *Anuraeopsis fissa* and *Brachionus rubens*, in test waters of moderate hardness and at a pH of 7.2. The measured LC<sub>50</sub> values were 315 and 554 mg/L, respectively. Based on these concentrations, the fraction of zinc as the free ion (about 89 % for both species) and the ameliorative cation concentrations, log  $K_{ZnBL}$  values of 5.75 and 5.50, respectively, were determined. These values are listed in Table S1.

Hernández-Flores et al. (2020) studied the toxicity of several metals to the rotifer, *Euchlanis dilatata*, using both 24 h mortality and 5 d reproduction assays. There was no trend in the two toxicity endpoints, with either the acute or chronic endpoint having the lower concentration for a particular metal. As such, both concentrations were used in the present study to determine binding constants, from which the average value was determined. For zinc, when speciation (the free ion was the dominant form of zinc) and ameliorative cation concentrations were considered, the average log  $K_{ZnBL}$  value of 6.12 was determined. This value is listed in Table S1.

The toxicity of zinc to the rotifer, *Lecane inermis*, in water of relatively high hardness and at pH 7.4 was studied by Klimek et al. (2013). From speciation calculations, it was determined that the free ion was the dominant form of zinc (85.7 %) in the test solutions. From the speciation, the determined LC<sub>50</sub> and the ameliorative cation concentrations, a log  $K_{ZnBL}$  value of 6.27 was determined. This value is listed in Table S1.

Guzman et al. (2010) studied the toxicity of zinc to another *Lecane* sp., *L. quadridentata*, at pH 7.5 and in test water of moderate hardness. Again, the free ion was found to be the dominant (84.6 %) form of zinc in the test solutions, and considering this and the observed toxicity (123 mg/L), as well as the ameliorative cation concentrations, a log  $K_{ZnBL}$  value of 6.18 was derived (Table S1).

#### S1.15. Insect Species

Only a single study of the toxicity of zinc to an insect species was reviewed. Anderson et al. (1980) studied the toxicity of several metals to the insect, *Tanytarsus dissimilis*. For zinc, an LC<sub>50</sub> concentration of 37 mg/L was determined, which is a relatively low concentration. The mortality concentrations of the other metals studied were also relatively low, which enabled a  $(f_c)_{M^{50\%}}$  value of 0.258 to be assigned to all four metals studied. Based on this and the LC<sub>50</sub> value, the zinc speciation and the ameliorative cation concentrations, a log  $K_{ZnBL}$  value of 6.09 was determined, as is listed in Table S1.

## S2. Nickel

### S2.1. Fish Species

Six studies are given for nickel toxicity to fish species, three for *O. mykiss* and three for *P. promelas*. The values determined for log  $K_{NiBL}$  from the six studies range between 4.47 and 5.14, with an average of 4.70. The range and binding constant are consistent with that found across all aquatic freshwater species.

Pane et al. (2006) studied the uptake of nickel in the renal brush border membrane vesicles (BBMVs) of rainbow trout (*O. mykiss*). The uptake rate of nickel in the BBMVs could be explained by an expression similar in part (transport term) to equation (2):

$$\text{Uptake Rate} = \frac{J_{\max}[\text{Ni}]}{K_m + [\text{Ni}]} + m[\text{Ni}] \quad (\text{S1})$$

where the first term on the right-hand side is the transport term;  $J_{\max}$  is the maximal rate of transport and  $K_m$  is the affinity constant, while the second term is a linear diffusive component where  $m$  is the slope of the component. Four separate conditions were studied (unacclimated control, unacclimated and acutely Ni-exposed, chronically acclimated control, chronically acclimated and acutely Ni-exposed), where it was found that the calculated affinity constant,  $K_m$ , within the measured uncertainties, was the same for all four conditions and averaged 19.6 mmol/L. The affinity constant is related to the Ni binding constant for cell surface sites, namely  $K_{\text{NiBL}} = 1 / K_m$ . The average binding constant ( $\log K_{\text{NiBL}} = 4.71$ ) determined from the study is listed in Table S1.

Deleebeeck et al. (2007a) also studied nickel toxicity to *O. mykiss*. Instead of uptake, however, this study determined the median 17-day lethal nickel concentrations to juvenile rainbow trout. The amelioration effect on nickel toxicity as a result of the presence of  $\text{Ca}^{2+}$  (3.64 to 110 mg/L),  $\text{Mg}^{2+}$  (2.85 to 72 mg/L) and  $\text{H}^+$  (pH 5.48 to 8.47) was also studied. The study determined  $\log K_{\text{NiBL}}$  values for twenty different conditions that included synthetic and five natural waters. The average binding constant (4.89) was calculated from the  $\log K_{\text{NiBL}}$  values listed in the study and is given in Table S1 (for the constant, three values were quoted as less-than values and, as such, were not included in this assessment). From their toxicity results in natural waters, a value of  $(f_c)_{\text{Ni}^{50\%}}$  equal to 0.22 has been determined for the fraction of cells bound by nickel at the  $\text{LC}_{50}$ .

A third study on nickel toxicity to *O. mykiss* was carried out by Brix et al. (2004). From acute toxicity measurements, a 96 h  $\text{LC}_{50}$  value of 20.8 mg/L was determined at a hardness of 91 mg/L (as  $\text{CaCO}_3$ ). The study listed data from two other studies (Nebeker et al., 1985; Pane et al., 2003) that also studied nickel toxicity to *O. mykiss* (with 96 h  $\text{LC}_{50}$  concentrations of 10 and 15.3 mg/L, respectively), but at differing hardness concentrations (33 and 140 mg/L (as  $\text{CaCO}_3$ )). The results from the three studies were combined to remove the effect of hardness (i.e., the data from the other two studies were corrected to the hardness (91 mg/L (as  $\text{CaCO}_3$ )) used by Brix et al. (2004)) and were then further corrected for the free nickel ( $\text{Ni}^{2+}$ ) concentration (which was found to be 25 % of total nickel) and also for the concentrations in the test solutions of  $\text{H}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  that can ameliorate the toxicity of metals to aquatic organisms. The obtained  $\log K_{\text{NiBL}}$  (4.47) from this evaluation is listed in Table S1.

Hoang et al. (2004) studied the toxicity of nickel to juvenile fathead minnows (*P. promelas*) with varying hardness (12 to 150 mg/L (as  $\text{CaCO}_3$ )), alkalinity (3.3 to 375 mg/L (as  $\text{CaCO}_3$ )), dissolved organic carbon (DOC: 0.5 to 10.3 mg/L) and pH (6.07 to 8.81) using a 96 h mortality test. Using changes in the water quality parameters outlined, a total of 56 test conditions were studied by Hoang et al. (2004), but only the first 12 were used in the present study to determine a  $\log K_{\text{NiBL}}$  value (the first 12 conditions had low alkalinity ( $\leq 27$  mg/L (as  $\text{CaCO}_3$ )), and a very low DOC (0.5 mg/L), and, therefore, the main Ni species in solution will be  $\text{Ni}^{2+}$ ; they also had variable hardness (20 to 150 mg/L (as  $\text{CaCO}_3$ )) and variable pH (6.53 to 8.20). In each of these 12 conditions, the  $\text{LC}_{50}$  concentration determined was modified for the concentrations of  $\text{H}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  using the  $\log K_{\text{NiBL}}$  values determined in this study (see below) to determine a value for  $\log K_{\text{NiBL}}$  (5.14; see Table S1).

Meyer et al. (1999) also studied the toxicity of nickel to *P. promelas* at varying calcium concentrations (0 to 361 mg/L) using a 96 h mortality test. The data were evaluated in the present study to determine both  $\log K_{\text{NiBL}}$  and  $(f_c)_{\text{Ni}^{50\%}}$ . The value determined for  $(f_c)_{\text{Ni}^{50\%}}$  (0.50) indicates, within uncertainty, that the  $\text{LC}_{50}$  occurs when 50 % of the cell surface sites are occupied by nickel. The value determined for  $\log K_{\text{NiBL}}$  (4.48) is listed in Table S1.

Schubauer-Berigan et al. (1993) measured the toxicity of nickel to four freshwater species that included *P. promelas* as a function of pH. As with their data for zinc, the toxicity to *P. promelas* was found to depend on pH and the  $\log K_{\text{NiBL}}$  value (4.52) was determined from the intercept of toxicity against the  $\text{H}^+$  concentration. This value is listed in Table S1. The toxicity values reported for *L. variegatus* in the study were large, suggesting the species may have a mechanism for protecting itself against exposures to nickel.

## S2.2. *Daphnia magna*

Six studies are reported with toxicity data for *D. magna* with respect to its response to nickel. The values determined for  $\log K_{\text{NiBL}}$  for *D. magna* range between 4.54 and 5.18, with an average of  $4.85 \pm 0.25$ . These values are in good agreement with the range and average  $\log K_{\text{NiBL}}$  values determined for other species.

Deleebeeck et al. (2008) studied the acute toxicity of nickel to *D. magna* that included the amelioration effect of increases in concentration of  $\text{H}^+$  (pH 5.95 to 8.13),  $\text{Na}^+$  (1.79 to 322 mg/L),  $\text{Ca}^{2+}$  (6.63 to 181 mg/L) and  $\text{Mg}^{2+}$  (5.18 to 111 mg/L) in synthetic water and also studied the toxicity in eight natural waters (in Europe) using 48 h immobilisation assays. The synthetic data were reanalysed in the present study to determine both the binding constant,  $\log K_{\text{NiBL}}$ , the stability constants of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  with cell surfaces (see below) and the fraction of cells bound by nickel at the  $\text{EC}_{50}$ ,  $(f_{\text{c}})_{\text{Ni}^{50\%}}$ . The  $\log K_{\text{NiBL}}$  value determined (4.67) is listed in Table S1 and the value found for  $(f_{\text{c}})_{\text{Ni}^{50\%}}$  was 0.45. The values found for both  $\log K_{\text{NiBL}}$  and  $(f_{\text{c}})_{\text{Ni}^{50\%}}$  are larger than those reported by Deleebeeck et al. (2008) [4.0 and 0.21, respectively], however, as might be expected, the values of  $(f_{\text{c}})_{\text{Ni}^{50\%}}/(1-(f_{\text{c}})_{\text{Ni}^{50\%}}) \cdot 1/K_{\text{NiBL}}$  (i.e., the nickel concentration at the  $\text{EC}_{50}$  when corrected for all amelioration effects) determined from the data given by Deleebeeck et al. (2008) and in the present study are quite similar.

Mano and Shinohara (2020) also studied the acute toxicity of nickel to *D. magna* in natural waters (five Japanese rivers) using a 48 h immobilisation endpoint (they also determined a 48 h mortality endpoint). Their immobilisation data were re-evaluated in the present study to determine both  $\log K_{\text{NiBL}}$  and  $(f_{\text{c}})_{\text{Ni}^{50\%}}$  values, using  $\log K_{\text{MBL}}$  values from the studies of Deleebeeck et al. (2008) [Ca and Mg] and Heijerick et al. (2005a) [Na and H]. The value obtained for  $(f_{\text{c}})_{\text{Ni}^{50\%}}$  (0.45) is identical to that obtained from the study of Deleebeeck et al. (2008), whereas the value of  $\log K_{\text{NiBL}}$  (5.01; Table S1) is slightly larger. Mano and Shinohara (2020) used a  $\log K_{\text{NiBL}}$  value of 4.0 from the work of Deleebeeck et al. (2008) and determined a value for  $(f_{\text{c}})_{\text{Ni}^{50\%}}$  of 0.090 (they determined a value of 0.11 from their mortality experiments). Again, the lower values of both  $\log K_{\text{NiBL}}$  and  $(f_{\text{c}})_{\text{Ni}^{50\%}}$  when compared to the values determined in the present study lead to similar values of  $(f_{\text{c}})_{\text{Ni}^{50\%}}/(1-(f_{\text{c}})_{\text{Ni}^{50\%}}) \cdot 1/K_{\text{NiBL}}$ .

Okamoto et al. (2015) studied the toxic effects of 50 metals to *D. magna*, including Ni, Zn, Cu, Cd, Pb and U at a water hardness of 72 mg/L (as  $\text{CaCO}_3$ ) using a 48 h acute immobilisation test. They also listed metal toxicity values from other studies (Biesinger and Christensen, 1972; Khangarot and Ray, 1989; Rathore, 2001) at hardness values of 45 and 240 mg/L (as  $\text{CaCO}_3$ ). The data from these studies were used to determine an average nickel toxicity at the average hardness, as there was not a reasonable relationship between hardness and toxicity from the studies (although the toxicity decreased with increasing hardness, as would be expected). From the average values,  $\log K_{\text{NiBL}}$  was determined (4.98) by correcting for the concentrations of  $\text{H}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . The obtained value is listed in Table S1. The value is in good agreement with the two previous nickel toxicity values given for *D. magna*, even though the analysis methodology used on and, therefore, the  $\log K_{\text{NiBL}}$  value obtained from, the data cited by Okamoto et al. (2015) is more uncertain.

Nys et al. (2016a) also studied the toxicity of nickel to *D. magna* in test waters that had mildly alkaline pH and elevated hardness. In the study, *D. magna* was found to be relatively sensitive to nickel, and a low  $(f_{\text{c}})_{\text{Ni}^{50\%}}$  value (0.028) was derived. The nickel ion was the dominant form (56.2 %) of nickel in the test solutions. Accounting for the speciation, the fraction of cells bound at the  $\text{EC}_{50}$  and the ameliorative cation concentrations led to a  $\log K_{\text{NiBL}}$  value of 4.54. This value is listed in Table S1.

The toxicity of nickel to *D. magna* was also studied by He et al. (2023) in several Chinese river waters. The toxicity to *D. magna* was greater (i.e., lower  $\text{EC}_{50}$  concentrations) than was found in the study for *R. subcapitata*. Using the water quality (pH and other ameliorative cation concentrations) for each river, together with the measured  $\text{EC}_{50}$  concentration and the  $(f_{\text{c}})_{\text{Ni}^{50\%}}$  value found for the study of Nys et al. (2016a), a  $\log K_{\text{NiBL}}$  value of 4.76 was determined (Table S1).

Pavlaki et al. (2011) also studied the toxicity of nickel to *D. magna* in hard water. The EC<sub>50</sub> concentration (289 mg/L) was adjusted for the ameliorative cation concentrations in the test water and the  $(f_c)_{Ni^{50\%}}$  value from the study of Van Regenmortel et al. (2017) for the same species. The derived log  $K_{NiBL}$  value was 5.18, as is listed in Table S1.

### S2.3. Other *Daphnia* Species

Three other studies are listed that have reported nickel toxicity data to other *Daphnia* species. The selected log  $K_{NiBL}$  values range from 4.62 to 4.89, with an average value of 4.76. The average value is consistent with that found for *D. magna*.

Kozlova et al. (2009) studied the effect of varying pH (6.3 to 8.3) and Ca<sup>2+</sup> (0.8 to 59 mg/L), Mg<sup>2+</sup> (0.24 to 35 mg/L), Na<sup>+</sup> (4.6 to 23 mg/L) and K<sup>+</sup> (1.1 to 31 mg/L) concentrations on the toxicity of nickel to *D. pulex* using a 48 h immobilisation test. Only Ca<sup>2+</sup> and Mg<sup>2+</sup> were found to ameliorate the nickel toxicity. However, the full set of data was used to determine values for log  $K_{NiBL}$  and  $(f_c)_{Ni^{50\%}}$ . The value determined for  $(f_c)_{Ni^{50\%}}$  was 0.41 which is similar to the values derived for  $(f_c)_{Ni^{50\%}}$  for *D. magna*. The log  $K_{NiBL}$  value (4.62) calculated is listed in Table S1. Kozlova et al. (2009) determined a log  $K_{NiBL}$  value of 4.87, slightly higher than that found in the present study, but it is assumed they also used a value for  $(f_c)_{Ni^{50\%}}$  of 0.5.

The toxicity of nickel to *D. pulex* (48 h mortality and then uptake) was also studied by Leonard and Wood (2013). The study used both uptake (using an expression equivalent to the first term in equation (S1)) and toxicity to determine values for log  $K_{NiBL}$ . For *D. pulex*, log  $K_{NiBL}$  values were determined in both soft and hard waters for both toxicity and uptake, with the average of the four values (4.77; the average of the two uptake values was 4.59 and the two toxicity values was 4.96) determined by Leonard and Wood (2013) listed in Table S1.

Reproduction assays (48 hours) of ten freshwater cladoceran species including *D. longispina* were utilised by Deleebeeck et al. (2007b) to study the sensitivity of the species to exposures to nickel. The log  $K_{NiBL}$  value derived from the study for *D. longispina* was 4.89. This value is listed in Table S1. The free nickel ion was the dominant species in solution. A value for  $(f_c)_{Ni^{50\%}}$  was used for the species from De Schamphalaere et al. (2007) for the same species.

### S2.4. *Ceriodaphnia dubia*

There have been four studies examined for the toxicity of nickel to *C. dubia*. In all four, the  $(f_c)_{Ni^{50\%}}$  value derived for the study of Keithly et al. (2004) was utilised. The log  $K_{NiBL}$  values derived ranged between 4.85 and 5.48, with an average value of 5.18. It is possible that the average value may be skewed high, due to the use of a single rather than individual (and lower) values (for  $(f_c)_{Ni^{50\%}}$ ) for data from two of the studies.

The study of Deleebeeck et al. (2008) examined the earlier work of Keithly et al. (2004) in which the toxicity of nickel to *C. dubia* was studied in synthetic water with hardness between 50 and 253 mg/L (as CaCO<sub>3</sub>). Deleebeeck et al. (2008) noted that *C. dubia* was more sensitive to nickel than *D. magna* and determined a value for  $(f_c)_{Ni^{50\%}}$  of 0.065 using the same log  $K_{NiBL}$  value (4.0) they determined for *D. magna*. As shown in Table S1, the present study found a larger log  $K_{NiBL}$  (4.67) from the study of Deleebeeck et al. (2008) for *D. magna* and an even larger average value for all nickel exposures (4.84: see Table S1). In assessing the Keithly et al. (2004) data in the present study, a log  $K_{NiBL}$  of 4.85 was derived together with a value for  $(f_c)_{Ni^{50\%}}$  of 0.052, similar to that derived by Deleebeeck et al. (2008), but larger than that reported by Keithly et al. (2004). The lower value of  $(f_c)_{Ni^{50\%}}$  than found for *D. magna* illustrates that *C. dubia* is more sensitive to nickel (and, as will be shown, also for other metals) than is *D. magna* and that  $(f_c)_{Ni^{50\%}}$  (and  $(f_c)_{M^{50\%}}$ , in general) can be considered a sensitivity parameter (Deleebeeck et al., 2008).

The toxicity of nickel to four freshwater species including *C. dubia* was measured by Schubauer-Berigan et al. (1993) as a function of pH. Two LC<sub>50</sub> values were determined for *C. dubia* and, as such, the average value was used to determine the log  $K_{NiBL}$  value (5.00) which included the use of the  $(f_c)_{Ni^{50\%}}$  value determined from the data of Keithly et al. (2004). The value is listed in Table S1.

Nys et al. (2016b) studied the toxicity of nickel to *C. dubia* and found an average EC<sub>50</sub> nickel concentration of 512 nmol/L. The use of this concentration, together with the  $(f_c)_{Ni^{50\%}}$  value



derived from the study of Keithly et al. (2004) for the same species, leads to the log  $K_{NiBL}$  value (5.39) listed in Table S1. Peters et al. (2018) determined  $EC_{50}$  values for several species, including *C. dubia*. Of the species studied, *C. dubia* was found to be the most sensitive. Utilisation of the data from the study of Peters et al. (2018) and again using the  $(f_c)_{Ni^{50\%}}$  value determined from the data of Keithly et al. (2004) gives the log  $K_{NiBL}$  value (5.48) listed in Table S1. It should be noted that the two log  $K_{NiBL}$  values determined for *C. dubia* are the two largest of all the log  $K_{NiBL}$  values determined for nickel. This might imply that the  $(f_c)_{Ni^{50\%}}$  value used for the data from Nys et al. (2016b) and Peters et al. (2018) should be lower than that found for the study of Keithly et al. (2004). In fact, the value determined for  $(f_c)_{Ni^{50\%}}$  by Keithly et al. (2004) for *C. dubia*, and by Schubauer-Berigan et al. (1993) for the same species, were both lower (0.011 and 0.0047, respectively) than found in the present study (0.052). A lower value for  $(f_c)_{Ni^{50\%}}$  will lead to a reduced value for log  $K_{NiBL}$ .

### S2.5. Other Ceriodaphnia Species

Reproduction assays (48 hours) of ten freshwater cladoceran species that also included *C. pulchella* and *C. quadrangula* were utilised by Deleebeeck et al. (2007b) to study the sensitivity of the species to exposures to nickel. For the two *Ceriodaphnia* species the log  $K_{NiBL}$  values obtained were 4.81 (*C. pulchella*) and 4.82 (*C. quadrangula*). These values are listed in Table S1. The free nickel ion was the dominant species in solution. Values for  $(f_c)_{Ni^{50\%}}$  were used for several of the ten species studied, utilising data determined from other studies for the same or similar species. For the two *Ceriodaphnia* species, the  $(f_c)_{Ni^{50\%}}$  value from Keithly et al. (2004) was used for *C. quadrangula* (that was derived for *C. dubia*) and for *C. pulchella*, a  $(f_c)_{Ni^{50\%}}$  value of 0.24 was used, as derived from De Schamphalaere et al. (2007).

### S2.6. Other Crustacean Species

The other seven species utilised by Deleebeeck et al. (2007b) to study the sensitivity of exposures to nickel using reproduction assays (48 hours) were *Alona affinis*, *Bosmina coregoni*, *Camptocercus lilljeborgi*, *Chydorus ovalis*, *Peracantha truncata*, *Sinocephalus serrulatus* and *Sinocephalus vetulus*. For these seven cladoceran species, the log  $K_{NiBL}$  values ranged between 4.58 and 4.99. These values are listed in Table S1. The free nickel ion was the dominant species in solution. As noted above, values for  $(f_c)_{Ni^{50\%}}$  were used for several species, utilising data determined from other studies for the same or similar species. For *S. vetulus*, *C. lilljeborgi* and *B. coregoni*, a  $(f_c)_{Ni^{50\%}}$  value of 0.24 was also used, as derived from De Schamphalaere et al. (2007).

Schroeder (2008) studied the toxic response of *H. azteca* to nickel in synthetic waters with varying pH (6.4 to 8.9) and  $Ca^{2+}$  (9.9 to 300 mg/L) and  $Mg^{2+}$  (2.6 to 47 mg/L) concentrations. A log  $K_{NiBL}$  value (5.34) was derived from the observed toxicities, correcting for the ameliorative effects of  $H^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$  (Table S1). The response of *H. azteca* to changes in pH was quite variable (see below in Protons ( $H^+$ ) section), but the use of the full range of pH data still led to a reasonable value for log  $K_{NiBL}$ . A value was also determined for  $(f_c)_{Ni^{50\%}}$  from the toxicity data (0.184), a value similar to that derived for *O. mykiss* (Deleebeeck et al., 2007a), indicating that *H. azteca* is also relatively sensitive to exposure to nickel.

One of the four freshwater species studied by Schubauer-Berigan et al. (1993) with relation to the toxicity of nickel as a function of pH was *H. azteca*. The toxicity to this species was derived from a test water at pH 8.25 and the log  $K_{NiBL}$  value derived was 5.03. This value is listed in Table S1.

Keithly et al. (2004) also studied the toxicity of nickel to *H. Azteca*, which was found to be much less sensitive to nickel exposure than was *C. dubia*. Taking the measured  $EC_{50}$  from the study, and accounting for the ameliorative cation concentrations, led to a log  $K_{NiBL}$  value of 4.75, as is listed in Table S1.

A fourth study on the toxicity of nickel to *H. azteca* was conducted by Chan (2013). An  $LC_{50}$  concentration of 560 mg/L was found, and then adjusting for the ameliorative cation concentrations led to a log  $K_{NiBL}$  value of 5.19. This value is listed in Table S1. The four log  $K_{NiBL}$  values determined for *H. azteca* ranged between 4.75 and 5.34, with an average value of 5.08.

Lebrun et al. (2011) studied the uptake of nickel into *Gammarus pulex*. The study quoted a log  $K_{NiBL}$  value determined from the uptake measurements of 4.70. This value has been included in Table S1.

There were twelve values listed for the toxicity of nickel to other crustacean species, relating to eight species. The log  $K_{NiBL}$  values determined ranged from 4.58 to 5.34. The average value was  $4.89 \pm 0.14$ . This value and its associated 95 % uncertainty interval are in good agreement with other values and intervals derived for nickel toxicity to freshwater organisms.

### S2.7. *Raphidocelis subcapitata*

Six studies were assessed where the toxicity of nickel to *R. subcapitata* was examined. The log  $K_{NiBL}$  values derived ranged between 4.42 and 4.97. The average value is  $4.71 \pm 0.22$ . Although the average value is somewhat lower than found for other species, the uncertainty intervals still have substantial overlap.

Deleebeeck et al. (2009) studied the toxicity of nickel to *R. subcapitata* in synthetic water with varying pH (6.01 to 7.95) and concentrations of  $Mg^{2+}$  (2.76 to 115 mg/L) and  $Ca^{2+}$  (2.97 to 144 mg/L) and eight natural waters. The toxicity data from the study were re-evaluated in the present study to determine both the log  $K_{NiBL}$  and  $(f_c)_{Ni^{50\%}}$  values. The derived log  $K_{NiBL}$  value (4.93) is listed in Table S1 and the value determined for  $(f_c)_{Ni^{50\%}}$  was 0.11; therefore, it is less sensitive than *C. dubia*, but more so than *D. magna*, to exposures to nickel.

The toxicity of nickel to *R. subcapitata* was also studied by dos Reis et al. (2024a, 2024b), where  $IC_{50}$  concentrations of 490 and 400 mg/L, respectively, were determined. The free nickel ion was found to be the dominant form of nickel in the test waters. Using the  $IC_{50}$  concentrations and the nickel speciation, together with the ameliorative cation concentrations, led to log  $K_{NiBL}$  values of 4.64 and 4.73, respectively. These values are listed in Table S1. The  $(f_c)_{Ni^{50\%}}$  value from Deleebeeck et al. (2009) was also used for both sets of data.

One of the several metals studied by Filova et al. (2021) with respect to toxicity to *R. subcapitata* in waters of relatively low hardness was nickel. The study determined a 96 h growth endpoint of 500 mg/L where the nickel ion was the dominant form of zinc (85.8 %) of zinc in the test solutions. Using the  $EC_{50}$  concentration and the zinc speciation, together with the ameliorative cation concentrations, led to a log  $K_{NiBL}$  value of 4.42, as is listed in Table S1. Again, the  $(f_c)_{Ni^{50\%}}$  value from Deleebeeck et al. (2009) was also used.

He et al. (2023) also studied the toxicity of nickel to *R. subcapitata* in several Chinese river and lake waters. The toxicity to *D. magna* was greater (i.e., lower  $EC_{50}$  concentrations) than was found in the study for *R. subcapitata*. Using the water quality (pH and other ameliorative cation concentrations) for each river, together with the measured  $EC_{50}$  concentration and the  $(f_c)_{Ni^{50\%}}$  value found for the study of Deleebeeck et al. (2009), an average log  $K_{NiBL}$  value of 4.97 was determined (Table S1).

In another study, Nys et al. (2016a) measured the toxicity of nickel to several species, including *R. subcapitata*, in waters of high hardness. Although the free nickel ion was the dominant nickel species, its concentration was just over 50 % of total nickel. The observed  $EC_{50}$  was lower (285 mg/L) than found in other studies for nickel toxicity on the same species. A lower  $(f_c)_{Ni^{50\%}}$  value was derived (0.018) from the data provided in this study than was used for the other five studies. The derived log  $K_{NiBL}$  value was 4.58. This value is listed in Table S1.

### S2.8. *Chlorella* sp.

Toxicity data were also used from the study of Peters et al. (2018) for *Chlorella* sp. For this species, it was assumed that the value of  $(f_c)_{Ni^{50\%}}$  was 0.5, as a value could not be determined from any of the studies that examined the toxic effect of metals to the alga. The derived log  $K_{NiBL}$  value (4.80) from the study of Peters et al. (2018) is listed in Table S1.

McKnight et al. (2023) also studied the toxicity of nickel to *Chlorella* sp. The study found that the species was more sensitive to nickel than found in the earlier study of Peters et al. (2018). Using the measured  $EC_{50}$  concentration, adjusting for ameliorative cation concentrations and using a  $(f_c)_{Ni^{50\%}}$  value derived for the study of Macoustra et al. (2019) for the same species, led to a log  $K_{NiBL}$  value of 5.12. This value is listed in Table S1.



### S2.9. Other Microalgae Species

McKnight et al. (2023) also studied the toxicity of nickel to the microalga *Monoraphidium arcuatum*. The study found that the species had similar sensitivity to that found for *Chlorella* sp. in the study. Using the measured EC<sub>50</sub> concentration, adjusting for ameliorative cation concentrations and using the same  $(f_c)_{Ni^{50\%}}$  value as used for *Chlorella* sp. from the study, led to a log  $K_{NiBL}$  value of 4.67. This value is listed in Table S1.

### S2.10. Mollusc Species

Nine values are listed in Table S1 for the log  $K_{NiBL}$  for mollusc species, with three being for *Lymnaea stagnalis*. The values range from 4.58 to 5.21. The average value is  $4.89 \pm 0.13$ . This value is in good agreement with those found for other groups of species for nickel toxicity. The three values for *L. stagnalis* average 4.88.

Leonard and Wood (2013) also studied the toxicity and uptake of nickel by the gastropod, *L. stagnalis*. From their toxicity studies (96 h mortality), they determined a log  $K_{NiBL}$  value of 5.21, as shown in Table 1. The log  $K_{NiBL}$  of 5.72 from their uptake studies is out of the range of the large number of values determined in the present study (Table S1) and is not considered.

Schlekat et al. (2010) studied the toxicity of nickel to several species, including *L. stagnalis*. The study measured EC<sub>50</sub> concentrations for *L. stagnalis* using four North American natural waters. The study found that *L. stagnalis* was extremely sensitive to nickel, with EC<sub>50</sub> concentrations as low as 6.2 mg/L. The toxicity data were reanalysed in the present study, with a  $(f_c)_{Ni^{50\%}}$  value of 0.0033 calculated for *L. stagnalis*. This value is one of the lowest found for any species, and consequently, the species is very sensitive to nickel exposure. Without adjustment using  $(f_c)_{Ni^{50\%}}$ , the unadjusted log  $K_{NiBL}$  would be substantially larger than that derived by Leonard and Wood (2013) for nickel uptake by *L. stagnalis*. Using the  $(f_c)_{Ni^{50\%}}$  given above, the derived log  $K_{NiBL}$  value is 4.58. This value is listed in Table S1.

Mattsson (2023) used 3 h uptake measurements to study the bioaccumulation of nickel by *L. stagnalis*. Using the data in the study at 18 °C and same weight, a log  $K_{NiBL}$  value of 4.84 was derived. This value lies between that determined from the toxicity studies of Leonard and Wood (2013) and Schlekat et al. (2010). The binding constant is listed in Table S1.

Mortality (24 to 72 hours) studies of six freshwater mussels (glochidia: *Alathyria profuga*, *Cucumerunio novaehollandiae*, *Hyridella australis*, *Hyridella depressa*, *Hyridella drapeta* and *Velesunio ambiguus*) were undertaken by Markich (2017) to study the sensitivity of the mussel glochidia to exposures of metals, including nickel. The study found the quite large average value for log  $K_{NiBL}$  of 6.15, with a range of 6.02 to 6.29 (48 h mortality data). The free nickel ion (96.7 %) was the dominant species in solution. Reanalysis of the toxicity data in the present study led to smaller log  $K_{NiBL}$  values, ranging between 4.80 and 5.00, with an average of 4.89, by using a  $(f_c)_{Ni^{50\%}}$  value of 0.072 for all six species. All six values are listed in Table S1.

### S2.11. Macrophyte Species

Schlekat et al. (2010) also studied the toxicity of nickel to *Lemna minor*, also reporting EC<sub>50</sub> concentrations for the species. Nickel toxicity to the species was studied using four North American natural waters. The study found that *L. minor* was quite sensitive to nickel, but more than an order of magnitude less sensitive than *L. stagnalis*. The toxicity data were reanalysed in the present study, with a  $(f_c)_{Ni^{50\%}}$  value of 0.035 calculated for *L. minor*. The calculated log  $K_{NiBL}$  value was found to be 4.52. This value is included in Table S1.

Naumann et al. (2007) also studied the toxicity of nickel to *L. minor*. The reported EC<sub>50</sub> was 329 mg/L. This value was used together with the ameliorative cation concentrations and the  $(f_c)_{Ni^{50\%}}$  value from Schlekat et al. (2010) to determine a log  $K_{NiBL}$  value of 4.82. This value is listed in Table S1.

In another study of *L. minor*, Nys et al. (2016a) measured the toxicity of nickel to several species in waters of high hardness. Although the free nickel ion was the dominant nickel

species, its concentration was just over 50 % of total nickel. The observed EC<sub>50</sub> was lower (286 mg/L) and was similar to those found in other studies for nickel toxicity to the same species. A similar  $(f_c)_{Ni^{50\%}}$  value was derived (0.023) to those utilised in the other two studies on nickel toxicity to *L. minor*. The derived log  $K_{NiBL}$  value was 4.69. This value is listed in Table S1.

There are three studies examined in relation to the toxicity of nickel to *L. minor*. The log  $K_{NiBL}$  values derived range from 4.52 to 4.82, with an average value of 4.64. In this analysis, two  $(f_c)_{Ni^{50\%}}$  values were used. If the average value was used for all three sets of data, the log  $K_{NiBL}$  values would range from 4.44 to 4.74, with an average value of 4.63. Given that there is virtually no effect of the use of two  $(f_c)_{Ni^{50\%}}$  values on the observed average, the two values have been retained.

### S2.12. Cnidaria Species

A third species examined in the study of Peters et al. (2018) was the toxicity of nickel to *Hydra viridissima*. For this species, it was also assumed that the value of  $(f_c)_{Ni^{50\%}}$  was 0.5, as this was the only study assessed on the toxicity of nickel to a cnidaria species. The derived log  $K_{NiBL}$  value (5.15) from the study of Peters et al. (2018) is listed in Table S1.

### S2.13. Rotifer Species

Of the several species studied by Nys et al. (2016a), for the toxicity of nickel in waters of high hardness, *Brachionus calyciflorus* (a rotifer) was studied. The free nickel ion was the dominant nickel species (58.1 %) of total nickel in the test solutions. The observed EC<sub>50</sub> was lower (2459 mg/L). From this concentration, and adjusting for the ameliorative cation concentrations, led to a derived log  $K_{NiBL}$  value was 4.60. This value is listed in Table S1.

## S3. Cadmium

### S3.1. *Oncorhynchus mykiss*

Thirteen studies that examined the toxicity of cadmium to *O. mykiss* have been reviewed. Two of the studies used both uptake and toxicity, three used uptake only and the remaining eight toxicity only. A  $(f_c)_{Cd^{50\%}}$  value was derived from the study of Niyogi et al. (2008) that studied both uptake and toxicity (see main text). This value was used to adjust the log  $K_{CdBL}$  values in all the toxicity studies. The values found for log  $K_{CdBL}$  had the relatively narrow range of 7.33 to 7.70, with an average of  $7.47 \pm 0.07$ . The four uptake studies had a similar range of 7.33 to 7.52, with an average of 7.38.

Niyogi et al. (2004, 2008) conducted two studies examining the toxicity of cadmium to *O. mykiss* using 3 h uptake assays. In the earlier uptake study, Niyogi et al. (2004) determined a log  $K_{CdBL}$  value of 7.34. This binding constant was quite similar to the value they found for yellow perch, *Perca flavescens*, namely 7.20 (see discussion in main text). The values for both fish species are retained in this study and are listed in Table S1. In their later study (Niyogi et al., 2008) they determined a slightly larger log  $K_{CdBL}$  value of 7.52. Again, this value is retained in the present study (Table S1).

Three-hour uptake assays were also used by Bircneau et al. (2008) to study cadmium toxicity to *O. mykiss*. The study reported a log  $K_{CdBL}$  value of 7.33. This value is consistent with the studies of Niyogi et al. (2004, 2008) for the same species and using the same toxicity assay. The reported log  $K_{CdBL}$  value is listed in Table S1.

Hollis et al. (2000) also studied the uptake of cadmium in rainbow trout using a 3 h assay. The study determined log  $K_{CdHL}$  values in control tests in both soft and hard water as well as low Cd-containing solutions (3 mg/L), with values determined of 7.3, 7.6 and 7.3, respectively. The average log  $K_{CdBL}$  value from the study, namely 7.37, has been accepted in the present study and is listed in Table S1. Again, this value is in good agreement with the previously discussed studies.

Liao et al. (2010) also studied the uptake of cadmium in *O. mykiss*, but used a much longer assay period of 4 days. Analysis of the data obtained, however, led to a very small value for  $(f_c)_{Cd^{50\%}}$  of 0.007 and an associated log  $K_{CdBL}$  value of 7.35 (Table S1). This latter value is in very good agreement with the other four values obtained for *O. mykiss* that used much shorter

uptake periods, but the  $(f_c)_{Cd^{50\%}}$  value is very different. The study used a larger log  $K_{CdBL}$  value of 8.6, which was adopted from the earlier study of Playle et al. (1993), but they also calculated a low value for  $(f_c)_{Cd^{50\%}}$  of 0.013.

Naddy et al. (2015) also studied the toxicity of several metals to rainbow trout, including cadmium. The effect of varying hardness on the toxic response was also considered. The average toxicity in soft water was utilised together with that in hard water. The hard water also had elevated alkalinity and pH compared to the soft water. The  $Cd^{2+}$  ion dominated cadmium speciation (91.6 %) in the soft water, but was a much smaller percentage (48.6 %) of the hard water. When accounting for the speciation, the effects of the ameliorative cations and the  $(f_c)_{Cd^{50\%}}$  derived from the study of Niyogi et al. (2008), an average log  $K_{CdBL}$  of 7.59 was derived (Table S1).

The toxicity of several metals to rainbow trout was studied by Calfee et al. (2014) in test waters of moderate hardness at a reasonably high pH (8.1). Again, the metals studied included cadmium. The observed 96 h  $LC_{50}$  (3.9 mg/L) is reasonably consistent with those found in other studies for the same species. In the test water used in this study, the free cadmium ion also predominated the metal's speciation. Considering the  $LC_{50}$  and speciation together with the concentrations of the ameliorative cations as well as the  $(f_c)_{Cd^{50\%}}$  value of Niyogi et al. (2008) led to a log  $K_{CdBL}$  value of 7.48. This value is listed in Table S1.

Besser et al. (2007) studied the toxicity to *O. mykiss* using the same metals as the previous two studies and found almost the same cadmium  $LC_{50}$  concentration (3.7 mg/L) as the study of Calfee et al. (2014). The test water was also similar to that of the Calfee et al. (2014) study and the derived log  $K_{CdBL}$  value was 7.50. Again, the  $(f_c)_{Cd^{50\%}}$  value derived from the Niyogi et al. (2008) study was also used.

The study of Chapman (1978) also examined the toxicity of cadmium to the swim-up stage of rainbow trout that led to a log  $K_{CdBL}$  value of 7.44 (Table S1), using 96 h  $LC_{50}$  concentrations and the  $(f_c)_{Cd^{50\%}}$  value from the study of Niyogi et al. (2008). Water in the tests was supplied from wells on the banks of the Willamette River and the derived log  $K_{CdBL}$  was adjusted from the inverse of the  $LC_{50}$  using the ameliorative cation concentrations and the fraction of  $Cd^{2+}$  in the test water (the free ion dominated cadmium speciation (92.3 %)).

Davies et al. (1993) also studied the toxicity of cadmium to rainbow trout in waters of varying hardness. The measured 96 h  $LC_{50}$  concentrations were used together with the ameliorative cation concentrations in each of the test waters and the  $(f_c)_{Cd^{50\%}}$  value from Niyogi et al. (2008) to derive an average log  $K_{CdBL}$  value of 7.46. This value is listed in Table S1.

The toxicity of cadmium to rainbow trout was also studied by Mebane et al. (2008). An  $LC_{50}$  of 0.87 mg/L was determined that is somewhat lower than found in other studies. In the test water,  $Cd^{2+}$  was the dominant (96.9 %) cadmium species. The measured toxicity and derived speciation was used together with the ameliorative cation concentrations and the  $(f_c)_{Cd^{50\%}}$  value from Niyogi et al. (2008) to derive an average log  $K_{CdBL}$  value of 7.70 (this value is marginally larger than other values derived for this parameter, but is still retained in Table S1).

Another study that examined the toxicity of cadmium to *O. mykiss* was that of Spehar and Carlson (1984). A 96 h  $LC_{50}$  concentration of 2.3 mg/L was determined, similar to those found in other studies on the same species. The test water used was relatively soft and in the water, cadmium was the dominant species (93.9 %) of total cadmium. Using these data, a log  $K_{CdBL}$  value of 7.40 was determined (Table S1). The  $(f_c)_{Cd^{50\%}}$  value from the study of Niyogi et al. (2008) was also used.

A twelfth study on the toxicity of cadmium to rainbow trout was conducted by Hansen et al. (2002) using water of moderate hardness and circumneutral pH (7.5). The 120 h mortality ( $LC_{50}$ ) concentration determined was 2.07 mg/L, again similar to the concentration found in other studies. Based on this  $LC_{50}$  concentration and the concentrations of the ameliorative cation concentrations in the test waters, as well as the  $(f_c)_{Cd^{50\%}}$  value from Niyogi et al. (2008), a log  $K_{CdBL}$  value of 7.59 was determined (Table S1).

### S3.2. Other *Oncorhynchus* Species

Chapman (1978) also studied the toxicity of cadmium to the swim-up stage of chinook salmon (*O. tshawytscha*), from which a log  $K_{CdBL}$  value of 7.57 (Table S1) was determined using 96 h LC<sub>50</sub> concentrations and the ameliorative cation concentrations. Again, the  $(f_c)_{Cd^{50\%}}$  value from the study of Niyogi et al. (2008) was also used. Water in the tests was supplied from wells on the banks of the Willamette River. The derived log  $K_{CdBL}$  was also adjusted for the fraction of Cd<sup>2+</sup> in the test water (the free ion dominated cadmium speciation (93.0 %)).

### S3.3. Other Fish Species

There are nine studies reviewed in relation to the toxicity of cadmium to other fish species, including three to *P. promelas*. The calculated values for log  $K_{CdBL}$  range between 6.99 and 7.62, with an average value of  $7.20 \pm 0.15$ . This value is marginally lower than that found for *O. mykiss*, but still consistent with values found for fish species. For trout and salmon species, the  $(f_c)_{Cd^{50\%}}$  value from Niyogi et al. (2008) was used, whereas for other fish species, the standard value of 0.5 was used.

Vardy et al. (2014) also studied the toxicity of cadmium to *A. transmontanus* in both laboratory and Columbia River water. The data for the youngest life-stage (8 days post-hatch) and laboratory water were used in the present analysis, together with the cadmium speciation and ameliorative cation concentrations, to determine a log  $K_{CdBL}$  value of 7.32. This value is included in Table S1. A  $(f_c)_{Cd^{50\%}}$  value was not used to adjust the derived log  $K_{CdBL}$  value calculated from this study. Nevertheless, the derived log  $K_{CdBL}$  value is in good agreement with that found for other fish species.

Besser et al. (2007) also studied the toxicity of cadmium to *C. bairdi* and found a larger LC<sub>50</sub> concentration (7.9 mg/L) than was found by the study for *O. mykiss*. A log  $K_{CdBL}$  value from the LC<sub>50</sub> concentration and the ameliorative cation concentrations was calculated to be 7.18. For this species, the  $(f_c)_{Cd^{50\%}}$  value derived from the Niyogi et al. (2008) study was also used.

As described in the *O. mykiss* section, Niyogi et al. (2004) also studied uptake into *P. flavescens*. The study derived a log  $K_{CdBL}$  value of 7.20 (as listed in Table S1). This value is not inconsistent with the log  $K_{CdBL}$  values derived for *O. mykiss*.

The toxicity of cadmium to *Oreochromis mossambicus* was studied by Chang et al. (1998) in relatively soft water. Based on the 96 h LC<sub>50</sub> concentration determined (21.4 mg/L) and the ameliorative cation concentrations, a log  $K_{CdBL}$  value of 7.00 was calculated (Table S1). As also found for *C. bairdi*, this log  $K_{CdBL}$  was not further adjusted for  $(f_c)_{Cd^{50\%}}$ .

Schubauer-Berigan et al. (1993) also measured the toxicity of cadmium to *P. promelas* as a function of pH. The toxicity was found to depend on pH and the log  $K_{CdBL}$  value (7.17) was determined from the relationship of toxicity with respect to the H<sup>+</sup> concentration and adjusted for the ameliorative cation concentrations. The log  $K_{CdBL}$  value is listed in Table S1.

Diamond et al. (1997) also studied the toxicity of cadmium to *P. promelas* in both laboratory water and Lehigh River water sampled at different times of year. The geometric average of the LC<sub>50</sub> data for the laboratory water was used in the present analysis and gave a concentration of 31 mg/L. The use of this toxicity concentration, together with the cadmium speciation (Cd<sup>2+</sup> is 89.2 % of total cadmium) and the ameliorative cation concentrations, resulted in a log  $K_{CdBL}$  value of 6.99 (Table S1).

A third study that examined the toxicity of cadmium to *P. promelas* was that of Spehar and Carlson (1984). A 96 h LC<sub>50</sub> concentration of 17.4 mg/L was determined, consistent with those found in other studies on the same species. The test water used was relatively soft and in the water, cadmium was the dominant species (93.8 %) of total cadmium. Using these data, a log  $K_{CdBL}$  value of 7.09 was determined (Table S1). None of the data from the three studies of cadmium toxicity to *P. promelas* had a  $(f_c)_{Cd^{50\%}}$  value used to further adjust the log  $K_{CdBL}$  value.

Hansen et al. (2002) also studied the toxicity of cadmium to bull trout (*Salvelinus confluentus*) conducted by using water of moderate hardness and circumneutral pH (7.5). The 120 h mortality (LC<sub>50</sub>) concentration of 5.23 mg/L was similar to that found in the study for rainbow trout. Based on this LC<sub>50</sub> concentration and the concentrations of the ameliorative cation concentrations in the test waters, as well as the  $(f_c)_{Cd^{50\%}}$  value from Niyogi et al. (2008), a log  $K_{CdBL}$  value of 7.19 was determined (Table S1).

Spehar and Carlson (1984) also studied the toxicity of cadmium to brown trout (*Salmo trutta*). A 96 h LC<sub>50</sub> concentration of 1.4 mg/L was determined. The test water used was relatively soft and in the water, cadmium was the dominant species (93.8 %) of total cadmium. Using these data, a log  $K_{\text{CdBL}}$  value of 7.62 was determined (Table S1). The  $(f_{\text{c}})_{\text{Cd}^{50\%}}$  value from Niyogi et al. (2008) was also used to further adjust the log  $K_{\text{CdBL}}$  value.

### S3.4. *Daphnia magna*

Seven log  $K_{\text{CdBL}}$  values are given for the binding constant for cadmium to cell surface sites in *D. magna*. The values range between 6.25 and 7.22, a relatively large range, with an average of  $6.86 \pm 0.30$ . This value overlaps the range in values (95 % uncertainty range) of the fish species.

Tan and Wang (2011) examined the toxicity of cadmium to *D. magna* using a 48 h immobilisation assay at varying pH and Ca<sup>2+</sup> concentrations. The study found that the free Cd ion was the dominant cadmium species in the test solutions, ranging from 59 to 86 % of total cadmium. The reported EC<sub>50</sub> concentrations were related to the free ion concentration in each solution. From the reported EC<sub>50</sub> concentrations and correcting for the pH and Ca<sup>2+</sup> concentrations in the test solutions, the calculated value for log  $K_{\text{CdBL}}$  was 6.97 (Table S1).

The toxic effect of cadmium to *D. magna* was also studied by Okamoto et al. (2015) at a water hardness of 72 mg/L (as CaCO<sub>3</sub>), using a 48 h acute immobilisation test. Unlike the previous metals, a cadmium toxicity value was only available from Biesinger and Christensen (1972) at a hardness value of 45 mg/L (as CaCO<sub>3</sub>), which was cited in the study. The data from the two studies were used to determine an average cadmium toxicity at the average hardness, as there was not a reasonable relationship between hardness and toxicity from the studies. From the average values, a log  $K_{\text{CdBL}}$  value of 7.14 was determined by correcting for the concentrations of H<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>. The obtained value is listed in Table S1.

Kim et al. (2017) studied the toxicity of cadmium to *D. magna* in hard water. An LC<sub>50</sub> concentration of 21 mg/L was determined. The cadmium ion (Cd<sup>2+</sup>) was the dominant form (71.4 %) of cadmium in the test solutions. Based on the LC<sub>50</sub>, the cadmium speciation, the concentrations of the ameliorative cations and the  $(f_{\text{c}})_{\text{Cd}^{50\%}}$  value determined by Van Regenmortel et al. (2017) for the same species, a log  $K_{\text{CdBL}}$  value of 6.89 was determined. This value is listed in Table S1.

Three different forms of light (white fluorescent, UV-B and sunlight) were used by Lee et al. (2009) in a study of the toxicity of cadmium to *D. magna*. The average toxicity (19 mg/L) was used in the present study, together with the ameliorative cation concentrations (moderate hardness), to determine a log  $K_{\text{CdBL}}$  value of 7.22 (Table S1). The binding constant was not further adjusted using a  $(f_{\text{c}})_{\text{Cd}^{50\%}}$  value.

In another study of the toxicity of cadmium to *D. magna*, Liu and Wang (2015) used a 48 h mortality endpoint in relatively soft water to derive an LC<sub>50</sub> concentration of 36.8 mg/L. The Cd<sup>2+</sup> ion was the dominant form (88.8 %) of cadmium in the test solutions. The LC<sub>50</sub> concentration was used with the ameliorative cation concentrations and Cd speciation to derive a log  $K_{\text{CdBL}}$  value of 6.79 (Table S1).

Test solutions of variable pH were used by Schuytema et al. (1984) to also study the toxicity of cadmium to *D. magna*. There was no dependence found in the study between the toxicity and pH. As such, the four separate pH conditions studied were assessed independently and an average value was determined for log  $K_{\text{CdBL}}$ . The value determined was 6.74 and is listed in Table S1.

Cadmium was studied by Mount and Norberg (1984) in evaluating the toxicity of several metals to *D. magna*. The study used a pH of 7.3 and test waters of relatively low hardness. The free cadmium ion accounted for the vast majority (92.2 %) of total cadmium. For cadmium, a relatively high 48 h LC<sub>50</sub> of 118 mg/L was determined, which led to the low log  $K_{\text{CdBL}}$  value of 6.25 (Table S1) when the ameliorative cation concentrations were considered.

### S3.5. Other *Daphnia* Species

Clifford and McGeer (2010) studied the acute toxicity of cadmium to *D. pulex* using a 48 h immobilisation assay. The toxicity was studied with varying pH (6.10 to 8.02) and concentrations of  $\text{Ca}^{2+}$  (1.2 to 64.5 mg/L),  $\text{Mg}^{2+}$  (0.24 to 34 mg/L) and  $\text{Na}^+$  (12.4 to 37.9 mg/L). Using the data obtained, a log  $K_{\text{CdBL}}$  value of 6.79 was derived and is listed in Table S1.

In addition to studying the toxicity of cadmium to *D. magna*, Mount and Norberg (1984) also evaluated the toxicity of the metal to *D. pulex*. The study used a pH of 7.3 and test waters of relatively low hardness. The free cadmium ion accounted for the vast majority (92.1 %) of total cadmium. A 48 h  $\text{LC}_{50}$  of 68 mg/L was determined that led to a log  $K_{\text{CdBL}}$  value of 6.49 (Table S1) when the ameliorative cation concentrations were considered.

### S3.6. *Ceriodaphnia dubia*

The toxicity of cadmium to *C. dubia* was studied by Sofyan et al. (2007) using both 7 d reproduction and mortality assays. The  $\text{IC}_{50}$  and  $\text{LC}_{50}$  values determined for waterborne cadmium were 7.24 and 10.67 mg/L, respectively. The average of these two concentrations was used to derive a log  $K_{\text{CdBL}}$  value of 7.10. This value is listed in Table S1. The  $(f_{\text{c}})_{\text{M}^{50\%}}$  value from Keithly et al. (2004) was not used to adjust this value.

Cadmium toxicity to four freshwater species including *C. dubia* was measured by Schubauer-Berigan et al. (1993) as a function of pH. A relatively weak relationship of  $\text{LC}_{50}$  was determined for *C. dubia* with respect to  $\text{H}^+$  and, as such, the log  $K_{\text{CdBL}}$  value was derived from the intercept and adjusted for the ameliorative cation concentrations and cadmium speciation. The  $\text{Cd}^{2+}$  was found to be the dominant cadmium species at all pH values studied, but decreased in dominance as the pH increased. The log  $K_{\text{CdBL}}$  value determined was 6.60. The value is listed in Table S1.

Naddy et al. (2015) also studied the toxicity of cadmium to *C. dubia*. The effect of varying hardness on the toxic response was also considered. The average toxicity in soft water was utilised together with that in hard water. The hard water also had elevated alkalinity and pH compared to the soft water. The  $\text{Cd}^{2+}$  ion dominated cadmium speciation in the soft water, but was a much smaller fraction (0.486) of the hard water. When accounting for the speciation and the effects of the ameliorative cations, an average log  $K_{\text{CdBL}}$  of 7.35 was derived (Table S1); there was good agreement between the two log  $K_{\text{CdBL}}$  values derived from the two very different water qualities.

The average log  $K_{\text{CdBL}}$  values reviewed for *C. dubia* is 7.02. This value is in relatively good agreement with that found for *D. magna* and for fish species. It is also in excellent agreement with the average of all species listed in Table 1.

### S3.7. Other *Ceriodaphnia* Species

Mount and Norberg (1984) also evaluated the toxicity of cadmium to *C. reticulata*. The study used a pH of 7.3 and test waters of relatively low hardness. Again, the free cadmium ion accounted for the vast majority (92.1 %) of total cadmium. A 48 h  $\text{LC}_{50}$  of 66 mg/L was determined that leads to a log  $K_{\text{CdBL}}$  value of 6.50 (Table S1) when the ameliorative cation concentrations are considered.

Raymundo et al. (2024) studied the toxicity of cadmium to both *C. rigaudi* and *C. silvestri* in relatively soft water. The  $\text{EC}_{50}$  concentrations reported in the study were 20.7 and 26.6 mg/L, respectively. The use of these concentrations and adjusting for the ameliorative cation concentrations led to log  $K_{\text{CdBL}}$  values of 7.02 and 6.91. Both these values have been listed in Table S1.

There are three log  $K_{\text{CdBL}}$  values listed for other *Ceriodaphnia* species in Table S1. The average of the three values is 6.81, which is reasonably consistent with the average value determined for *C. dubia*.

### S3.8. Other Crustacean Species

There are seven studies reported for other crustacean species, two for *H. azteca* and two for *Simocephalus* species. The log  $K_{\text{CdBL}}$  values derived range from 6.83 to 7.43, with an average value of 7.07. The average value determined is in quite good agreement with the values

determined for other crustacean species and the average value reported for all species in Table 1.

Schroeder (2008) also studied the toxic response of *H. azteca* to cadmium in synthetic waters with varying pH (7.1 to 8.9) and  $\text{Ca}^{2+}$  (11 to 300 mg/L) concentrations. A log  $K_{\text{CdBL}}$  value was derived (7.16) from the observed toxicities, correcting for the ameliorative effects of  $\text{H}^+$  and  $\text{Ca}^{2+}$  (Table S1). The response of *H. azteca* to changes in pH was variable, but the use of the full range of pH data still led to a reasonable value for log  $K_{\text{CdBL}}$ . A value was also determined for  $(f_c)_{\text{Cd}^{50\%}}$  from the toxicity data (0.184), which was the same value found for the study for *H. azteca* to exposures to nickel.

Jackson et al. (2000) also studied the toxicity of cadmium to *H. azteca* using 96 h mortality in variable concentrations of  $\text{Ca}^{2+}$  (2 to 150 mg/L) and  $\text{Mg}^{2+}$  (1.2 to 83.2 mg/L). The data obtained in the study were recalculated to determine the effect of the hardness cations on toxicity, from which a log  $K_{\text{CdBL}}$  value of 6.83 was calculated using the  $(f_c)_{\text{Cd}^{50\%}}$  value derived from the study of Schroeder (2008). This value is listed in Table S1.

Zou and Bu (2004) studied the toxicity of cadmium to *Moina irrasa* in very soft water at pH 8. The toxicity found in the study was 15.27 mg/L, using a 48 h mortality endpoint. The magnitude of the adjustment of this value is quite small due to the relatively high pH and very soft water that led to a selected log  $K_{\text{CdBL}}$  value of 6.95. This value is listed in Table S1.

The toxicity of cadmium to the copepod, *Notodiaptomus iheringi*, was studied by Rocha et al. (2024). The relatively low  $\text{EC}_{50}$  concentration of 8 mg/L was reported and, adjusting for the ameliorative cation concentrations, leads to a log  $K_{\text{CdBL}}$  value of 7.43 that is relatively large. This value has not been further adjusted using a  $(f_c)_{\text{Cd}^{50\%}}$  value due to the lack of appropriate data. The log  $K_{\text{CdBL}}$  value is listed in Table S1.

Freitas and Rocha (2011) studied the toxicity of cadmium to *Pseudosida ramosa*. The test water was reasonably soft and the  $\text{Cd}^{2+}$  ion dominated (94.7 %) the cadmium speciation in the test solutions. Using the measured  $\text{EC}_{50}$  concentration, the ameliorative cation concentrations and the cadmium speciation, led to a derived log  $K_{\text{CdBL}}$  value of 7.24. This value is listed in Table S1.

Other species studied by Spehar and Carlson (1984) in relation to the toxicity of cadmium were *S. serrulatus* and the amphipod *Gammarus pseudolimnaeus*. The 96 h  $\text{LC}_{50}$  concentrations of 24.5 and 68.3 mg/L, respectively, were determined and were an order of magnitude larger than those found for the trout species. The test water used was relatively soft and in the water, cadmium was the dominant species (93.9 % for both species) of total cadmium. Using these data, log  $K_{\text{CdBL}}$  values of 6.94 and 6.49 were determined (Table S1).

Another species studied by Mount and Norberg (1984) in relation to the toxicity of cadmium was *S. vetulus*. The study used a pH of 7.3 and test waters of relatively low hardness. The free cadmium ion accounted for the vast majority (92.0 %) of total cadmium. A 48 h  $\text{LC}_{50}$  of 24 mg/L was determined, which led to a log  $K_{\text{CdBL}}$  value of 6.94 (Table S1) when the ameliorative cation concentrations are considered.

### S3.9. *Raphidocelis subcapitata*

Seven log  $K_{\text{CdBL}}$  values have been derived for the toxicity of cadmium to *R. subcapitata*. The seven values cover the relatively large range of 6.49 to 7.46, the largest observed for a single species to the toxicity of a single metal. The average value is 6.99 and due to the large range, a large 95 % confidence interval of 0.39 is also derived. However, the average value is consistent with those found for other freshwater species.

Cadmium toxicity to *R. subcapitata* was studied by Paquet et al. (2015) using a 96 h growth (cell yield and cell volume) assay. Two initial cell densities (2,500 and 10,000 cells/mL) were used in the study and the results from the latter were used in the present study. Reported  $\text{EC}_{50}$  concentrations (as  $\text{Cd}^{2+}$ ) were 35 (cell yield) and 50 (cell volume) nM. The average concentration was used to determine a log  $K_{\text{CdBL}}$  value of 7.37, as given in Table S1.

Källqvist (2009) also studied the toxicity of cadmium to *R. subcapitata* using a 72 h growth assay in an artificial medium and in natural lake waters. The lake with the lowest hardness (3.4 mg/L (as  $\text{CaCO}_3$ )) gave the lowest  $\text{EC}_{50}$  concentration of 9.4 mg/L. Correction of this

concentration for the pH and low concentrations of the hardness ions in the lake water led to a log  $K_{CdBL}$  value of 7.18 (Table S1). This value is in good agreement with that found by Paquet et al. (2015) for the same species.

The toxicity of cadmium to *R. subcapitata* was also studied by dos Reis et al. (2024a), where an  $IC_{50}$  concentration of 80 mg/L was determined. The free cadmium ion was found to be the dominant form (84.0 %) in the test waters. Using the  $IC_{50}$  concentration and the cadmium speciation, together with the ameliorative cation concentrations, led to a log  $K_{CdBL}$  values of 6.61.

Alves et al. (2017) also studied the toxicity of cadmium to *P. subcapitata* in waters of relatively low hardness and at a pH of 7. A 72 h growth endpoint of 36 mg/L was determined in the study. The correction for ameliorative cations was quite small and the cadmium ion was the dominant form (90.9 %) of cadmium in the test water, leading to a log  $K_{CdBL}$  value of 6.71, as is listed in Table S1.

In another study, Blaise et al. (1986) examined the toxicity of cadmium to *P. subcapitata* and found similar  $EC_{50}$  concentrations that varied between 22.5 and 37 mg/L, with an average value of 29.9 mg/L. The pH of the test solutions was 7 and the hardness was relatively low, with the cadmium ion (94.1 %) dominating the cadmium speciation. These conditions led to a log  $K_{CdBL}$  value of 6.49 (Table S1).

Franklin et al. (2001) also studied the toxicity of cadmium to *R. subcapitata* using a 72 h growth endpoint. An  $EC_{50}$  value of 15 mg/L was determined at pH 7.5 and in waters of very low hardness. Taking the measured  $EC_{50}$  concentration, the ameliorative cation concentrations and the cadmium speciation ( $Cd^{2+}$  was the dominant species (92.5 %)) led to a log  $K_{ZnBL}$  value of 6.97 (Table S1).

The toxicity of cadmium to *R. subcapitata* was also studied by Alho et al. (2018), also using a 72 h growth endpoint. The test solution has a relatively low hardness and a pH of 7.4, also with  $Cd^{2+}$  as the dominant form (94.7 %) of cadmium. The  $EC_{50}$  concentration determined in the study, namely 4.5 mg/L, is relatively low. This concentration, the concentrations of the ameliorative cations and the cadmium speciation leads to the high log  $K_{CdBL}$  value of 7.64. This value is listed in Table S1.

### S3.10. Mollusc Species

Markich (2017) also used mortality (24 to 72 hours) to study the toxicity of cadmium to six freshwater mussels (glochidia: *A. profuga*, *C. novaehollandiae*, *H. australis*, *H. depressa*, *H. drapeta* and *V. ambiguus*). The study found an average value for log  $K_{CdBL}$  of 6.78, with a range of 6.65 to 6.78 (48 h mortality data). The free cadmium ion (90.8 %) was the dominant species in the solution. Reanalysis of the toxicity data in the present study led again to marginally smaller log  $K_{CdBL}$  values, ranging between 6.56 and 6.77. The six values for the binding constant are listed in Table S1.

Croteau and Luoma (2007) studied the uptake of cadmium in *L. stagnalis* using a 24 h assay. The study determined log  $K_{CdBL}$  values for four water types (deionised, soft, moderately hard and hard) with values of 7.0, 6.8, 6.6 and 6.0, respectively. It might be thought that these results show a decrease in log  $K_{CdBL}$  with an increase in hardness; however, this relationship was not observed for copper, which was also studied. As such, the average log  $K_{CdBL}$  value from the four waters is retained in the present study, namely 6.60 (Table S1).

The toxicity of cadmium to *L. stagnalis* was studied by Capela et al. (2024) using 7 d growth and development endpoints. The average  $EC_{50}$  was 35.49 mg/L for the two endpoints. The  $Cd^{2+}$  ion was determined to be the dominant form (74.1 %) of cadmium in the test solutions. The test solutions had very low hardness and, based on this and the average  $EC_{50}$  and cadmium speciation, a log  $K_{CdBL}$  value of 6.64 was determined. This value is listed in Table S1; it is in excellent agreement with the value derived from the uptake measurements of Croteau and Luoma (2007).

Das and Khangarot (2010) studied the toxicity of cadmium to the Indian pond snail, *Lymnaea luteola*. The test water was relatively hard with an elevated alkalinity at a pH of 7.5. A 14 d  $LC_{50}$  concentration of 337 mg/L was determined, which decreased to 43 mg/L after 49 d of



exposure. Using the 14 d LC<sub>50</sub>, the ameliorative cation concentrations and the cadmium speciation (Cd<sup>2+</sup> was 52.7 % of total cadmium) led to a log K<sub>CdBL</sub> value of 6.46. This value is listed in Table S1 and is in reasonable agreement with the value determined from the studies of Croteau and Luoma (2007) and Capela et al. (2024) on *L. stagnalis*.

Wang et al. (2010b) studied the toxicity of metals, including cadmium, to the early life stages of freshwater mussels using various mortality assays (1 to 28 days). For juvenile fatmucket, *L. siliquioidea*, a 48 h mortality assay gave an LC<sub>50</sub> concentration of 16 mg/L; this endpoint was also used for zinc. Free cadmium (86.7 %) was the dominant cadmium species and correcting for the effect of the ameliorative cations led to a log K<sub>CdBL</sub> value of 7.47 (Table S1). The study utilised the same assay for *L. rafinesqueana* and determined an LC<sub>50</sub> concentration of 20 mg/L. Using the same methodology, a log K<sub>CdBL</sub> value of 7.37 is calculated (Table S1).

The toxicity of cadmium to the snail, *Potamopyrgus antipodarum*, was studied by Ruppert et al. (2016) for both juvenile and adult life stages. The toxicity was found to be relatively similar between the two life stages (15 and 11.3 mg/L, respectively) and the average toxicity was used. Using the average 28 d EC<sub>50</sub>, the ameliorative cation concentrations and the cadmium speciation (Cd<sup>2+</sup> accounted for 81.3 % of total cadmium), led to a log K<sub>CdBL</sub> value of 7.32. This value is listed in Table S1.

### S3.11. Macrophyte Species

Naumann et al. (2007) studied the toxicity of a range of metals to *Lemna minor* in relatively hard water. In their study using cadmium, Cd<sup>2+</sup> was found to be only 39 % (the same as that for zinc) of total cadmium in the test solutions, where the observed toxicity occurred at a concentration of 152 mg/L. Utilising the EC<sub>50</sub> concentration and the cadmium speciation, together with the ameliorative cation concentrations, led to a log K<sub>CdBL</sub> value of 7.07 (Table S1).

### S3.12. Cnidaria Species

Clifford (2009) studied the toxicity of cadmium to *Hydra attenuata* using a 96 h growth assay in variable concentrations of Ca<sup>2+</sup> (8.0 to 48.5 mg/L) and Mg<sup>2+</sup> (4.4 to 15 mg/L). The data obtained in the study were recalculated to determine the effect of the hardness cations on toxicity, from which a log K<sub>CdBL</sub> value of 7.12 was calculated. This value is listed in Table S1.

### S3.13. Insect Species

Again, only a single study of the toxicity of cadmium to an insect species was reviewed. Anderson et al. (1980) studied the toxicity of several metals to the insect *T. dissimilis*. For cadmium, an LC<sub>50</sub> concentration of 3.8 mg/L was determined, which is a relatively low concentration. Based on all four LC<sub>50</sub> values being relatively low, a (f<sub>C</sub>)<sub>M</sub><sup>50%</sup> value of 0.258 was assigned to all metals studied. Based on this and the LC<sub>50</sub> value, the cadmium speciation and the ameliorative cation concentrations, a log K<sub>CdBL</sub> value of 7.29 was determined, as is listed in Table S1.

## S4. Cobalt

Stubblefield et al. (2020) studied the toxicity of cobalt to several species, including *R. subcapitata*, *L. minor*, *C. dubia*, *D. magna*, *H. azteca*, *Aeolosoma* sp., *L. stagnalis*, *P. promelas*, *Danio rerio*, *C. tentans* and *O. mykiss*, using acute and chronic assays. The acute and chronic toxicity data were similar for only three of the species tested (*R. subcapitata*, *L. minor* and *O. mykiss*), with the chronic toxicity being more sensitive for all other species. The chronic toxicity data have been used in the present study. Differing water quality conditions were used for each species, and although the free cobalt ion (Co<sup>2+</sup>) was the dominant species in all test waters, the concentration of Co<sup>2+</sup> ranged between 43 and 94.4 % of total cobalt. The pH and Ca<sup>2+</sup>, Mg<sup>2+</sup> and Na<sup>+</sup> concentrations were also used in the analysis of the binding constants for each test species. *Chironomus* species are insects that are not fully aquatic (i.e., they are not submersed permanently and can live above water) and, as such, the data for this species were not processed in this study.

#### S4.1. Fish Species

Three fish species were studied by Stubblefield et al. (2020), *O. mykiss*, *P. promelas* and *Danio rerio*. Based on the measured toxicity concentrations, the cobalt speciation and ameliorative cation concentrations, the log  $K_{CoBL}$  values determined were 5.27 (*O. mykiss*) and 5.68 (*P. promelas*). These values are listed in Table S1. From the study, only the toxicity concentration derived for *D. rerio* was not used in the present assessment.

Marr et al. (1998) studied the toxicity of cobalt to rainbow trout using a 14 d mortality assay. Speciation calculations showed that the free ion,  $Co^{2+}$ , was the dominant cobalt species (91.8 %) in the test solutions. Further adjustment for the concentration of the ameliorative cations  $H^+$ ,  $Na^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$  led to a derivation of the log  $K_{CoBL}$  value of 5.42 (Table S1). The value is in good agreement with the other two log  $K_{CoBL}$  values determined for *O. mykiss* (Alsop and Wood, 2000; Stubblefield et al., 2020), being between the two other values.

Alsop and Wood (2000) quoted a log  $K_{CoBL}$  value of 5.1 from the earlier study of cobalt uptake in *O. mykiss* by Richards and Playle (1998). This value for log  $K_{CoBL}$  (5.10) was retained and is listed in Table S1. These uptake data were based on 2- to 3-hour exposures of the organisms to cobalt. Consequently, it might appear that for *O. mykiss*, the log  $K_{CoBL}$  decreases with the length of the exposure time. When comparing these data to those from other species, it would appear that they are consistent with the data from other species across a wide range of endpoint durations.

Three log  $K_{CoBL}$  values have been derived for *O. mykiss* that range from 5.10 to 5.42, with an average of 5.26. Overall, the average log  $K_{CoBL}$  value for fish species (four values) is 5.37. These average values are in reasonable accord with values for other species.

#### S4.2. Crustacean Species

Stubblefield et al. (2020) also studied three cladoceran species, *C. dubia*, *D. magna* and *H. azteca*. For *C. dubia*, the  $(f_c)_{M^{50\%}}$  value from Keithly et al. (2004) was also used. For these three species, the calculated log  $K_{CoBL}$  values are 5.48 (*C. dubia*), 5.67 (*D. magna*) and 5.83 (*H. azteca*). The three values are listed in Table S1.

The toxic effect of cobalt to *D. magna* was also studied by Okamoto et al. (2015) at a water hardness of 72 mg/L (as  $CaCO_3$ ) using a 48 h acute immobilisation test. Again, cobalt toxicity values from three other studies (Biesinger and Christensen, 1972; Khangarot and Ray, 1989; Rathore, 2001) at hardness values of 45 and 240 mg/L (as  $CaCO_3$ ), were cited in the study. The data from the four studies were used to determine an average cobalt toxicity at the average hardness, as there was not a reasonable relationship between hardness and toxicity from the studies. From the average values, log  $K_{CoBL}$  was determined by correcting for the concentrations of  $H^+$ ,  $Na^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$ . The obtained value (5.11) is listed in Table S1.

#### S4.3. Microalgae Species

Stubblefield et al. (2020) also studied the toxicity of cobalt to *R. subcapitata*. For this species, an  $EC_{50}$  concentration of 144 mg/L was determined. Analysis of the provided data led to a log  $K_{CoBL}$  value of 5.68. This value is consistent with the average value found for cobalt toxicity to freshwater species (Table 1) and other individual species.

The toxicity of cobalt to *R. subcapitata* was also studied by dos Reis et al. (2024b), where an  $IC_{50}$  concentration of 380 mg/L was determined. The free cobalt ion was found to be the dominant form of cobalt in the test waters. Using the  $IC_{50}$  concentration and the cobalt speciation, together with the ameliorative cation concentrations, led to a log  $K_{CoBL}$  value of 5.65 (Table S1). This value is in excellent agreement with the value derived from the study of Stubblefield et al. (2020) for the same species.

#### S4.4. Mollusc Species

Markich (2017) also used mortality (24 to 72 hours) to study the toxicity of cobalt to six freshwater mussels (glochidia: *A. profuga*, *C. novaehollandiae*, *H. australis*, *H. depressa*, *H. drapeta* and *V. ambiguus*). The study found an average value for log  $K_{CoBL}$  of 6.16, with a range of 6.04

to 6.29 (48 h mortality data). The free cobalt ion (97.0 %) was the dominant species in the solution. Reanalysis of the toxicity data in the present study led again to marginally smaller log  $K_{CoBL}$  values, ranging between 5.93 and 6.12. Table S1 lists all six values.

The snail, *L. stagnalis*, was also studied by Stubblefield et al. (2020). Based on the toxicity data provided, a log  $K_{CoBL}$  value of 5.91 was determined (Table S1). This value is in good agreement with values determined for other mollusc species.

#### S4.5. Macrophyte Species

Another species studied by Stubblefield et al. (2020) was *L. minor*. From the toxicity data provided for this species and the water chemistry, a log  $K_{CoBL}$  value of 5.89 was determined. This value is listed in Table S1.

Naumann et al. (2007) studied the toxicity of a range of metals to *L. minor* in relatively hard water. In their study using cobalt,  $Co^{2+}$  was found to be 54.4 % of total cobalt in the test solutions, where the observed toxicity occurred at a concentration of 292 mg/L. Utilising the  $EC_{50}$  concentration and the cobalt speciation, together with the ameliorative cation concentrations, led to a log  $K_{CoBL}$  value of 6.36. This value is somewhat larger than that derived from the study of Stubblefield et al. (2020), but has still been included in Table S1.

#### S4.6. Annelid Species

The final species studied by Stubblefield et al. (2020) was an *Aeolosoma* species. Again, based on the toxicity data provided for this species and the water chemistry, a log  $K_{CoBL}$  value of 5.36 was determined. This value is listed in Table S1.

### S5. Copper

#### S5.1. *Oncorhynchus mykiss*

Twelve studies have been reviewed with respect to the toxicity of copper to rainbow trout (*O. mykiss*). The derived values of log  $K_{CuBL}$  ranged between 7.63 and 8.28, with an average of  $7.92 \pm 0.13$ . If only  $Cu^{2+}$  was responsible for toxicity, the average log  $K_{CuBL}$  would increase to 8.17 (the range is more than one log unit: 7.68 to 8.73) with a 95 % confidence interval of 0.29.

The toxicity of copper to *O. mykiss* was studied by Crémazy et al. (2017) using both 96 h and 30 d mortality assays. Toxicity in both series of tests was studied at varying pH, DOC, calcium and magnesium concentrations; in the 30 d tests, the ranges used were as follows: pH (5.1 to 8.6), DOC (0.42 to 11 mg/L), Ca (2.4 to 124 mg/L) and Mg (0.97 to 75 mg/L). From these data, a log  $K_{CuBL}$  value of 8.18 was determined. This value is listed in Table S1.

The toxicity of copper to rainbow trout was studied by Marr et al. (1998) using a 14 d mortality assay. Speciation calculations showed that the free ion,  $Cu^{2+}$  (4.8 %) and  $CuOH^+$  (4.1 %) accounted for a similar proportion of total copper in the test solutions. Further adjustment for the concentration of the ameliorative cations  $H^+$ ,  $Na^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$  led to a derivation of the log  $K_{CuBL}$  value of 7.97 (Table S1). If only  $Cu^{2+}$  causes toxicity, the value of log  $K_{CuBL}$  increases to 8.09.

In another study of copper toxicity to *O. mykiss*, Cusimano et al. (1986) used 96 h mortality tests at a variable pH (4.7 to 7.0). The  $LC_{50}$  values determined were linearly dependent on the  $H^+$  concentration, with the solution speciation being dominated by  $Cu^{2+}$  at all three pH values (71-97 % of total copper; only at pH 7 did  $CuOH^+$  account for measurable copper (8 %)). Based on the toxicity results obtained, a log  $K_{CuBL}$  value of 7.77 was determined (Table S1).

Naddy et al. (2015) also studied the toxicity of copper to rainbow trout. The effect of varying hardness on the toxic response was also considered. The average toxicity in soft water was utilised together with that in hard water. The hard water also had elevated alkalinity and pH compared to the soft water. The  $Cu^{2+}$  ion was a relatively small percentage (10.6 %) in the soft water, and an even smaller percentage (0.3 %) of the hard water, whereas  $CuOH^+$  was 8.2 % of total copper in the soft water and 2.2 % in the hard water. When accounting for the speciation and the effects of the ameliorative cations, an average log  $K_{CuBL}$  of 7.79 was derived (Table S1). If only  $Cu^{2+}$  was responsible for toxicity, the log  $K_{CuBL}$  value would increase to 8.19.

The toxicity of copper to rainbow trout was studied by Calfee et al. (2014). The test solution had a pH of 7.9, a hardness of 104 mg/L as  $\text{CaCO}_3$  and an alkalinity of 94 mg/L as  $\text{CaCO}_3$ . The  $\text{Cu}^{2+}$  ion was only a small percentage (1.0 %) of the total copper in the test solution, whereas  $\text{CuOH}^+$  was a larger percentage (3.2 %), and accounting for the speciation and the ameliorative cations led to a log  $K_{\text{CuBL}}$  value of 8.09 (Table S1). The log  $K_{\text{CuBL}}$  value increases to 8.51 if only  $\text{Cu}^{2+}$  is responsible for toxicity.

Besser et al. (2007) studied the toxicity of copper to rainbow trout from populations in the Minnesota and Missouri Rivers. The acute swim-up life stage test results were used in the present analysis. The speciation of the test solutions indicated that the free copper ion was only a small portion (0.8 %) of total copper, with  $\text{CuOH}^+$  accounting for a larger amount (3.6 %), and using the speciation together with consideration of the effects of the ameliorative cations led to a log  $K_{\text{CuBL}}$  value of 8.07 (Table S1). If only  $\text{Cu}^{2+}$  is responsible for toxicity, the log  $K_{\text{CuBL}}$  value increases to 8.59.

The study of Chapman (1978) on the toxicity of copper to the swim-up stage of rainbow trout also led to a log  $K_{\text{CuBL}}$  value of 7.79 (Table S1) using 96 h  $\text{LC}_{50}$  concentrations. For copper, as was found for zinc, the 96 h  $\text{LC}_{50}$  was identical to the 200 h  $\text{LC}_{50}$ . Water in the tests was supplied from wells on the banks of the Willamette River. The  $\text{Cu}^{2+}$  ion was 6.3 % and  $\text{CuOH}^+$  3.4 % of total copper in the test solutions. The log  $K_{\text{CuBL}}$  value was derived from the  $\text{LC}_{50}$  and adjusted using the ameliorative cation concentrations and the copper speciation. As  $\text{CuOH}^+$  was a smaller fraction of total copper than  $\text{Cu}^{2+}$ , the change to log  $K_{\text{CuBL}}$  would only be relatively small if only  $\text{Cu}^{2+}$  accounted for toxicity (i.e., increasing to 7.89).

Another study examining the toxicity of copper to rainbow trout was by Ng et al. (2010) and used a pH of 7 and test waters of relatively low hardness, alkalinity and dissolved organic carbon. Consequently,  $\text{Cu}^{2+}$  was a reasonable percentage (19.5 %) in the test waters, with  $\text{CuOH}^+$  accounting for a further 5.0 % of total copper. Using the measured  $\text{LC}_{50}$  concentration (9.2 mg/L), the speciation and the ameliorative cation concentrations led to a log  $K_{\text{CuBL}}$  value of 7.63 (Table S1). Only a small change occurs in the log  $K_{\text{CuBL}}$  value if only  $\text{Cu}^{2+}$  is responsible for toxicity (i.e., increasing to 7.68).

Morris et al. (2019) also studied the toxicity of copper to *O. mykiss*. An  $\text{LC}_{50}$  concentration of 16 mg/L was measured in test solutions where  $\text{Cu}^{2+}$  was a reasonable percentage (15.5 %) of total copper and  $\text{CuOH}^+$  was much less (1.4 %). Based on the measured  $\text{LC}_{50}$  concentration, the copper speciation and the ameliorative cation concentrations, a log  $K_{\text{CuBL}}$  value of 7.66 was calculated. This value only increased by 0.02 (to 7.68) if only  $\text{Cu}^{2+}$  was responsible for toxicity.

Welsh et al. (2008) studied the toxicity of copper to rainbow trout in laboratory test waters of differing compositions. In the test waters, both  $\text{Cu}^{2+}$  and  $\text{CuOH}^+$  were only a small fraction of total copper. Taking account of the measured  $\text{LC}_{50}$  concentrations (16.6 and 17.0 mg/L), the copper speciation and water composition led to an average log  $K_{\text{CuBL}}$  value of 7.92 (Table S1). This value increased to 8.19 if only  $\text{Cu}^{2+}$  was responsible for toxicity.

Grosell and Wood (2002) used 2 h uptake measurements on *O. mykiss*. They determined an affinity constant ( $K_m$ ) in sodium-insensitive systems of 9.6 nmol/L. From this value, a log  $K_{\text{CuBL}}$  value of 8.02 is determined. This value is included in Table S1.

Naddy et al. (2002) also studied the toxicity of copper to *O. mykiss* using test solutions of varying  $\text{Ca}^{2+}$  concentration (10 to 68 mg/L). As the  $\text{Ca}^{2+}$  concentration increased, so too did the toxicity concentration. The  $\text{Cu}^{2+}$  ion was only a small percentage (1.0 %) of total copper, with  $\text{CuOH}^+$  an additional 3.6 %. Based on the available data and the copper speciation, the calculated log  $K_{\text{CuBL}}$  value was 8.28 (which increases to 8.73 if only  $\text{Cu}^{2+}$  is responsible for toxicity). The calculated log  $K_{\text{CuBL}}$  value is listed in Table S1.

### S5.2. Other *Oncorhynchus* Species

The toxicity of copper to *O. clarkii* was studied by Chakoumakos et al. (1979) in test waters of varying hardness, alkalinity and pH. As might be expected, as the alkalinity increased, so the fraction of copper as either  $\text{Cu}^{2+}$  or  $\text{CuOH}^+$  decreased. However, using the measured  $\text{LC}_{50}$  concentrations, the copper speciation and other water chemistry conditions in each test water led to very similar log  $K_{\text{CuBL}}$  values, with a calculated average value of 7.79 (Table S1). When

only considering the  $\text{Cu}^{2+}$  being responsible for toxicity, the individual log  $K_{\text{CuBL}}$  became more variable, with an average of 8.08.

Copper toxicity to three *Oncorhynchus* species (*O. kisutch*, *O. nerka* and *O. tshawytscha*) was studied by Porter et al. (2023) in Alaskan river/creek waters. The test waters were relatively soft and marginally acidic. The observed toxicities ranged between 6.3 and 35.2 mg/L. Copper speciation in the test waters was variable, with  $\text{Cu}^{2+}$  ranging between 6.2 and 21.5 % of total copper and  $\text{CuOH}^+$  between 0.2 and 1.8 %. Taking account of the  $\text{LC}_{50}$  concentrations, the copper speciation and ameliorative cation concentrations led to log  $K_{\text{CuBL}}$  values of 7.53 (*O. tshawytscha*), 7.66 (*O. nerka*) and 7.82 (*O. kisutch*). These values are listed in Table S1. In these test waters,  $\text{CuOH}^+$  is only a relatively small fraction of total copper and much less than  $\text{Cu}^{2+}$ . As such, the log  $K_{\text{CuBL}}$  values do not change substantially if only  $\text{Cu}^{2+}$  is considered to cause toxicity (7.54, 7.66 and 7.83, respectively).

The study of Chapman (1978) on the toxicity of copper to the swim-up stage of *O. tshawytscha* led to a log  $K_{\text{CuBL}}$  value of 7.82 (Table S1) using 96 h  $\text{LC}_{50}$  concentrations. Again, the 96 h  $\text{LC}_{50}$  was identical to the 200 h  $\text{LC}_{50}$ . Water in the tests was supplied from wells on the banks of the Willamette River. The  $\text{Cu}^{2+}$  ion was 7.1 % and  $\text{CuOH}^+$  2.4 % of total copper in the test solutions. The log  $K_{\text{CuBL}}$  value was derived from the  $\text{LC}_{50}$  and adjusted using the ameliorative cation concentrations and the copper speciation. As  $\text{CuOH}^+$  was a smaller fraction of total copper than  $\text{Cu}^{2+}$ , the change to log  $K_{\text{CuBL}}$  would only be relatively small if only  $\text{Cu}^{2+}$  accounts for toxicity (i.e., increasing to 7.89).

### S5.3. *Pimephales promelas*

Eleven studies are available in relation to the toxicity of copper to *P. promelas*. The derived log  $K_{\text{CuBL}}$  values range from 7.40 to 7.78, with an average of  $7.59 \pm 0.08$ . The average value is less than that derived for *O. mykiss* and the 95 % uncertainty intervals do not overlap. Interestingly, the average value for other *Oncorhynchus* species (7.72) is intermediate between the values for *P. promelas* and *O. mykiss*, with the range of other *Oncorhynchus* species overlapping the range in values of both *P. promelas* and *O. mykiss*.

Meyer et al. (1999) studied the toxicity of copper to *P. promelas* at varying calcium concentrations (11.0 to 55.3 mg/L) using a 96 h mortality test. In the study, they also assessed the earlier data of Erickson et al. (1996) in relation to the toxicity of copper to *P. promelas*. The data presented by Meyer et al. (1999) were evaluated in the present study to determine the log  $K_{\text{CuBL}}$ . The value was adjusted for the pH and the concentrations of magnesium and sodium (as listed in the original study (Erickson et al., 1996)). The value determined for  $K_{\text{CuBL}}$  is 7.44 and is listed in Table S1. Interestingly, Di Toro et al. (2001) also assessed the work of Erickson et al. (1996). Evaluation of the data presented by Di Toro et al. (2001) led to a larger value for log  $K_{\text{CuBL}}$  of 7.78 (Table S1). The differences in the interpretation of the two studies would appear to be related to differing speciation calculations, where the study of Di Toro et al. (2001) has lower free copper ion concentrations than the earlier study of Meyer et al. (1999). Evaluation of the data of Erickson et al. (1996) in the present study led to an intermediate log  $K_{\text{CuBL}}$  value of 7.65. This value is also listed in Table S1.

The toxicity of copper to *P. promelas* was also studied by Playle et al. (1993) using 2 to 3 h uptake assays. The study determined a log  $K_{\text{CuBL}}$  value of 7.4. This value has been retained (Table S1).

Schubauer-Berigan et al. (1993) measured the toxicity of copper to four freshwater species, including that of *P. promelas* as a function of pH. Two  $\text{LC}_{50}$  values were determined for *P. promelas*, and the average value was used to calculate the log  $K_{\text{CuBL}}$  value (7.67). The log  $K_{\text{CuBL}}$  value is listed in Table S1.

Diamond et al. (1997) also studied the toxicity of copper to *P. promelas* in both laboratory water and Lehigh River water sampled at different times of year. The geometric average of the  $\text{LC}_{50}$  data for the laboratory water was used in the present analysis and gave a concentration of 36 mg/L. The use of this toxicity concentration, together with the copper speciation ( $\text{Cu}^{2+}$  is 18.4 % and  $\text{CuOH}^+$  is 5.6 % of total copper) and the ameliorative cation concentrations, resulted in

a log  $K_{CuBL}$  value of 7.55 (Table S1). If only  $Cu^{2+}$  is responsible for toxicity, the log  $K_{CuBL}$  value increases marginally to 7.61.

The toxicity of copper to *P. promelas* was studied by Sciera et al. (2004) using a large range of conditions including variable alkalinity, pH, dissolved organic carbon (DOC) concentration, hardness and  $Na^+$  concentration. In the absence of DOC, at low pH (around 6) and low alkalinity (4 mg/L as  $CaCO_3$ ), copper speciation was dominated by  $Cu^{2+}$ , whereas at high pH (around 8) and higher alkalinity (52 to 55.3 mg/L as  $CaCO_3$ ),  $Cu^{2+}$  was a much smaller fraction of total copper and was less than the fraction of  $CuOH^+$ . A log  $K_{CuBL}$  value was determined for each condition studied, of which there were 39, that led to an average value of  $7.74 \pm 0.10$ . This value is listed in Table S1. If only  $Cu^{2+}$  is responsible for toxicity, the average value increases to 7.87.

Naddy et al. (2002) also studied the toxicity of copper to *P. promelas* using test solutions of varying  $Ca^{2+}$  concentration (28.5 to 44.8 mg/L). As the  $Ca^{2+}$  concentration increased, so too did the toxicity concentration. The  $Cu^{2+}$  ion was only a small percentage (1.0 %) of total copper, with  $CuOH^+$  an additional 3.6 %. Based on the available data and the copper speciation, the calculated log  $K_{CuBL}$  value was 7.53 (which increases to 7.98 if only  $Cu^{2+}$  is responsible for toxicity). The calculated log  $K_{CuBL}$  value is listed in Table S1.

The toxicity of copper to *P. promelas* was studied by Welsh et al. (1996) in 18 lake waters from Ontario, Canada. Copper speciation in the lake waters was dependent on each lake's water chemistry. Using the measured  $LC_{50}$  concentrations, the copper speciation and ameliorative cation concentrations led to an average log  $K_{CuBL}$  value of 7.62. This value is listed in Table S1.

Morris et al. (2019) also studied the toxicity of copper to *P. promelas*. An  $LC_{50}$  concentration of 29 mg/L was measured in test solutions. The  $Cu^{2+}$  ion was a reasonable percentage (10.2 %) of total copper and  $CuOH^+$  was much less (3.0 %). Based on the measured  $LC_{50}$  concentration, the copper speciation and the ameliorative cation concentrations, a log  $K_{CuBL}$  value of 7.52 was calculated. This value increased by 0.06 (to 7.58) if only  $Cu^{2+}$  was responsible for toxicity.

In another study, Nimmo et al. (2006) examined the toxicity of copper to *P. promelas* in reconstituted water and found an  $LC_{50}$  concentration of 31.7 mg/L. In the water,  $Cu^{2+}$  was 6.7 % and  $CuOH^+$  11.0 % of total copper. Taking account of the  $LC_{50}$ , the copper speciation and other water quality data led to a log  $K_{CuBL}$  value of 7.59. This value is listed in Table S1. It increased to 7.85 if only  $Cu^{2+}$  is responsible for toxicity.

#### S5.4. Other Fish Species

Five studies have been reviewed with respect to the toxicity of copper to other fish species. The log  $K_{CuBL}$  values determined range between 7.34 and 8.29, with an average of 7.92. This is the same average log  $K_{CuBL}$  value that was found for *O. mykiss* and is consistent with the average found for all freshwater species (Table 1).

Trenfield et al. (2022) studied the toxicity of copper to several species, including *Mogurnda mogurnda* (7 d growth assay) in waters of low hardness. Speciation calculations indicated that the free copper ion was 14.2 % of total copper and  $CuOH^+$  a further 1.6 %. The  $EC_{50}/LC_{50}$  concentrations determined for all species studied ranged between 7.6 and 22.5 mg/L only, a relatively narrow range, with *M. mogurnda* having the largest  $EC_{50}$  concentration of 22.5 mg/L. Combining this copper toxicity concentration with the copper speciation and adjusting for the ameliorative cation concentrations leads to a log  $K_{CuBL}$  value of 7.34 (Table S1).

Vardy et al. (2014) studied the toxicity of copper to white sturgeon (*A. transmontanus*) in both laboratory and Columbia River water. The data for the youngest life-stage (8 days post-hatch) and laboratory water were used in the present analysis, together with the copper speciation and ameliorative cation concentrations, to determine a log  $K_{CuBL}$  value of 8.14. This value is included in Table S1. The log  $K_{CuBL}$  value increases to 8.32 if only  $Cu^{2+}$  is responsible for toxicity.

The toxicity of copper to white sturgeon was also studied by Ivey et al. (2019) in water of relatively high hardness and pH. An  $LC_{50}$  concentration of 21.5 mg/L was determined where  $Cu^{2+}$  and  $CuOH^+$  were 2.0 and 6.0 %, respectively, of total copper. Using the  $LC_{50}$  and adjusting

for the copper speciation and ameliorative cation concentrations leads to a log  $K_{CuBL}$  value of 8.29. This value is listed in Table S1; it increases to 8.68 if only  $Cu^{2+}$  is responsible for toxicity.

Besser et al. (2007) also studied the toxicity of copper to *C. bairdi* from populations in the Minnesota and Missouri Rivers. The acute swim-up life stage test results were used in the present analysis. The speciation of the test solutions had only relatively small amounts of the free copper ion (0.8 %) as well as the  $CuOH^+$  ion (3.6 %) and, together with consideration of the effects of the ameliorative cations, led to a log  $K_{CuBL}$  value of 8.13 (Table S1). If only  $Cu^{2+}$  was considered to be responsible for toxicity, the log  $K_{CuBL}$  value would increase to 8.64.

The toxicity of copper to *D. rerio* was studied by Abdel-monein et al. (2015) using a 48 h growth endpoint. An  $EC_{50}$  concentration of 34 mg/L was determined, where  $Cu^{2+}$  was 8.2 % and  $CuOH^+$  3.8 % of total copper. Using the  $EC_{50}$  and copper speciation together with the ameliorative cation concentrations led to a log  $K_{CuBL}$  value of 7.70. This value increased marginally to 7.79 if only  $Cu^{2+}$  accounts for toxicity.

### S5.5. *Daphnia magna*

There are twelve values listed related to the toxicity of copper to *D. magna*. The log  $K_{CuBL}$  values that have been determined range between 7.27 and 8.05, with an average value of  $7.77 \pm 0.13$ . This value and its associated 95 % uncertainty interval overlap with those of both *O. mykiss* and *P. promelas*.

De Schamphalaere and Janssen (2002) conducted a comprehensive study of the toxicity of copper to *D. magna* using a 48 h immobilisation assay at variable pH (5.98 to 7.92) and concentrations of calcium (10 to 160 mg/L), magnesium (6.1 to 122 mg/L), sodium (1.8 to 347 mg/L) and potassium (3.0 to 78 mg/L). The toxicity results from this study have been extensively reviewed in the present work. The data as a function of pH were assessed to determine a binding constant for  $CuOH^+$ ; this analysis used a zero ionic strength constant for the species from Brown and Ekberg (2016), updated using the data from Palmer (2017);  $\log K(Cu^{2+} + OH^- \rightleftharpoons CuOH^+) = 6.363$ . This analysis derived an average stability constant in the test solutions where pH was varied in the De Schamphalaere and Janssen (2002) study of 6.245 (range: 6.225 to 6.254). In the study (their Figure 1f), the reciprocal of the free copper concentration (from each of the test solutions) against the hydroxide ion concentration indicated a linear relationship, from which a slope/intercept value of 843,000 was obtained. When this value is divided by the  $CuOH^+$  stability constant (6.245) in the test solutions, a value of 0.48 is found. The inverse of this value is 2.084, which is the relative ratio of the effect of  $Cu^{2+}$  to that of  $CuOH^+$  in the variable pH test solutions. This value is essentially equivalent to that found by Markich et al. (2003) in a study of the response of bivalves to exposures to copper and confirms the use of this factor (2) to explain the response of organisms to exposure to both  $Cu^{2+}$  and  $CuOH^+$ . Analysis of the full dataset provided by De Schamphalaere and Janssen (2002) led to a value for log  $K_{CuBL}$  of 7.90 (Table S1) and a  $(f_c)_{Cu^{50\%}}$  value of 0.45. The latter value is identical to that derived for *D. magna* by studies from this laboratory for both nickel and zinc (Heijerick et al., 2005a; Deleebeeck et al., 2008) as well as by Mano and Shinohara (2020), as well as for nickel. Both values obtained are in reasonable agreement with those found in the study of De Schamphalaere and Janssen (2002), namely 8.02 and 0.39, respectively.

Long et al. (2004) also studied the toxicity of copper to *D. magna*, but used a 48 h mortality assay at a varying pH and hardness. The study provided speciation calculations for the contribution of copper species, in particular  $Cu^{2+}$  and  $CuOH^+$ . Data were provided for one or both of these copper species at pH 5.6 and 7. From these data, the  $LC_{50}$  values determined and the effects of the ameliorative cations, a log  $K_{CuBL}$  value of 7.85 was determined. This value is listed in Table S1.

The toxic effect of copper to *D. magna* was studied by Okamoto et al. (2015) at a water hardness of 72 mg/L (as  $CaCO_3$ ) using a 48 h acute immobilisation test. They also listed metal toxicity values from other studies (Biesinger and Christensen, 1972; Khangarot and Ray, 1989; Rathore, 2001) at hardness values of 45 and 240 mg/L (as  $CaCO_3$ ). From the toxicity concentration determined in the study, a log  $K_{CuBL}$  value of 7.93 was determined by correcting for the concentrations of  $H^+$ ,  $Na^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$  as well as the copper speciation in the test

solutions. The obtained value is listed in Table S1; if only  $\text{Cu}^{2+}$  is responsible for toxicity, the  $\log K_{\text{CuBL}}$  value increases to 8.05.

De Schamphalaere et al. (2007) also studied the toxicity of copper to *D. magna* with varying sodium concentrations (1.8 to 230 mg/L) using a 48 h immobilisation assay. The toxicity of copper to *D. magna* was found to increase as the concentration of sodium in the test solutions decreased. Using the relationship between toxicity and sodium concentration, and a derived value of 0.24 for  $(f_c)_{\text{Cu}^{50\%}}$ , led to a  $\log K_{\text{CuBL}}$  value of 7.27. This value is listed in Table S1. The value derived for  $(f_c)_{\text{Cu}^{50\%}}$  is half that derived by De Schamphalaere and Janssen (2002) for the same species. If the  $(f_c)_{\text{Cu}^{50\%}}$  value derived (0.45) from this latter study is used, the value determined for  $\log K_{\text{CuBL}}$  is 7.69, in much better agreement with the average  $\log K_{\text{CuBL}}$  determined in the present study.

Copper toxicity to *D. magna* was also studied by Mount and Norberg (1984) at pH 7.3 and with test waters of relatively low hardness. The free copper ion accounted for only 4.2 % of total copper and  $\text{CuOH}^+$  an additional 2 %. A toxicity of 54 mg/L was determined, which led to a  $\log K_{\text{CuBL}}$  value of 7.59 (Table S1) when the ameliorative cation concentrations and speciation are considered. The  $\log K_{\text{CuBL}}$  value increases to 7.68 if only  $\text{Cu}^{2+}$  is responsible for toxicity.

Naddy et al. (2002) also studied the toxicity of copper to several species that included *D. magna* using test solutions of varying alkalinity and pH. As the  $\text{Ca}^{2+}$  concentration increased, so too did the toxicity concentration. The  $\text{Cu}^{2+}$  ion was only a small fraction of total copper in the test solutions, with  $\text{CuOH}^+$ , in general, being a larger fraction. Based on the available data and the copper speciation, the average  $\log K_{\text{CuBL}}$  value was calculated to be 7.86 (which increases to 8.25 if only  $\text{Cu}^{2+}$  is responsible for toxicity). The calculated  $\log K_{\text{CuBL}}$  value is listed in Table S1.

The toxicity of copper to *D. magna* was also studied by Ryan et al. (2009) in test waters with low dissolved organic carbon contents, and two river waters of variable composition (alkalinity, hardness and pH). From the measured  $\text{LC}_{50}$  concentrations, the copper speciation and concentrations of the ameliorative cations, an average  $\log K_{\text{CuBL}}$  value of 7.77 was calculated (Table S1). This value increased to 8.03 if only  $\text{Cu}^{2+}$  resulted in toxicity.

Another study examining the toxicity of copper to *D. magna* was that of De Schamphalaere et al. (2002) that used test waters of variable composition (dissolved organic carbon, hardness, alkalinity,  $\text{Na}^+$  concentration and pH) as well as natural surface waters. The study used 48 h immobilisation endpoints, and when adjusted for copper speciation and the concentrations of the ameliorative cations, led to a  $\log K_{\text{CuBL}}$  value of 8.05 (Table S1). If only  $\text{Cu}^{2+}$  caused toxicity, this value increased to 8.27.

Villavicencio et al. (2005) studied the toxicity of copper to *D. magna* in several Chilean river and lake waters. The speciation of copper was quite variable, with  $\text{Cu}^{2+}$  increasing as the pH decreased and  $\text{CuOH}^+$  becoming important at a higher pH. Using the measured  $\text{LC}_{50}$  concentrations, the calculated copper speciation and the concentrations of the ameliorative cations led to a  $\log K_{\text{CuBL}}$  value of 7.95 (Table S1), which increases to 8.20 if only  $\text{Cu}^{2+}$  is used to explain toxicity.

The toxicity of copper to a large range of cladoceran species was tested by Bossuyt and Janssen (2005). The study included copper toxicity to *D. magna*. The average toxicity determined for *D. magna* at the  $\text{EC}_{50}$  was found to be 37.7 mg/L. Of the total copper in the test solution,  $\text{Cu}^{2+}$  was 8.8 % and  $\text{CuOH}^+$  was 12.6 %. Based on the average  $\text{EC}_{50}$ , the copper speciation and the ameliorative cation concentrations, a  $\log K_{\text{CuBL}}$  value of 7.86 was determined (Table S1). This value increased to 8.09 if only  $\text{Cu}^{2+}$  causes toxicity.

Koivisto et al. (1992) also studied the toxicity of copper to several cladoceran species including *D. magna*. The  $\text{LC}_{50}$  determined in the study was 49.5 mg/L. The  $\text{Cu}^{2+}$  was 2.2 % of total copper and  $\text{CuOH}^+$  was an additional 14 %. Taking account of the  $\text{LC}_{50}$ , the copper speciation and the concentrations of the ameliorative cations led to a  $\log K_{\text{CuBL}}$  value of 7.42 (Table S1). If only  $\text{Cu}^{2+}$  causes toxicity, this value increases to 8.04.

The toxicity of copper to *D. magna* in laboratory water and several surface waters was studied by Kramer et al. (2004). The  $\text{EC}_{50}$  concentrations were found to increase as the dissolved organic carbon (DOC) content of the test waters increased. The intercept of  $\text{EC}_{50}$  concentration



against DOC content was identical to the EC<sub>50</sub> concentration from laboratory test water (30 mg/L). The Cu<sup>2+</sup> ion was 2.8 % of total copper and the CuOH<sup>+</sup> ion was a further 6.6 %. Taking account of the EC<sub>50</sub>, the copper speciation, the ameliorative cation concentrations and the (f<sub>C</sub>)<sub>Cu</sub><sup>50%</sup> value from De Schamphalaere et al. (2007) led to a log K<sub>CuBL</sub> value of 7.75 (Table S1). This value increased to 8.08 if only Cu<sup>2+</sup> causes toxicity.

#### S5.6. Other *Daphnia* Species

Thirteen data points are reported for other *Daphnia* species. Three are for *D. galeata*, two for *D. longispina*, four for *D. pulex* and one single datum for four other *Daphnia* species. The log K<sub>CuBL</sub> values range between 7.42 and 8.14, with an average of 7.77 ± 0.12. The average value is identical to that found for *D. magna*. The need to include CuOH<sup>+</sup> in the calculation of log K<sub>CuBL</sub> is illustrated when considering the data of Winner (1985). When CuOH<sup>+</sup> is considered, the calculated log K<sub>CuBL</sub> value is consistent with other values; however, when it is not considered, the calculated log K<sub>CuBL</sub> increases substantially to 8.78, around 0.3 (or double for K<sub>CuBL</sub>) more than any other value and more than 1 log unit greater than some of the other values calculated (see below). Thus, there is far more variation in log K<sub>CuBL</sub> when CuOH<sup>+</sup> is not considered.

The toxicity of copper to cladoceran species studied by De Schamphalaere et al. (2007) using a 48 h immobilisation assay with varying sodium concentrations (1.8 to 230 mg/L) also included two more *Daphnia* sp., *D. galeata* and *D. longispina*. The toxicity of copper to the species was always found to increase as the concentration of sodium in the test solutions decreased. Using the relationship between toxicity and sodium concentration led to the following two log K<sub>CuBL</sub> values: 7.66 (*D. galeata*) and 7.87 (*D. longispina*). These values are listed in Table S1. For each species, all the copper-versus-sodium data for a given species were analysed together to derive the log K<sub>CuBL</sub> values. Also derived from the data in the study were the following (f<sub>C</sub>)<sub>Cu</sub><sup>50%</sup> values: 0.20 (*D. galeata*) and 0.17 (*D. longispina*). As indicated, the value of (f<sub>C</sub>)<sub>Cu</sub><sup>50%</sup> found for *D. magna* was 0.24, indicating that the values obtained were somewhat similar for the three *Daphnia* sp. The results from this study are discussed in further detail below in the Sodium section.

The toxicity of copper to *D. galeata* and *D. longispina* was also studied by Bossuyt and Janssen (2005). The EC<sub>50</sub> concentrations determined for *D. galeata* and *D. longispina* were found to be 22.6 and 10.8 mg/L, respectively. The copper speciation was the same as that found for *D. magna*. Based on the average EC<sub>50</sub> concentrations, the copper speciation and the ameliorative cation concentrations, log K<sub>CuBL</sub> values of 7.48 and 7.70, respectively, were determined (Table S1) using the (f<sub>C</sub>)<sub>Cu</sub><sup>50%</sup> values derived for the species from the study of De Schamphalaere et al. (2007). These values increase to 7.71 and 7.93 if only Cu<sup>2+</sup> causes toxicity.

Koivisto et al. (1992) also studied the toxicity of copper to *D. galeata* and *D. pulex*. The LC<sub>50</sub> concentrations determined in the study were 7.4 and 12.2 mg/L, respectively. The Cu<sup>2+</sup> was 2.2 % of total copper and CuOH<sup>+</sup> was an additional 14 %. Taking account of the LC<sub>50</sub>, the copper speciation and the concentrations of the ameliorative cations as well as the (f<sub>C</sub>)<sub>Cu</sub><sup>50%</sup> values from De Schamphalaere et al. (2007) for *D. galeata* and those of Kozlova et al. (2009) for *D. pulex* led to log K<sub>CuBL</sub> values of 7.64 and 7.87 (Table S1), respectively. If only Cu<sup>2+</sup> causes toxicity, these values increase to 8.27 and 8.49.

Cooper et al. (2009) studied the toxicity of copper to *D. carinata* using 48 h mortality assays. The LC<sub>50</sub> concentration determined for *D. carinata* was 37.3 mg/L. The Cu<sup>2+</sup> ion was 10.8 % of copper present in the test solutions and CuOH<sup>+</sup> was a further 8.1 %. The derived log K<sub>CuBL</sub> value determined from the study was 7.42 when the concentrations of the ameliorative cations were considered. The derived binding constant is listed in Table S1.

Harmon et al. (2003) studied the toxicity of copper to *D. ambigua* by using a 48 h immobilisation assay. The study found an EC<sub>50</sub> concentration of 30.4 mg/L. Taking this value into account and the calculated amounts of Cu<sup>2+</sup> (2.4 %) and CuOH<sup>+</sup> (6.0 %) of total copper, the derived value for log K<sub>CuBL</sub> is 7.88 (Table S1) when adjusting for the ameliorative cation concentrations; if CuOH<sup>+</sup> does not induce a toxic response, then the log K<sub>CuBL</sub> value increases to 8.23, due to the larger fraction of copper present as CuOH<sup>+</sup> in the test water.

Hernández-Zamora et al. (2023) studied the toxicity of copper to *D. exilis* and determined an LC<sub>50</sub> of 13.5 mg/L. The concentrations of Cu<sup>2+</sup> and CuOH<sup>+</sup> were similar, representing 11.9 and 8.2 %, respectively, of total copper. Using the LC<sub>50</sub> and adjusting for copper speciation and ameliorative cation concentrations leads to a log  $K_{CuBL}$  value of 7.84. This value is listed in Table S1; it increases to 7.97 if only Cu<sup>2+</sup> is responsible for copper toxicity.

Villavicencio et al. (2005) also studied the toxicity of copper to *D. obtusa* and *D. pulex* in several Chilean river and lake waters. The speciation of copper was quite variable, with Cu<sup>2+</sup> increasing as the pH decreased and CuOH<sup>+</sup> becoming important at a higher pH. Using the measured LC<sub>50</sub> concentrations, the calculated copper speciation and the concentrations of the ameliorative cations led to log  $K_{CuBL}$  values of 7.84 and 8.14, respectively (Table S1), which increases to 8.09 and 8.39 if only Cu<sup>2+</sup> is used to explain toxicity.

Mount and Norberg (1984) also studied copper toxicity to *D. pulex* at pH 7.3 and test waters of relatively low hardness. The free copper ion accounted for 4.2 % of total copper, with CuOH<sup>+</sup> representing a further 2 %. For this *Daphnia* species, a similar toxicity of 53 mg/L (compared to their value for *D. magna*) was determined, which led to a log  $K_{CuBL}$  value of 7.60 (Table S1) when the ameliorative cation concentrations are considered.

The toxicity of copper to *D. pulex* was also studied by Winner (1985). A 72 h LC<sub>50</sub> of 25.9 mg/L was determined. The Cu<sup>2+</sup> ion was only a small fraction (0.9 %) of total copper, with CuOH<sup>+</sup> having a larger content (8.4 %) at a pH of 8.6. Accounting for the LC<sub>50</sub> and adjusting for copper speciation and the ameliorative cation concentrations led to a log  $K_{CuBL}$  value of 8.02 (Table S1); given the low Cu<sup>2+</sup> concentration relative to CuOH<sup>+</sup>, if only the former is responsible for toxicity, the log  $K_{CuBL}$  value increases substantially to 8.78.

#### S5.7. *Ceriodaphnia dubia*

Fifteen studies are given for the toxicity of copper to *C. dubia*. The values determined for log  $K_{CuBL}$  range between 7.37 and 8.36, with an average of  $7.96 \pm 0.16$ . Within the uncertainty intervals, the average log  $K_{CuBL}$  value is consistent with that derived for *Daphnia* species.

Markich et al. (2005) studied the toxicity of copper to *C. dubia* using a 48 h immobilisation assay at various hardness concentrations, with the Cu<sup>2+</sup> and CuOH<sup>+</sup> fractions of total copper determined by the study at each hardness. Using equation (16) gave consistent EC<sub>50</sub> concentrations across the full range of hardness studied, which led to a log  $K_{CuBL}$  value of 8.02 (Table S1). If only Cu<sup>2+</sup> was responsible for toxicity, log  $K_{CuBL}$  increased fractionally to 8.04. In the conditions studied, the fraction of CuOH<sup>+</sup> was very much less than Cu<sup>2+</sup>, and, therefore, its effect on toxicity was only minor. The  $(f_c)_M^{50\%}$  value determined by Keithly et al. (2004) was not used to modify the EC<sub>50</sub> determined in the Markich et al. (2005) study.

The toxicity of copper to *C. dubia* was also tested by Hyne et al. (2005), where a 48 h immobilisation assay was utilised. The effect of pH (5.5 to 7.5) was studied and when corrected for pH, and the concentrations of the major ions, the EC<sub>50</sub> was found to range between 0.57 (pH 7.5) and 1.49 (pH 5.5) mg/L, with an average of 1.09 mg/L. This latter value, together with the speciation of copper as Cu<sup>2+</sup> and CuOH<sup>+</sup> in the test solutions, leads to a log  $K_{CuBL}$  of 7.76 (Table S1), again assuming that  $(f_c)_{Cu}^{50\%}$  is 0.50.

Schubauer-Berigan et al. (1993) measured the toxicity of copper to four freshwater species, including that of *C. dubia* as a function of pH. Only two LC<sub>50</sub> values were determined for *C. dubia* and, as such, the average value was used to determine the log  $K_{CuBL}$  value (7.87; Table S1) when accounting for the fractions of copper present as Cu<sup>2+</sup> and CuOH<sup>+</sup>.

De Schamphalaere et al. (2007) studied the toxicity of copper to a large number of cladoceran species, including *C. dubia* with varying sodium concentrations (1.8 to 230 mg/L), using a 48 h immobilisation assay. The toxicity of copper to *C. dubia* was found to increase as the concentration of sodium in the test solutions decreased. Using the relationship between toxicity and sodium concentration, and also utilising the  $(f_c)_M^{50\%}$  value from the study of Keithly et al. (2004), led to a log  $K_{CuBL}$  value of 7.77. This value is listed in Table S1.

Cooper et al. (2009) also studied the toxicity of copper to *C. dubia* using a 7 d reproduction assay and found an EC<sub>50</sub> concentration of 1.8 mg/L. The 7 d reproduction assay was found to be much more sensitive than a 48 h mortality assay (18.0 mg/L). The cations, Cu<sup>2+</sup> and CuOH<sup>+</sup>,

were found to be 10.8 and 8.5 %, respectively, of total copper. These data, together with the effect of the ameliorative cations, led to a calculated log  $K_{CuBL}$  value of 7.47 (Table S1); this value increases to 7.61 if only  $Cu^{2+}$  causes toxicity. The  $(fc)_{M^{50\%}}$  value from Keithly et al. (2004) for *C. dubia* was also used with the toxicity data from this study.

The toxicity of copper to *C. dubia* was also studied by Harmon et al. (2003) using a 48 h immobilisation assay. The study found an  $EC_{50}$  concentration of 36.2 mg/L. Taking this value into account and the calculated amounts of  $Cu^{2+}$  (2.4 %) and  $CuOH^+$  (6.0 %) of total copper, the derived value for log  $K_{CuBL}$  is 7.81 (Table S1); if  $CuOH^+$  does not induce a toxic response, then the log  $K_{CuBL}$  value increases to 8.16, due to the larger fraction of copper present as  $CuOH^+$  in the test water. For this study, the  $(fc)_{M^{50\%}}$  value determined by Keithly et al. (2004) was not used to modify the derived  $EC_{50}$ .

Another species Naddy et al. (2002) studied relating to the toxicity of copper was *C. dubia*, using test solutions with an alkalinity of 115-116 mg/L (as  $CaCO_3$ ) and a pH of 8.2. The  $Cu^{2+}$  ion was only a small fraction (about 1 %) of total copper in the test solutions, with  $CuOH^+$  being a larger fraction (3.6 %). Based on the available data and the copper speciation, the average log  $K_{CuBL}$  value was calculated to be 8.25 (which increases to 8.70 if only  $Cu^{2+}$  is responsible for toxicity). The calculated log  $K_{CuBL}$  value is listed in Table S1.

In a later study, Naddy et al. (2015) also examined the toxicity of copper to *C. dubia*. The effect of varying hardness on the toxic response was also considered. The average toxicity in soft water was utilised together with that in hard water. The hard water also had elevated alkalinity and pH compared to the soft water. The  $Cu^{2+}$  ion was 10.4 % of total copper and  $CuOH^+$  a further 8.2 % in the soft water, but both were much smaller fractions (0.2 and 2.4 %, respectively) of the hard water. When accounting for the speciation and the effects of the ameliorative cations, an average log  $K_{CuBL}$  of 7.97 was derived (Table S1).

Gensemer et al. (2002) studied the toxicity of copper to *C. dubia* in test waters of varying hardness. The test waters became more toxic as the hardness decreased, with an intercept toxicity of 14.1 mg/L. The  $Cu^{2+}$  ion was 2.6 % of total copper and  $CuOH^+$  a further 5.8 %. Using the derived  $EC_{50}$ , the copper speciation and the concentrations of the remaining ameliorative cations ( $Na^+$  and  $H^+$ ) leads to a log  $K_{CuBL}$  value of 8.07 (Table S1). This value increases to 8.40 if only  $Cu^{2+}$  is responsible for toxicity.

Bossuyt and Janssen (2005) also studied copper toxicity to *C. dubia*. The  $EC_{50}$  was found to be 11.8 mg/L for *C. dubia*. Of the total copper in the test solution,  $Cu^{2+}$  was 8.8 % and  $CuOH^+$  was 12.6 %. Based on the  $EC_{50}$ , the copper speciation and the ameliorative cation concentrations, a log  $K_{CuBL}$  value of 8.36 was determined (Table S1). This value increased to 8.59 if only  $Cu^{2+}$  causes toxicity.

The study of Diamond et al. (1997) examined the toxicity of copper to *C. dubia* in both laboratory water and Lehigh River water sampled at different times of year. The geometric average of the  $LC_{50}$  data for the laboratory water was used in the present analysis, which gave a concentration of 6.4 mg/L. The use of this toxicity concentration, together with the copper speciation ( $Cu^{2+}$  is 18.2 % and  $CuOH^+$  is 5.6 % of total copper) and the ameliorative cation concentrations, resulted in a log  $K_{CuBL}$  value of 8.30 (Table S1). If only  $Cu^{2+}$  accounted for toxicity, the log  $K_{CuBL}$  value would increase to marginally to 8.36.

Stauber et al. (2023) studied the toxicity of copper to *C. dubia* in a very soft test water. A 7 d reproduction endpoint was used. The free copper ion was 5.5 % of total copper and  $CuOH^+$  a further 13.4 % in the test waters studied. Based on the speciation and ameliorative cation concentrations and the measured  $EC_{50}$  (6.6 mg/L), a value of log  $K_{CuBL}$  of 7.98 is derived (Table S1). This value increases to 8.33 if only  $Cu^{2+}$  is responsible for toxicity.

In another study, Nimmo et al. (2006) examined the toxicity of copper to *C. dubia* in reconstituted water and found an  $LC_{50}$  concentration of 7.43 mg/L. In the water,  $Cu^{2+}$  was 6.7 % and  $CuOH^+$  11.0 % of total copper. Taking account of the  $LC_{50}$ , the copper speciation and other water quality data led to a log  $K_{CuBL}$  value of 8.22. This value is listed in Table S1. It increases to 8.48 if only  $Cu^{2+}$  is responsible for toxicity.

Wang et al. (2011b) studied the toxicity of copper to *C. dubia* in a reference water and other waters of varying DOC. Analysis of the data indicated that the relationship between  $LC_{50}$  and

the DOC content led to an intercept  $LC_{50}$  (i.e.,  $DOC = 0$ ) of 20.8 mg/L that was consistent with the  $LC_{50}$  determined in the reference water (22.8 mg/L). In both test waters,  $Cu^{2+}$  was a small proportion of total copper, with  $CuOH^+$  about three times greater than  $Cu^{2+}$ . Based on the  $LC_{50}$  values, the speciation and the ameliorative cation concentrations, an average  $\log K_{CuBL}$  value of 8.23 was derived (Table S1). If only  $Cu^{2+}$  accounts for toxicity, then the  $\log K_{CuBL}$  value increases to 8.64.

The toxicity of copper to *C. dubia* was also studied by Ivey et al. (2019) in water of relatively high hardness and pH. An  $LC_{50}$  concentration of 10.2 mg/L was determined, where  $Cu^{2+}$  and  $CuOH^+$  were 1.9 and 5.8 %, respectively, of total copper. Using the  $LC_{50}$  and adjusting for the copper speciation and ameliorative cation concentrations as well as the  $(f_c)_{Cu^{50\%}}$  value from Keithly et al. (2004) leads to a  $\log K_{CuBL}$  value of 7.37. This value is quite small compared to other values, but is still listed in Table S1; it increases to 7.77 if only  $Cu^{2+}$  is responsible for toxicity.

#### S5.8. Other Ceriodaphnia Species

There are six  $\log K_{CuBL}$  values for other *Ceriodaphnia* species that range between 7.56 and 8.24, with an average of 7.93. The average is consistent with that found for fish species and for *C. dubia*.

Choeri et al. (2009) studied the toxicity of copper to *C. cornuta* both with and without the addition of exudates. The data without exudate addition was used in the present study. A 48 h  $EC_{50}$  of 5.14 mg/L was determined in the study. Of total copper in solution at the  $EC_{50}$ ,  $Cu^{2+}$  was 22.1 % and  $CuOH^+$  was 15 %. Taking account of the  $EC_{50}$  and adjusting for the copper speciation and concentrations of the ameliorative cations led to a  $\log K_{CuBL}$  value of 7.92 (Table S1). This value increased to 8.05 if only  $Cu^{2+}$  causes toxicity.

The testing of the toxicity of copper to cladoceran species studied by De Schamphalaere et al. (2007), using a 48 h immobilisation assay with varying sodium concentrations (1.8 to 230 mg/L), also included two more *Ceriodaphnia* sp., *C. reticulata* and *C. pulchella*. The toxicity of copper to the species was found to increase as the concentration of sodium in the test solutions decreased. Using the relationship between toxicity and sodium concentration led to the following  $\log K_{CuBL}$  values, 7.84 (*C. reticulata*) and 7.56 (*C. pulchella*). These two values are listed in Table S1. For each species, all the copper-versus-sodium data for a given species were analysed together to derive the  $\log K_{CuBL}$  values. Also derived from the data in the study was the  $(f_c)_{Cu^{50\%}}$  value for *C. pulchella* (0.0020), whereas 0.50 was assumed for *C. reticulata*. The results from this study are discussed in further detail below in the Sodium section.

Bossuyt and Janssen (2005) also studied copper toxicity to both *C. pulchella* and *C. reticulata*. Average  $EC_{50}$  values of 14.2 and 15.5 mg/L were determined, respectively. Of the total copper in the test solution,  $Cu^{2+}$  was 8.8 % and  $CuOH^+$  was 12.6 %. Based on the  $EC_{50}$  values, the copper speciation and the ameliorative cation concentrations,  $\log K_{CuBL}$  values of 7.78 and 8.24 were determined (Table S1). These values increase to 8.01 and 8.48 if only  $Cu^{2+}$  causes toxicity. A  $(f_c)_{Cu^{50\%}}$  value derived from the study of De Schamphalaere et al. (2007) was used for *C. pulchella*.

Mount and Norberg (1984) also studied copper toxicity to *C. reticulata* at pH 7.3 and with test waters of relatively low hardness. The free copper ion accounted for 4.0 % of total copper, with  $CuOH^+$  representing a further 1.8 %. For this *Ceriodaphnia* species, a toxicity of 17 mg/L was determined, which led to a  $\log K_{CuBL}$  value of 8.12 (Table S1) when the ameliorative cation concentrations are considered. This value only increases marginally (8.21) if only the free copper ion causes toxicity.

#### S5.9. Other Crustacean Species

There are 26  $\log K_{CuBL}$  values that have been determined for other crustacean species relating to 21 individual species. The  $\log K_{CuBL}$  values range from 7.42 to 8.38, with an average of  $7.94 \pm 0.11$ . This value is in good agreement with that found for all *Ceriodaphnia* species and is consistent with values found for all *Daphnia* species.

The remaining cladoceran species studied by De Schamphalaere et al. (2007) in relation to copper toxicity were the two *Sinocephalus* sp., *S. vetulus* and *S. expinosus*. The study used a 48 h

immobilisation assay with varying sodium concentrations (1.8 to 230 mg/L). The toxicity of copper to the species was always found to increase as the concentration of sodium in the test solutions decreased. Using the relationship between toxicity and sodium concentration led to the following log  $K_{CuBL}$  values: 7.89 (*S. vetulus*) and 7.42 (*S. exspinosus*). These two values are listed in Table S1. A value of  $(f_c)_{Cu^{50\%}}$  of 0.50 was also used for both *Sinocephalus* sp. The results from this study are discussed in further detail below in the Sodium section.

Bossuyt and Janssen (2005) studied the toxicity of copper to a large number of cladoceran species. The species included *Acantholebris curvirostris*, two *Acroperus* sp., *A. elongatus* and *A. harpae*, *Alona quadrangularis* and another *Alona* sp., *Bosmina longirostris*, two *Chydorus* sp., *C. ovalis* and *C. sphearicus*, *Disparalona rostrata*, *Eurycercus lamellatus*, *Pleuroxus truncatus*, two *Scapholebris* sp., *S. microcephala* and *S. mucronata*, and two *Simocephalus* sp., *S. exspinosus* and *S. vetulus*. The test water was at pH 7.8 (except for *B. longirostris* that was at 8.4) which also had an elevated hardness. The ions  $Cu^{2+}$  and  $CuOH^+$  only accounted for a relatively small portion of total copper. Using the measured 48 h  $LC_{50}$  values, the copper speciation and the concentrations of the ameliorative cations led to the following log  $K_{CuBL}$  values: 7.75 (*A. curvirostris*), 8.22 (*A. elongatus*), 8.32 (*A. harpae*), 7.98 (*A. quadrangularis*), 8.08 (*Alona* sp.), 7.87 (*B. longirostris*), 7.91 (*C. ovalis*), 8.05 (*C. sphearicus*), 7.68 (*D. rostrata*), 8.05 (*E. lamellatus*), 7.72 (*P. truncatus*), 8.23 (*S. microcephala*), 8.11 (*S. mucronata*), 8.15 (*S. exspinosus*) and 8.16 (*S. vetulus*). These values are listed in Table S1. A  $(f_c)_{Cu^{50\%}}$  value of 0.2 was used for three species (*A. curvirostris*, *B. longirostris* and *S. mucronata*).

Another species studied by Mount and Norberg (1984) was *S. vetulus*. Copper toxicity was studied at pH 7.3 and test waters of relatively low hardness. The free copper ion accounted for 4.2 % of total copper, with  $CuOH^+$  representing a further 2 %. For this species, a toxicity of 57 mg/L was determined that led to a log  $K_{CuBL}$  value of 7.57 (Table S1) when the ameliorative cation concentrations are considered. This value only increases marginally (7.66) if only the free copper ion causes toxicity.

Another species studied by Schubauer-Berigan et al. (1993) in relation to the toxicity of copper was *H. azteca* as a function of pH. Three  $LC_{50}$  values were determined for *H. azteca* at a varying pH and, as such, a log  $K_{CuBL}$  value was determined for each pH, accounting for the fractions of copper present as  $Cu^{2+}$  and  $CuOH^+$ . The average log  $K_{CuBL}$  derived was 7.76. This value is listed in Table S1.

Koivisto et al. (1992) also studied the toxicity of copper to *B. longirostris* and *C. sphearicus*. The  $LC_{50}$  concentrations determined in the study were 3.7 and 7.6 mg/L, respectively. The  $Cu^{2+}$  was 2.2 % of total copper and  $CuOH^+$  was an additional 14 %. Taking account of the  $LC_{50}$ , the copper speciation and the concentrations of the ameliorative cations as well as an estimated  $(f_c)_{Cu^{50\%}}$  value of 0.2 for *B. longirostris* (this value was also used for this species in the evaluation of the study of Bossuyt and Janssen (2005)), led to log  $K_{CuBL}$  values of 7.94 and 8.23 (Table S1), respectively. If only  $Cu^{2+}$  causes toxicity, these values increase to 8.56 and 8.85.

Another species Naddy et al. (2002) studied relating to the toxicity of copper was a *Gammarus* sp., using test solutions with varying calcium concentrations, a pH of 8.2 and an alkalinity of 115 mg/L (as  $CaCO_3$ ). The  $Cu^{2+}$  ion was only a small fraction (about 1 %) of total copper in the test solutions, with  $CuOH^+$  being a larger fraction (3.6 %). Based on the available data and the copper speciation, the average log  $K_{CuBL}$  value was calculated to be 7.48 (which increases to 7.93 if only  $Cu^{2+}$  is responsible for toxicity). The calculated log  $K_{CuBL}$  value is listed in Table S1.

The toxicity of copper to the copepod, *Notodiaptomus iheringi*, was studied by Rocha et al. (2024). An  $EC_{50}$  concentration of 28 mg/L was reported and adjusting for the ameliorative cation concentrations and copper speciation (both  $Cu^{2+}$  and  $CuOH^+$  were 8 % of total copper) leads to a log  $K_{CuBL}$  value of 7.52. The log  $K_{CuBL}$  value is listed in Table S1.

Trenfield et al. (2022) studied the toxicity of copper to several species including *Moinadaphnia macleayi* (6 d reproduction) in waters of low hardness. Speciation calculations indicated that the free copper ion was 10.5 % of total copper and  $CuOH^+$  a further 1.0 %. The  $EC_{50}$  concentration determined for *M. macleayi* was 13.1 mg/L. Combining this copper toxicity

concentration with the copper speciation and adjusting for the ameliorative cation concentrations leads to a log  $K_{CuBL}$  value of 7.72 (Table S1).

Zou and Bu (2004) studied the toxicity of copper to *M. irrasa* in very soft water at pH 8. The toxicity found in the study was 5.93 mg/L, using a 48 h mortality endpoint. The  $Cu^{2+}$  ion was 4.8 % of total copper and  $CuOH^+$  another 12.6 %. The adjustment of this value is quite small due to the relatively high pH and very soft water, leading to a selected log  $K_{CuBL}$  value of 8.02. This value is listed in Table S1. The log  $K_{CuBL}$  value increases to 8.38 if only  $Cu^{2+}$  accounts for toxicity.

Gutierrez et al. (2011) studied the toxicity of copper to *Pseudosida variabilis* using a 48 h immobilisation endpoint. The  $Cu^{2+}$  and  $CuOH^+$  ions were only small fractions (1.0 and 8.0 %, respectively) of total copper. The derived  $EC_{50}$  concentration was 12 mg/L. Using this value and adjusting for copper speciation and the ameliorative cation concentrations led to a log  $K_{CuBL}$  value of 8.33 (Table S1).

#### S5.10. *Chlorella* sp.

There are eleven log  $K_{CuBL}$  values for *Chlorella* sp., with values that range from 7.49 to 8.18 and an average of  $7.84 \pm 0.14$ . This average value is consistent with that found for both fish and crustacean species.

Wilde et al. (2006) measured copper uptake in, and toxicity to, *Chlorella* sp. as a function of pH using a 48 h growth assay. At a pH of 6.5, the study reported a log  $K_{CuBL}$  value of 7.4, and a value of 7.49 was determined from the Cu concentration of the  $EC_{50}$  at this pH, with these values being consistent with the values for this parameter determined from the data of other studies of copper toxicity to *Chlorella* sp. At a pH of 7.0, a value for log  $K_{CuBL}$  of 7.88 was determined from the measured  $EC_{50}$ . A value for log  $K_{CuBL}$  was determined from the two  $EC_{50}$  concentrations (as  $Cu^{2+} + CuOH^+/2$ ) at pH 6.5 and 7.0; the derived value was 7.69, which is marginally larger than the value quoted in the study. This value is believed to be consistent for all pH values studied; it is listed in Table S1.

Markich et al. (2005) also used a 48 h growth assay with *Chlorella* sp. to study the toxicity of copper at various hardness concentrations. The study determined the  $Cu^{2+}$  and  $CuOH^+$  fractions of total copper at each hardness. Using equation (16) gave consistent  $EC_{50}$  concentrations across the full range of hardness studied, which led to a log  $K_{CuBL}$  value of 7.65 (Table S1). If only  $Cu^{2+}$  is responsible for toxicity, log  $K_{CuBL}$  increases fractionally to 7.67. In the conditions studied, the fraction of  $CuOH^+$  was very much less than  $Cu^{2+}$ , and, therefore, its effect on toxicity was only minor.

The toxicity of copper to *Chlorella* sp. was also studied by Franklin et al. (2002a) using 48 h and 72 h growth assays. The pH of the synthetic water used in the study was 7.5. Using the average of the  $EC_{50}$  concentrations determined at the two endpoints, and the speciation of copper ( $Cu^{2+}$  and  $CuOH^+$ ) in the test solutions, a log  $K_{CuBL}$  value of 7.82 (Table S1) was calculated, in good agreement with the study of Wilde et al. (2006) at the same pH; the log  $K_{CuBL}$  value increases to 7.97 if only the free copper ion is responsible for toxicity. In another study (Franklin et al., 2002b) using the longer duration growth assay, an average  $EC_{50}$  (initial cell densities of 100 and 1000 cells/mL) concentration of 6.4 mg/L was found. Speciation calculations indicated that  $Cu^{2+}$  and  $CuOH^+$  were 10.7 and 8.6 %, respectively, of total copper at the  $EC_{50}$ . Using these data leads to a log  $K_{CuBL}$  value of 7.97 (Table S1); this value increases to 8.12 if only the free ion causes the toxicity. In an earlier study, Franklin et al. (2000) also studied the toxicity of copper to *Chlorella* sp. using the 72 h growth assay and variable pH. The study found  $EC_{50}$  concentrations of 35 (pH 5.7) and 1.5 (pH 6.5) mg/L. At both pH values,  $CuOH^+$  (1.6 and 8.9 %, respectively) is substantially less dominant than the free copper ion,  $Cu^{2+}$  (97.5 and 85.6 %, respectively). Determining the effect of the variable  $H^+$  concentrations led to a log  $K_{CuBL}$  value of 7.93 (Table S1) when both species are considered to cause toxicity or the marginally larger value of 7.96 if only  $Cu^{2+}$  is responsible. Franklin et al. (2001) also reported data for the toxicity of copper to *Chlorella* sp. and again at pH 7.5 using a 72 h growth assay. At the  $EC_{50}$  determined in this study (7.3 mg/L),  $Cu^{2+}$  accounts for 9.3 % of total copper, with  $CuOH^+$  accounting for a



further 6.8 %. Using the  $EC_{50}$  and adjusting for the copper speciation and ameliorative cation concentrations led to a log  $K_{CuBL}$  value of 8.18.

Johnson et al. (2007) used a 72 h growth assay to examine the toxicity of copper to *Chlorella* sp. using synthetic solutions, also with a pH of 7.5. Based on the  $IC_{50}$  value found for copper (7.3 mg/L) and the fraction of copper determined to be present as  $Cu^{2+}$  (10.8 %) and  $CuOH^+$  (8.5 %), a log  $K_{CuBL}$  value of 7.76 was determined (Table S1). This value is in good agreement with the previous five studies on this species. If only  $Cu^{2+}$  is responsible for toxicity, the log  $K_{CuBL}$  value increases to 8.12.

Angel et al. (2017) studied the toxicity of copper to two microalgae, including *Chlorella* sp., in relatively soft water at a pH around 7.9. Using a 72 h growth assay, an  $EC_{50}$  of 3.3 mg/L was determined. Using this value, the copper speciation ( $Cu^{2+}$  and  $CuOH^+$  were 10.9 and 16 % of total copper, respectively) and the ameliorative cation concentrations led to a log  $K_{CuBL}$  value of 8.10. This value is listed in Table S1.

McKnight et al. (2023) also studied the toxicity of copper to *Chlorella* sp. The species was isolated from Lake Aesake in Papua New Guinea. The study found that the species was more sensitive to copper than was found for many other studies on this species. Using the measured  $EC_{50}$  concentration, adjusting for ameliorative cation concentrations and using a  $(fc)_{Cu^{50\%}}$  value used for nickel for the same species led to a log  $K_{CuBL}$  value of 7.64. This value is listed in Table S1.

Macoustra et al. (2019) also studied the toxicity of copper to *Chlorella* sp., again isolated from Lake Aesake in Papua New Guinea. The toxicity was found to decrease (increasing  $EC_{50}$ ) with an increase in DOC. From the relationship between  $EC_{50}$  and DOC concentration, an intercept concentration of 5.49 mg/L was determined. Using this value, the copper speciation and ameliorative cation concentrations as well as a  $(fc)_{Cu^{50\%}}$  value of 0.12 led to a log  $K_{CuBL}$  value of 7.49.

The toxicity of copper to *Chlorella* sp. was also studied by Trenfield et al. (2022) in relatively soft water. An  $EC_{50}$  of 9.1 mg/L was determined in the study. The free copper ion was about 10 % of total copper, with  $CuOH^+$  being less than 1 %. Based on the  $EC_{50}$  and the copper speciation, together with adjusting for the ameliorative cation concentrations, a log  $K_{CuBL}$  value of 7.95 was derived (Table S1).

#### S5.11. *Raphidocelis subcapitata*

There are eight studies listed that have reported data for the toxicity of copper to *R. subcapitata*. The values obtained for log  $K_{CuBL}$  range between 7.64 and 8.20, with an average value of  $7.82 \pm 0.16$ . This value is in excellent agreement with the average log  $K_{CuBL}$  value derived for *Chlorella* sp.

Heijerick et al. (2005b) studied the toxicity of copper to *R. subcapitata* in natural waters and in a standard (ISO) medium. In the standard medium, the measured  $EC_{50}$  concentration averaged 16.5 mg/L. Speciation calculations showed that the free copper ion (7.5 %) and  $CuOH^+$  (12.0 %) were both relatively important species. Based on the  $EC_{50}$ , the copper speciation and the concentrations of the ameliorative cations, the log  $K_{CuBL}$  value was found to be 7.64 (Table S1); if only  $Cu^{2+}$  causes toxicity, this value increases to 7.90.

Franklin et al. (2002b) also studied the toxicity of copper to *R. subcapitata* using a 72 h growth assay. An average  $EC_{50}$  (initial cell densities of 100 and 1000 cells/mL) concentration of 4.5 mg/L was found. Speciation calculations indicated that  $Cu^{2+}$  and  $CuOH^+$  were 10.0 and 8.2 %, respectively, of total copper at the  $EC_{50}$ . Using these data leads to a log  $K_{CuBL}$  value of 7.85 (Table S1); this value increases to 8.00 if only the free ion causes the toxicity. Franklin et al. (2001) also reported data for the toxicity of copper to *R. subcapitata* at pH 7.5 using a 72 h growth assay. At the  $EC_{50}$  determined in this study (7.5 mg/L),  $Cu^{2+}$  accounts for 16 % of total copper, with  $CuOH^+$  a further 12 %. Using the  $EC_{50}$  and adjusting for the copper speciation and ameliorative cation concentrations led to a log  $K_{CuBL}$  value of 7.65.

Filova et al. (2021) studied the toxicity of several metals, including copper to *R. subcapitata*, in waters of relatively low hardness. The study determined a 96 h growth endpoint of 150 mg/L, where the copper ion was only 0.7 % of copper in the test solutions and  $CuOH^+$  a further 1 %.

Using the EC<sub>50</sub> concentration and the copper speciation, together with the ameliorative cation concentrations, led to a log  $K_{CuBL}$  value of 7.72, as is listed in Table S1.

The toxicity of copper to *P. subcapitata* was also studied by Alves et al. (2017) in waters of relatively low hardness and at a pH of 7. A 72 h growth endpoint of 29 mg/L was determined in the study. The correction for ameliorative cations was quite small and the copper ion was 2.7 % and CuOH<sup>+</sup> 0.8 % of copper in the test water at the EC<sub>50</sub>, leading to a log  $K_{CuBL}$  value of 8.02, as is listed in Table S1.

Blaise et al. (1986), in studying the toxicity of copper to *P. subcapitata*, found an average EC<sub>50</sub> concentration of 29.9 mg/L. The pH of the test solutions was 7 and the hardness was relatively low, with the copper ion (3.2 %) and CuOH<sup>+</sup> (3.4 %) only small proportions of the copper speciation. These conditions led to a log  $K_{CuBL}$  value of 7.75 (Table S1).

Pascual et al. (2022) studied the toxicity of copper to *R. subcapitata* at varying temperature and pH values as well as in the presence of EDTA. The presence of EDTA was found to reduce bioavailability and with an increase in pH, toxicity increased (i.e., lower EC<sub>50</sub>). The data used in the present study was for a temperature of 24 °C (although temperature did not appear to have much effect on toxicity, except at the lowest temperature (15 °C) studied). Using the EC<sub>50</sub> concentrations and the copper speciation led to a log  $K_{CuBL}$  value of 7.76. This value is listed in Table S1.

Angel et al. (2017) also studied the toxicity of copper to *R. subcapitata* in relatively soft water at a pH around 7.9. Utilising the 72 h growth assay, an EC<sub>50</sub> of 3.0 mg/L was determined. Using this value, the copper speciation (Cu<sup>2+</sup> and CuOH<sup>+</sup> were 8.1 and 16.6 % of total copper, respectively) and the ameliorative cation concentrations led to a log  $K_{CuBL}$  value of 8.20. This value is listed in Table S1.

#### S5.12. Other Microalgae Species

McKnight et al. (2023) also studied the toxicity of copper to *M. arcuatum*. The species was also isolated from Lake Aesake in Papua New Guinea. Using the measured EC<sub>50</sub> concentration, adjusting for ameliorative cation concentrations and using the same  $(f_c)_{Cu^{50\%}}$  as was used for *Chlorella* sp. led to a log  $K_{CuBL}$  value of 8.05. This value is listed in Table S1.

#### S5.13. Mollusc Species

A total of 41 data points are reported for copper toxicity to mollusc species that relate to 32 separate species. The derived log  $K_{CuBL}$  values range from 7.37 to 8.32, with an average of  $7.90 \pm 0.09$ . This average value is consistent with those found for other freshwater species groups.

The toxicity of copper to *Corbicula fluminea* was studied by Liao et al. (2007), using up to 5 h valve closure assays. The study used the speciation of copper to explain its toxicity to *C. fluminea* and reported a value for log  $K_{CuBL}$  of 7.70. This value is listed in Table S1.

Clearwater et al. (2014) also studied the toxicity of copper to *E. menziesii* using three natural waters and a 48 h survival assay. In the test waters, the free copper ion (Cu<sup>2+</sup>; 12.1 %) and CuOH<sup>+</sup> (14.6 %) were important copper species. The average LC<sub>50</sub> concentration from the three lake waters studied was found to be 2.83 mg/L and was used together with the speciation, and adjusting for the concentrations of the ameliorative cations, to derive a log  $K_{CuBL}$  value of 8.06 (Table S1). If only Cu<sup>2+</sup> accounts for toxicity, then the log  $K_{CuBL}$  value increases to 8.27.

Croteau and Luoma (2007) studied the uptake of copper in *L. stagnalis* using a 24 h assay. As with cadmium, the study determined log  $K_{CuBL}$  values for four water types (deionised, soft, moderately hard and hard) with values of 7.2, 8.4, 8.5 and 8.6, respectively, being reported. The average log  $K_{CuBL}$  value from the four waters is retained in the present study, namely, 8.18 (Table S1).

Markich (2017) also used mortality assays (24 to 72 hours) to study the toxicity of copper to six freshwater mussels (glochidia: *A. profuga*, *C. novaehollandiae*, *H. australis*, *H. depressa*, *H. drapeta* and *V. ambiguus*). The study found an average value for log  $K_{CuBL}$  of 7.30, with a range of 7.17 to 7.46 (from the 48 h mortality data). The free copper ion (53.1 %) was the dominant species in the solution, with CuOH<sup>+</sup> accounting for 9.7 % of total copper. Reanalysis of the



toxicity data in the present study led again to larger log  $K_{CuBL}$  values, ranging between 7.37 and 7.66. These six values are listed in Table S1.

Besser et al. (2016) studied the toxicity of copper to six snail species (*Fluminicola* sp., *Fontigens aldrichi*, *L. stagnalis*, *Physa gyrina*, *Pyrgulopsis robusta* and *Taylorconcha serpenticola*) using 28 d mortality assays. The  $LC_{50}$  concentrations obtained for the six species were similar and ranged between 15 and 38 mg/L. The species,  $Cu^{2+}$  (0.5 to 1.0 %) and  $CuOH^+$  (2.6 %), were only small components of total copper. Based on the  $LC_{50}$  concentrations and the calculated speciation, the log  $K_{CuBL}$  values derived ranged between 7.97 and 8.37 (all six values are listed in Table S1). The average value was 8.21. If only  $Cu^{2+}$  was responsible for toxicity, the average log  $K_{CuBL}$  value increased to 8.73.

The toxicity of copper to juvenile *Planorbella pilsbryi* was studied by Osborne et al. (2020), using a 72 h mortality assay following the exposure of adult species to a 7 d exposure to sub-lethal doses of copper. In the test where the adults were not exposed to copper in the 7 d pre-juvenile exposure, the  $LC_{50}$  of copper was found to be 29.25 mg/L. The  $LC_{50}$  decreased if the adults had been exposed to small (9.4 or 18.8 mg/L) non-lethal doses of copper. Speciation modelling indicated that  $Cu^{2+}$  (0.7 %) and  $CuOH^+$  (1.6 %) were only small components of the total copper concentration. Based on the data provided in the study and the speciation calculations, the log  $K_{CuBL}$  value determined was 8.16 (Table S1). This value increased to 8.49 if only the free ion causes toxicity.

Gillis et al. (2008) used 48 h mortality assays to study the toxicity of copper to the glochidia of seven mussels. The species studied were *Actinonaias ligamentina*, two *Epioblasma* species, *E. rangiana* and *E. triquetra*, two *Lampsilis* species, *L. fasciola* and *L. siliquioidea*, *Obovaria subrotunda* and *Villosa fabalis*. The observed toxicity ranged from 4.6 to 21.6 mg/L, with the fraction of  $Cu^{2+}$  (9.1 to 10.1 %) and  $CuOH^+$  (9.4 to 10.2 %) increasing with increasing  $EC_{50}$ . Taking account of the  $EC_{50}$  and adjusting for the copper speciation and the ameliorative cation concentrations led to the following log  $K_{CuBL}$  values: 7.65 (*A. ligamentina*), 7.93 (*E. rangiana*), 8.17 (*E. triquetra*), 7.76 (*L. fasciola*), 7.52 (*L. siliquioidea*), 7.81 (*O. subrotunda*) and 8.23 (*V. fabalis*). These values are listed in Table S1.

In a similar study, Wang et al. (2007) used 48 h mortality assays to study the toxicity of copper to the glochidia of eight mussels. In the study, the following were used: *A. ligamentina*, four *Lampsilis* species, *L. abrupta*, *L. fasciola*, *L. rafinesquana* and *L. siliquioidea*, *Potamilus ohioensis*, *Venustaconcha ellipsiformis* and *Villosa iris*. The observed toxicity ranged from 6.9 to 27 mg/L, with the fraction of  $Cu^{2+}$  (1.6 %) and  $CuOH^+$  (8.6 %) being relatively small fractions of total copper. These data led to the following log  $K_{CuBL}$  values: 7.60 (*A. ligamentina*), 7.92 (*L. abrupta*), 8.19 (*L. fasciola*), 7.75 (*L. rafinesquana*), 7.69 (*L. siliquioidea*), 7.92 (*P. ohioensis*), 8.10 (*V. ellipsiformis*) and 7.69 (*V. iris*). These values are listed in Table S1.

Wang et al. (2011b) studied the toxicity of copper to *V. iris* in a reference water and other waters of varying DOC. Analysis of the data indicated that the relationship between  $LC_{50}$  and the DOC content led to an intercept  $LC_{50}$  (i.e., DOC = 0) of 16.1 mg/L that was reasonably consistent with the  $LC_{50}$  determined in the reference water (23.1 mg/L). In both test waters,  $Cu^{2+}$  was a small proportion of total copper, with  $CuOH^+$  about three times greater than  $Cu^{2+}$ . Based on the  $LC_{50}$  values, the speciation and the ameliorative cation concentrations, an average log  $K_{CuBL}$  value of 8.24 was derived (Table S1). If only  $Cu^{2+}$  accounts for toxicity, then the log  $K_{CuBL}$  value increases to 8.60.

Salerno et al. (2020) also studied the toxicity of copper to *V. iris* and derived an  $EC_{50}$  of 14.2 mg/L. The  $Cu^{2+}$  and  $CuOH^+$  ions were only a small percentage of total copper (1.9 and 7.4 %, respectively). Using the  $EC_{50}$ , the copper speciation and accounting for the ameliorative cation concentrations led to a log  $K_{CuBL}$  of 8.32. This value is listed in Table S1.

Trenfield et al. (2022) also studied the toxicity of copper to *A. cumungi* (96 h reproduction) and a *Velesunio* species in waters of low hardness. Speciation calculations indicated that the free copper ion was 10.1 and 8.7 %, respectively, of total copper and  $CuOH^+$  a further 1.2 and 0.3 %. The  $EC_{50}$  concentrations determined were 8.0 mg/L for *A. cumungi* and 6.7 mg/L for *Velesunio* sp. Combining these copper toxicity concentrations with the copper speciation and adjusting

for the ameliorative cation concentrations leads to log  $K_{CuBL}$  values of 7.94 and 8.21, respectively (Table S1).

Jorge et al. (2013) used a 28 d mortality endpoint to study the toxicity of copper to *L. siliquoides*. The calculated  $LC_{50}$  was 7.3 mg/L. Both  $Cu^{2+}$  and  $CuOH^+$  were about 10 % of total copper. Using the  $LC_{50}$  and adjusting for the copper speciation and the ameliorative cation concentrations led to a log  $K_{CuBL}$  value of 8.14 (Table S1). This value increases to 8.31 if only  $Cu^{2+}$  accounts for toxicity.

Copper toxicity to *Lymnaea luteola* was studied by Das and Khangarot (2011). Using the measured  $EC_{50}$ , the copper speciation and adjusting for the ameliorative cation concentrations led to a log  $K_{CuBL}$  of 8.22. This value is listed in Table S1.

Ng et al. (2011) studied the toxicity of copper to *L. stagnalis* in hard water. The derived  $EC_{50}$  was 24.9 mg/L, where  $Cu^{2+}$  (2.6 %) and  $CuOH^+$  (8 %) were relatively small proportions of total copper. From the  $EC_{50}$ , the copper speciation and the ameliorative cation concentrations, a log  $K_{CuBL}$  of 8.14 was derived (Table S1). This value increases to 8.55 if only  $Cu^{2+}$  is responsible for toxicity.

Brix et al. (2011) also studied the toxicity of copper to *L. stagnalis*. The  $LC_{50}$  of 30.7 mg/L was similar to that derived in the study of Ng et al. (2011). The  $Cu^{2+}$  (2.9 %) and  $CuOH^+$  (4.4 %) ions were only a relatively small proportion of total copper. Using the  $LC_{50}$  and adjusting for copper speciation and the ameliorative cation concentrations led to a log  $K_{CuBL}$  value of 7.98 (Table S1).

The toxicity of copper to the snail, *Physa acuta*, was studied by Gao et al. (2017). The ions  $Cu^{2+}$  (2.4 %) and  $CuOH^+$  (6.2 %) were only small fractions of total copper. The derived  $LC_{50}$  was found to be 23.8 mg/L. The  $LC_{50}$  and copper speciation, accounting for the ameliorative cation concentrations, led to a log  $K_{CuBL}$  of 8.29. This value is listed in Table S1. If only  $Cu^{2+}$  is responsible for toxicity, this value increases to 8.65.

Gupta et al. (1981) studied the toxicity of copper to the snail, *Viviparus bengalensis*, at varying temperatures and pH conditions. The  $Cu^{2+}$  ion ranged between 1.4 and 7.1 % of total copper, whereas  $CuOH^+$  was between 2.6 and 2.8 %. The measured  $LC_{50}$  values were between 60 and 88 mg/L. A log  $K_{CuBL}$  value was determined for each condition and an average value of 7.86 was calculated (Table S1). If only  $Cu^{2+}$  is responsible for toxicity, this value increases to 8.04.

#### S5.14. Macrophyte Species

Markich et al. (2006) studied the toxicity of copper to *Ceratophyllum demersum* at pH 7 with varying hardness using a 96 h growth assay. At each hardness examined, the study determined the copper speciation, with  $Cu^{2+}$  being dominant (54 to 60 %), but  $CuOH^+$  was also important (13 to 14 %), at all three hardness concentrations studied. Using these data and equation (16), an  $EC_{50}$  concentration of 5.07 mg/L was determined for zero hardness. This concentration was further adjusted to take account of the test pH and sodium concentration. A log  $K_{CuBL}$  value of 7.19 (Table S1) was determined; if only  $Cu^{2+}$  caused toxicity, this value would increase marginally to 7.24.

Trenfield et al. (2022) studied the toxicity of copper to several species, including *Lemna aequinoctialis* (96 h growth) in waters of low hardness. Speciation calculations indicated that the free copper ion was 10.4 % of total copper and  $CuOH^+$  a further 1.5 %. The  $EC_{50}$  concentration determined for *L. aequinoctialis* was 12 mg/L. Combining this copper toxicity concentration with the copper speciation and adjusting for the ameliorative cation concentrations leads to a log  $K_{CuBL}$  value of 7.73 (Table S1).

Naumann et al. (2007) studied the toxicity of a range of metals to *L. minor* in relatively hard water. In their study using copper,  $Cu^{2+}$  was found to be 9.6 % of total copper and  $CuOH^+$  a further 0.7 %, where the observed toxicity occurred at a concentration of 155 mg/L. Utilising the  $EC_{50}$  concentration and the copper speciation, together with the ameliorative cation concentrations, led to a log  $K_{CuBL}$  value of 7.42 (Table S1).

#### S5.15. Cnidaria Species

Reithmuller et al. (2000) studied the toxicity of copper to *H. viridissima* at pH 6 and a varying hardness (6.6 to 330 mg/L (as  $\text{CaCO}_3$ )) using a 96 h growth assay. The results were coupled with the earlier work of Markich and Camilleri (1997) that studied the same species with the same assay, but at a hardness of 4 mg/L (as  $\text{CaCO}_3$ ). At pH 6, the free copper ion (96 %) is the dominant species in the test solutions. The measured  $\text{EC}_{50}$  concentrations ranged from 4.0 (Markich and Camilleri, 1997) to 5.5 mg/L. Taking account of the copper speciation and adjustment for the pH of the test solutions led to a log  $K_{\text{CuBL}}$  value of 7.37 (Table S1). The  $\text{CuOH}^+$  species is minor in the conditions studied and does not influence log  $K_{\text{CuBL}}$ .

Trenfield et al. (2022) also studied the toxicity of copper to *H. viridissima* (96 h growth) in waters of low hardness. Speciation calculations indicated that the free copper ion was 10.1 % of total copper and  $\text{CuOH}^+$  a further 0.9 %. The  $\text{EC}_{50}$  concentration determined for *H. viridissima* was 7.6 mg/L. Combining this copper toxicity concentration with the copper speciation and adjusting for the ameliorative cation concentrations leads to a log  $K_{\text{CuBL}}$  value of 7.98 (Table S1). Again, the  $\text{CuOH}^+$  is a minor compared to  $\text{Cu}^{2+}$  and would not affect the value attained for log  $K_{\text{CuBL}}$ .

#### S5.16. Rotifer Species

Hernández-Flores et al. (2020) studied the toxicity of several metals to the rotifer, *E. dilatata*, using both 24 h mortality and 5 d reproduction assays. There was no trend in the two toxicity endpoints, with either the acute or chronic endpoint having the lower concentration for a particular metal. As such, both concentrations were used in the present study to determine binding constants, from which the average value was determined. For copper, when speciation (the free ion was 10.2 % and  $\text{CuOH}^+$  was 8.2 % of total copper) and ameliorative cation concentrations were considered, the average log  $K_{\text{CuBL}}$  value of 7.94 was determined. This value is listed in Table S1.

The toxicity of copper to the rotifer, *L. inermis*, in water of relatively high hardness and at pH 7.4, was studied by Klimek et al. (2013). From speciation calculations, it was determined that the free ion was 7.6 % and  $\text{CuOH}^+$  was 4.6 % in the test solutions at the  $\text{LC}_{50}$ . From the speciation, the determined  $\text{LC}_{50}$  and the ameliorative cation concentrations, a log  $K_{\text{CuBL}}$  value of 7.99 was determined. This value is listed in Table S1.

#### S5.17. Insect Species

The toxicity of copper to the insect *T. dissimilis* was studied by Anderson et al. (1980). An  $\text{LC}_{50}$  concentration of 3.8 mg/L was determined, which is a relatively low concentration. The mortality concentrations of the other metals studied were also relatively low, which enabled a  $(f_c)_{\text{M}^{50\%}}$  value of 0.258 to be assigned to all four metals studied. Based on this and the  $\text{LC}_{50}$  value, the copper speciation and the ameliorative cation concentrations, a log  $K_{\text{CuBL}}$  value of 7.53 was determined, as is listed in Table S1.

The toxicity of copper to another freshwater insect, *Chironomus tentans*, was studied by Gauss et al. (1985). An  $\text{LC}_{50}$  concentration of 16.7 mg/L was determined. The copper ions,  $\text{Cu}^{2+}$  and  $\text{CuOH}^+$ , were relatively small fractions of total copper (11.0 and 11.4 %, respectively). Using the  $\text{LC}_{50}$  and the copper speciation, and accounting for the ameliorative cation concentrations, led to a log  $K_{\text{CuBL}}$  value of 7.59. This value is listed in Table S1.

#### S5.18. Bacteria Species

Markich et al. (2005) also studied the toxicity of copper to *Erwinnia* sp. using a 4 h growth assay at various hardness concentrations, with the  $\text{Cu}^{2+}$  and  $\text{CuOH}^+$  fractions of total copper determined by the study at each hardness. Equation (16) was used and gave consistent  $\text{EC}_{50}$  concentrations across the full range of hardness studied, which led to a log  $K_{\text{CuBL}}$  value of 7.66 (Table S1). If only  $\text{Cu}^{2+}$  was responsible for toxicity, log  $K_{\text{CuBL}}$  increased to fractionally to 7.68. As with the other two species studied, in the conditions used, the fraction of  $\text{CuOH}^+$  was very much less than  $\text{Cu}^{2+}$ , and, therefore, its effect on toxicity was only minor.

## S6. Uranium

### S6.1. Fish Species

Trenfield et al. (2011) studied the toxicity of uranium to *M. mogurnda* using a 96 h mortality assay in two synthetic waters (DOC removed) with an average pH of 6.23. The LC<sub>50</sub> concentrations determined were 1520 and 1730 mg/L, which were similar to those found in previous studies (Markich and Camilleri, 1997; Chang et al., 2010) as well as in a later study (Pease et al., 2021), even though two of the other three studies used a longer duration endpoint (28 d mortality (Cheng et al., 2010) or 7 d growth (Pease et al., 2021)). The other three studies were performed at differing pH values (6, Markich and Camilleri (1997); 6.6, Pease et al. (2021) and 6.7, Cheng et al. (2010)) and the toxicity decreased as the pH also decreased. This behaviour was also observed when the toxicity was expressed in terms of ( $\text{UO}_2^{2+} + \text{UO}_2\text{OH}^+/2$ ). Based on the intercept value of the latter data as a function of the  $\text{H}^+$  concentration, and accounting for small concentrations of the ameliorative cations, the log  $K_{\text{UO}_2\text{BL}}$  value determined from the four studies was 7.38. This value is listed in Table S1.

The study of Goulet et al. (2015) investigated the toxicity of uranium to a range of freshwater species, including the two fish *O. mykiss* and *P. promelas*, using 30 d early-life stage renewal (*O. mykiss*), 96 h mortality (*O. mykiss* fry), 7 d growth (*P. promelas*) and 96 h mortality (*P. promelas*) assays. The effect of hardness (5 to 240 mg/L (as  $\text{CaCO}_3$ )) on uranium toxicity was studied for each species; there was no relationship of toxicity with increasing hardness for *P. promelas* or *O. mykiss*; therefore, average EC<sub>50</sub>/LC<sub>50</sub> values will be used for both fish species. The study provided the concentrations of  $\text{UO}_2^{2+}$  and  $\text{UO}_2\text{OH}^+$  that were the uranium species indicated to be responsible for the observed toxicity. Based on the average toxicity found in the hardness tests and the speciation found, log  $K_{\text{UO}_2\text{BL}}$  values of 7.38 (*P. promelas*) and 7.34 (*O. mykiss*) were determined (Table S1). If only  $\text{UO}_2^{2+}$  is responsible for the toxicity, the log  $K_{\text{UO}_2\text{BL}}$  values increases to 8.96 and 8.92.

### S6.2. Crustacean Species

A 48 h mortality assay was used by Zeman et al. (2008) to study the toxicity of uranium to *D. magna* in relatively hard water (250 mg/L (as  $\text{CaCO}_3$ )). Speciation calculations indicated that  $\text{UO}_2^{2+}$  (0.095 %) was only a small proportion of total uranium, with  $\text{UO}_2\text{OH}^+$  (5.34 %) being considerably greater. From the LC<sub>50</sub> concentration, the calculated speciation and the relatively large effect of hardness, a log  $K_{\text{UO}_2\text{BL}}$  value of 8.04 was determined. This value is listed in Table S1. If only the free uranyl ion is responsible for toxicity, log  $K_{\text{UO}_2\text{BL}}$  increases to 9.47.

Chen et al. (2022) studied the toxicity of uranium to the ostracod, *Cypridopsis vidua*, using a 96 h mortality assay. The study found a quite large LC<sub>50</sub> concentration of 14.2 mg/L. However, the proportions of  $\text{UO}_2^{2+}$  (0.0003 %) and  $\text{UO}_2\text{OH}^+$  (0.018 %) of total uranium were extremely low. Accounting for this speciation, the pH and moderate hardness used in the tests led to a log  $K_{\text{UO}_2\text{BL}}$  value of 7.80 (Table S1); this latter value increases to 10.25 if only  $\text{UO}_2^{2+}$  invokes toxicity.

The toxicity of uranium to *C. dubia* was also studied by Goulet et al. (2015) using both 7 d mortality and reproduction assays. Although hardness was varied (5 to 240 mg/L (as  $\text{CaCO}_3$ )), the results obtained for *C. dubia* indicated that hardness did not affect the toxicity. The study provided the concentrations of  $\text{UO}_2^{2+}$  and  $\text{UO}_2\text{OH}^+$  that were indicated to be responsible for the observed toxicity. Based on the average toxicity found in the hardness tests and the speciation found and, using the  $(f_c)_{\text{M}^{50\%}}$  value from Keithly et al. (2004), a log  $K_{\text{UO}_2\text{BL}}$  value of 7.36 was determined (Table S1). If only  $\text{UO}_2^{2+}$  were responsible for the toxicity, the log  $K_{\text{UO}_2\text{BL}}$  value would increase to 8.53.

Semaan et al. (2001) used 48 h immobilisation and mortality assays to study the toxicity of uranium to *M. macleayi* in one synthetic and three natural waters. The average EC<sub>50</sub> concentration was found to be 252 mg/L (as  $\text{UO}_2$ ), with  $\text{UO}_2^{2+}$  and  $\text{UO}_2\text{OH}^+$  accounting for 0.03 and 1.4 %, respectively, of the total uranium concentration. Taking these values into account,

the calculated log  $K_{\text{UO}_2\text{BL}}$  value was found to be 8.17 (Table S1); this value increases to 9.55 if only the free uranyl ion is responsible for the toxicity.

The study of Goulet et al. (2015) also investigated the toxicity of uranium to *H. azteca* using 14 d mortality assays. The effect of hardness (5 to 240 mg/L (as  $\text{CaCO}_3$ )) on uranium toxicity was studied for each species; there was no strong relationship between toxicity and hardness for *H. azteca*; therefore, average  $\text{EC}_{50}/\text{LC}_{50}$  values were used. The study provided the concentrations of  $\text{UO}_2^{2+}$  and  $\text{UO}_2\text{OH}^+$ , which were the uranium species indicated to be responsible for the observed toxicity. Based on the average toxicity found in the hardness tests and the speciation found, a log  $K_{\text{UO}_2\text{BL}}$  value of 7.42 was determined (Table S1). The value derived for *H. azteca* utilised the  $(f_c)_{\text{UO}_2^{50\%}}$  values derived for these species from the study of Schroeder (2008). If only  $\text{UO}_2^{2+}$  is responsible for the toxicity, the log  $K_{\text{UO}_2\text{BL}}$  value increases to 7.95. The increase in the stability constant for *H. azteca* if only  $\text{UO}_2^{2+}$  is responsible for toxicity is not as marked as for the other species studied because the free uranyl ion accounts for much more of the toxicity.

### S6.3. Microalgae Species

Charles et al. (2002) studied the toxicity of uranium to *Chlorella* sp. at various hardness concentrations using a 72 h growth assay at a pH of 7. The data from the study were used to determine an  $\text{EC}_{50}$  concentration (56 mg/L as U) with the free ion,  $\text{UO}_2^{2+}$ , only accounting for a small percentage (1.4 %) of total uranium and the hydrolysed species,  $\text{UO}_2\text{OH}^+$ , being present at a much larger concentration (9 %), with neither species being dominant in the test solutions. If both species are responsible for the toxic effect (i.e., using equation (16)), then the calculated log  $K_{\text{UO}_2\text{BL}}$  is 7.86 (this value is listed in Table S1). If only  $\text{UO}_2^{2+}$  causes the toxicity, then log  $K_{\text{UO}_2\text{BL}}$  is much larger, namely 8.48.

The toxicity of uranium to *Chlorella* sp. was also studied by Franklin et al. (2000) using a 72 h growth assay and variable pH. The study found  $\text{EC}_{50}$  concentrations (as U) of 78 (pH 5.7) and 44 (pH 6.5) mg/L. Again, at both pH values,  $\text{UO}_2\text{OH}^+$  (41.1 and 19.8 %, respectively) is more dominant than the free uranyl ion,  $\text{UO}_2^{2+}$  (10.6 and 0.82 %, respectively). Determining the effect of the variable  $\text{H}^+$  concentrations leads to a log  $K_{\text{UO}_2\text{BL}}$  value of 7.87 (Table S1) when both species are considered to cause toxicity or the very much larger value of 9.13 if only  $\text{UO}_2^{2+}$  is responsible.

Hogan et al. (2005) also used *Chlorella* sp. and a 72 h growth assay to study the toxicity of uranium in natural waters and a variable DOC. As with the previous two studies, at the pH (6.0) studied,  $\text{UO}_2\text{OH}^+$  (17.8 %) was more dominant than  $\text{UO}_2^{2+}$  (1.2 %). Using these data and correcting for the concentrations of the ameliorative cations, leads to a log  $K_{\text{UO}_2\text{BL}}$  value of 7.77 (Table S1). If only  $\text{UO}_2^{2+}$  is responsible for toxicity, then the calculated log  $K_{\text{UO}_2\text{BL}}$  value increases to 8.69.

A fourth study on the toxicity of uranium to *Chlorella* sp. was conducted by Trenfield et al. (2011) in synthetic waters simulating natural North Australian creek and billabong waters. Again, a 72 h growth assay was used in the study. Speciation calculations also indicated that the concentration of  $\text{UO}_2\text{OH}^+$  at the  $\text{EC}_{50}$  was greater than that of  $\text{UO}_2^{2+}$  in both synthetic waters. When both species are considered to cause toxicity, and adjusting for the concentrations of the ameliorative cations, a log  $K_{\text{UO}_2\text{BL}}$  value of 7.87 is calculated (Table S1). If only  $\text{UO}_2^{2+}$  results in toxicity, the value of log  $K_{\text{UO}_2\text{BL}}$  increases to 8.70.

The study of Goulet et al. (2015) also investigated the toxicity of uranium to *R. subcapitata* using 72 h growth assays. The effect of hardness (5 to 240 mg/L (as  $\text{CaCO}_3$ )) on uranium toxicity was studied for each species; there was no relationship of toxicity with increasing hardness for *R. subcapitata*; therefore, average  $\text{EC}_{50}/\text{LC}_{50}$  values were used. The study provided the concentrations of  $\text{UO}_2^{2+}$  and  $\text{UO}_2\text{OH}^+$ , the uranium species indicated to be responsible for the observed toxicity. Based on the average toxicity found in the hardness tests and the speciation found, a log  $K_{\text{UO}_2\text{BL}}$  value of 8.13 was determined (Table S1). The value derived for *R. subcapitata* utilised the  $(f_c)_{\text{UO}_2^{50\%}}$  values derived for the species from the study of Deleebeeck et al. (2009). If only  $\text{UO}_2^{2+}$  is responsible for the toxicity, the log  $K_{\text{UO}_2\text{BL}}$  values increase to 9.32.

Uranium toxicity to the unicellular alga *Chlamydomonas reinhardtii* was studied by Fortin et al. (2007) using 30 min uptake assays. The study examined the effect of uptake competition between the uranyl ion and competing cations ( $H^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$ ). A value for  $\log K_{UO_2BL}$  of 7.7 was determined by the study, although it was indicated that the value was somewhat uncertain. The value is retained in the present study and is listed in Table S1. In a later study from the same laboratories on the same species (Lavoie et al., 2014), uranium toxicity was observed using a 96 h growth assay at pH 5 and 7. The study reported  $EC_{50}$  concentrations (as  $UO_2^{2+}$ ) at the two pH values and using these values, a  $\log K_{UO_2BL}$  value of 7.68 was determined (Table S1).

Uranium toxicity to *Euglena gracilis* was studied by Trenfield et al. (2012) at varying DOC. The toxicity was determined in a background medium (150 mM aspartic acid) with and without the addition of 20 mg/L DOC. The study determined the uranium speciation in the two test waters; in the aspartic acid only medium, free uranyl and  $UO_2OH^+$  were 5.5 and 32 % of total uranium, respectively, whereas in the presence of DOC, their proportions decreased to 0.9 and 5 %, respectively. The measured  $EC_{50}$  concentrations were 57 (aspartic acid) and 254 (+ DOC) mg/L (as U). Accounting for the speciation, using equation (16), the  $EC_{50}$  concentrations decrease to 12.3 and 8.6 mg/L, respectively, with an average of 10.4 mg/L. Taking this concentration and the effect of the ameliorative cations led to a  $\log K_{UO_2BL}$  value of 7.67 (Table S1); with only the free uranyl ion explaining the toxicity, the  $\log K_{UO_2BL}$  value increases to 8.31.

#### S6.4. Mollusc Species

Hogan et al. (2010) used a 96 h egg production assay to also study the toxicity of uranium to *A. cumingi* and determined an  $EC_{50}$  concentration of 282 mg/L (as U; pooled data). The free ion,  $UO_2^{2+}$ , and  $UO_2OH^+$  were only a very small percentage of the total uranium at the  $EC_{50}$ , being 0.19 and 0.65 %, respectively. Accounting for the speciation and the concentrations of the ameliorative ions led to a  $\log K_{UO_2BL}$  value of 8.07 (Table S1). If only  $UO_2^{2+}$  is responsible for the toxicity, a very much larger  $\log K_{UO_2BL}$  value of 8.71 is derived.

Fournier et al. (2004) studied the valve closure response (5 h) of *C. fluminea* to uranium at a variable pH. At pH values of 5.5 and 6.5, the study found  $EC_{50}$  concentrations of 0.05 and 0.13 mmol/L, respectively. At the lower pH, the study found that the two species,  $UO_2^{2+}$  and  $UO_2OH^+$ , were the dominant species in solution, accounting for 35 and 48 % of total uranium, respectively. Conversely, at the higher pH, the two species account for much less of the uranium speciation (1 and 25 %, respectively). Taking the speciation into account at the two pH values leads to very similar  $EC_{50}$  concentrations (as  $[UO_2^{2+}] + [UO_2OH^+]/2$ ) of 29.5 and 17.6 nmol/L (as U). These values lead to an average  $\log K_{UO_2BL}$  value of 7.63 (Table S1); if only  $UO_2^{2+}$  is considered with respect to toxicity, the stability constant ( $\log$ ) increases to 8.03.

#### S6.5. Macrophyte Species

Uranium toxicity to duckweed, *L. aequinoctialis*, was studied by Charles et al. (2006) in synthetic water using a 96 h growth assay at pH 6.5. Speciation calculations indicated that both  $UO_2^{2+}$  (0.3 %) and  $UO_2OH^+$  (6 %) were only relatively small fractions of the total uranium in the test water. Taking the speciation into account, as well as the concentrations of the ameliorative cations, led to a  $\log K_{UO_2BL}$  value of 7.25 (Table S1). The larger  $\log K_{UO_2BL}$  value of 8.34 is derived if only  $UO_2^{2+}$  is responsible for the observed toxicity.

Hogan et al. (2010) also used a 96 h growth assay to study the toxicity of uranium to *L. aequinoctialis* and determined an  $EC_{50}$  concentration of 1479 mg/L. The free ion,  $UO_2^{2+}$ , and  $UO_2OH^+$  were only a very small percentage of the total uranium at the  $EC_{50}$ , being 0.03 and 1.2 %, respectively. Accounting for the speciation and the concentrations of the ameliorative ions led to a  $\log K_{UO_2BL}$  value of 7.47 (Table S1). If only  $UO_2^{2+}$  is responsible for the toxicity, a very much larger  $\log K_{UO_2BL}$  value of 8.79 is derived.

The toxicity of uranium to *L. aequinoctialis* was also studied by Pease et al. (2016) using synthetic water and a 96 h growth assay (surface area rather than frond number). Speciation calculations indicated that  $UO_2^{2+}$  and  $UO_2OH^+$  were 4 and 8 %, respectively, of the total uranium concentration at the  $EC_{50}$ . Using this speciation and accounting for the concentrations of  $H^+$ ,



$\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Na}^{+}$  in the test solutions led to a value for  $\log K_{\text{UO}_2\text{BL}}$  of 7.58 (Table S1). The value for  $\log K_{\text{UO}_2\text{BL}}$  only increases to 7.84 if only the free uranyl ion is responsible for the toxicity.

Goulet et al. (2015) also studied the toxicity of uranium to *L. minor* using 7 d growth assays (frond number, dry weight). The effect of hardness was studied at two concentrations (35 and 137 mg/L (as  $\text{CaCO}_3$ )), with the  $\text{EC}_{50}$  concentration increasing as hardness increased. Again, the study provided the concentrations of  $\text{UO}_2^{2+}$  and  $\text{UO}_2\text{OH}^{+}$  that were indicated to be responsible for the observed toxicity. Based on the toxicity found in the hardness tests, the speciation found and the relationship between toxicity and hardness, a  $\log K_{\text{UO}_2\text{BL}}$  value of 7.86 was determined (Table S1). If only  $\text{UO}_2^{2+}$  were responsible for the toxicity, the  $\log K_{\text{UO}_2\text{BL}}$  value would increase to 9.19.

The toxicity of uranium to *L. minor* was also studied by Horemans et al. (2016) using multiple 7 d growth assays (frond area, frond number, fresh weight, dry weight). The average  $\text{EC}_{50}$  concentration found using these assays in a synthetic freshwater was 24.8 mmol/L. Speciation calculations indicated that  $\text{UO}_2^{2+}$  (0.04 %) and  $\text{UO}_2\text{OH}^{+}$  (2.0 %) were only minor contributions to total uranium at the  $\text{EC}_{50}$ . Considering the  $\text{EC}_{50}$  concentration and the calculated speciation and, including the effect of the ameliorative cations (which is substantial; in particular, the effect of calcium), the  $\log K_{\text{UO}_2\text{BL}}$  value derived is 7.63 (Table S1; this value increases to 8.66 if only  $\text{UO}_2^{2+}$  is responsible for toxicity).

A 7 d growth assay was used by Markich (2013) to study the toxicity of uranium to *C. demersum* at a varying hardness. The  $\text{EC}_{50}$  concentrations increased linearly with respect to hardness, from which an  $\text{EC}_{50}$  of 134 mg/L (as U) was determined for zero hardness. At this condition, the calculated uranium speciation included  $\text{UO}_2^{2+}$  (0.6 %) and  $\text{UO}_2\text{OH}^{+}$  (5.6 %), being relatively minor species of total uranium. From these data, a  $\log K_{\text{UO}_2\text{BL}}$  value of 7.65 (also considering the pH and  $\text{Na}^{+}$  concentration: Table S1) was determined and, in addition, a  $(f_c)_{\text{UO}_2^{50\%}}$  value of 0.48 was also calculated. A  $\log K_{\text{UO}_2\text{BL}}$  value of 8.42 is calculated if only  $\text{UO}_2^{2+}$  is responsible for the observed toxicity.

### S6.6. Cnidaria Species

The toxicity of uranium to *H. viridissima* was studied by Reithmuller et al. (2000) at pH 6 and a varying hardness (6.6 to 330 mg/L (as  $\text{CaCO}_3$ )) using a 96 h growth assay. The uranium species was almost invariant with respect to the solution hardness, with  $\text{UO}_2\text{OH}^{+}$  (32 to 38 %) having a larger concentration than  $\text{UO}_2^{2+}$  (6 to 7.5 %). The measured  $\text{EC}_{50}$  concentrations ranged from 108 to 219 mg/L (as U). Taking account of the uranium speciation and adjustment for the pH of the test solutions led to a  $\log K_{\text{UO}_2\text{BL}}$  value of 7.15 (Table S1) when both uranium species are considered. If only the uranyl ion exerts a toxic response, then  $\log K_{\text{UO}_2\text{BL}}$  increases to 7.72. Analysis of the study of Markich and Camilleri (1997) [also on *H. viridissima* using a 96-h growth assay] led to a  $\log K_{\text{UO}_2\text{BL}}$  value of 7.19 (Table S1), as expected, very similar to that derived by Reithmuller et al. (2000). If only  $\text{UO}_2^{2+}$  causes toxicity, the  $\log K_{\text{UO}_2\text{BL}}$  value increases to 7.89.

Trenfield et al. (2011) also studied the toxicity of uranium to *H. viridissima* in synthetic waters simulating North Australian natural creek and billabong waters. This study also used a 96 h growth assay. Speciation calculations indicated that the concentration of  $\text{UO}_2\text{OH}^{+}$  (36.6 or 34.6 % of total uranium) at the  $\text{EC}_{50}$  was greater than that of  $\text{UO}_2^{2+}$  (3.6 or 3.4 %) in both synthetic waters. If both chemical species cause toxicity, and adjusting for the concentrations of the ameliorative cations, a  $\log K_{\text{UO}_2\text{BL}}$  value of 7.43 is calculated. This value is listed in Table S1. If only  $\text{UO}_2^{2+}$  results in toxicity, the value of  $\log K_{\text{UO}_2\text{BL}}$  increases to 8.21.

## S7. Lead

### S7.1. Pimephales promelas

There are five studies listed from which  $\log K_{\text{PbBL}}$  values have been derived relating to the toxicity of lead to *P. promelas*. The values range between 6.62 and 6.98, with an average of  $6.78 \pm 0.20$ . The 95 % confidence interval is relatively large, in part, because of the small amount of data available.

Esbaugh et al. (2011) conducted a study of lead toxicity to *P. promelas* using a 96 h mortality assay in natural waters. The study determined the speciation in the test solutions, from which an average  $LC_{50}$  of 105 nmol/L was determined (using equation (16)). Using this concentration leads to a log  $K_{PbBL}$  value of 6.98 (Table S1); if only  $Pb^{2+}$  is responsible for the toxicity, then the log  $K_{PbBL}$  value increases to 7.04.

Mager et al. (2011) also studied the toxicity of lead to *P. promelas* at varying pH (5.64 to 8.22) and Ca concentrations (6.4 to 118 mg/L). From the variation of the toxicity as a function of pH, where the ratio of the concentrations of  $Pb^{2+}$  and  $PbOH^+$  also vary, a log  $K_{PbBL}$  value of 6.63 has been determined. This value is listed in Table S1. If only  $Pb^{2+}$  causes toxicity, the log  $K_{PbBL}$  value increases only marginally to 6.67. Although  $PbOH^+$  is the more dominant lead species at the highest pH, its concentration still does not have a sufficient influence to change the stability constant substantially.

Schubauer-Berigan et al. (1993) measured the toxicity of copper to four freshwater species, including *P. promelas*, as a function of pH. Toxicity data were only available for *P. promelas* at the lowest pH studied. The  $LC_{50}$  value determined for *P. promelas*, taking account of the fraction of lead present as  $Pb^{2+}$  and  $PbOH^+$ , was used to calculate the log  $K_{PbBL}$  value (6.76). This value is listed in Table S1.

Diamond et al. (1997) also studied the toxicity of lead to *P. promelas* in both laboratory water and Lehigh River water sampled at different times of year. The geometric average of the  $LC_{50}$  data for the laboratory water was used in the present analysis and gave a concentration of 225 mg/L. The use of this toxicity concentration, together with the lead speciation ( $Pb^{2+}$  is 21.5 % and  $PbOH^+$  is 14.4 % of total lead) and the ameliorative cation concentrations, resulted in a log  $K_{PbBL}$  value of 6.62 (Table S1). If only  $Pb^{2+}$  is responsible for toxicity, the log  $K_{PbBL}$  value increases marginally to 6.75.

Grossell et al. (2006a) studied the toxicity of lead to *P. promelas* in waters of varying hardness and humic acid content. The toxicity decreased (i.e., greater  $LC_{50}$  values) as both hardness and humic acid content increased. Based on the measured  $LC_{50}$  values, the lead speciation and the concentrations of the ameliorative cation concentrations, an average log  $K_{PbBL}$  value of 6.90 was determined (Table S1). If only  $Pb^{2+}$  is responsible for toxicity, the log  $K_{PbBL}$  value increases to 7.08.

### S7.2. Other Fish Species

Three-hour uptake assays were used by Bircneau et al. (2008) to study lead toxicity to *O. mykiss*. The study reported a log  $K_{PbBL}$  value of 7.05; the reported value is listed in Table S1.

Mebane et al. (2008) studied the toxicity of lead to *O. mykiss* in soft waters and near neutral pH (6.75). The derived  $LC_{50}$  was 127 mg/L and in the test waters,  $Pb^{2+}$  accounted for 24.8 % of total lead and  $PbOH^+$  a further 3.8 %. Considering the  $LC_{50}$  and the lead speciation, together with the ameliorative cation concentrations, led to a log  $K_{PbBL}$  value of 6.92 (Table S1).

Alsop et al. (2016) also studied the toxicity of lead to *O. mykiss*. They determined an  $LC_{50}$  value of 487 mg/L, where  $Pb^{2+}$  was 3.5 % of total lead and  $PbOH^+$  another 7 %. The  $LC_{50}$  value with the lead speciation, as well as the ameliorative cation concentrations, led to a log  $K_{PbBL}$  value of 7.38 (Table S1). This value increases to 7.68 if only  $Pb^{2+}$  accounts for toxicity.

Vardy et al. (2014) studied the toxicity of lead to white sturgeon (*A. transmontanus*) in both laboratory and Columbia River water. The data for the youngest life-stage (8 days post-hatch) and laboratory water were used in the present analysis, together with the lead speciation and ameliorative cation concentrations to determine a log  $K_{PbBL}$  value of 7.26. This value is included in Table S1. The log  $K_{PbBL}$  value increases to 7.44 if only  $Pb^{2+}$  is responsible for toxicity.

### S7.3. *Ceriodaphnia dubia*

Seven studies have been reviewed in relation to the toxicity of lead to *C. dubia*. There is a large variation in the log  $K_{PbBL}$  values derived, ranging from 6.53 to 7.62, with an average of  $6.99 \pm 0.37$ . Nevertheless, the average value is still in good agreement with those values for other freshwater species.



Nys et al. (2014) studied the toxicity of lead to *C. dubia* at various calcium concentrations (9.6 to 72 mg/L) and pH (6.35 to 8.14) values using a 7 d reproduction assay. The concentrations of  $\text{Pb}^{2+}$  and  $\text{PbOH}^+$  were determined in the study for each test solution. Based on these concentrations, using equation (16), an  $\text{EC}_{50}$  concentration of 5.8 nmol/L was determined from the intercept of the toxicity as a function of the calcium concentration. This  $\text{EC}_{50}$  concentration was used together with the  $(f_c)_{\text{M}^{50\%}}$  value from Keithly et al. (2004) for the same species to derive a log  $K_{\text{PbBL}}$  value of 6.98 (Table S1). If only  $\text{Pb}^{2+}$  was responsible for toxicity, the log  $K_{\text{PbBL}}$  value would increase marginally to 7.02 (this is because  $\text{Pb}^{2+}$  is much more dominant than  $\text{PbOH}^+$  in the test solutions). Nys et al. (2016b) also studied lead toxicity to *C. dubia* using a 7 d reproduction assay. From the speciation and  $\text{EC}_{50}$  determined in the study, a log  $K_{\text{PbBL}}$  value of 6.65 was determined (Table S1).

The toxicity of lead to *C. dubia* was also studied by Mager et al. (2011) using a 48 h mortality assay with variable alkalinity, humic acid, calcium and sodium concentrations. For each test solution, the study determined the speciation of lead, from which an average  $\text{LC}_{50}$  of 12.9 nmol/L was determined, using equation (16). From this  $\text{LC}_{50}$  concentration, a log  $K_{\text{PbBL}}$  value of 6.63 was derived (Table S1), also using the  $(f_c)_{\text{M}^{50\%}}$  value from Keithly et al. (2004); if  $\text{PbOH}^+$  does not have a toxic effect, then the log  $K_{\text{PbBL}}$  value increases to 6.83.

Esbaugh et al. (2011) conducted a similar study with the same metal, species and assay endpoint, but in natural waters. This latter study also determined the speciation in the test solutions, from which an average  $\text{LC}_{50}$  of 16.4 nmol/L was determined (using equation (16)). Using this concentration leads to a log  $K_{\text{PbBL}}$  value of 6.53 (Table S1); if only  $\text{Pb}^{2+}$  is responsible for the toxicity, then the log  $K_{\text{PbBL}}$  value increases to 6.86.

Cooper et al. (2009) also studied the toxicity of lead to *C. dubia* using a 7 d reproduction assay and found an  $\text{EC}_{50}$  concentration of 5.1 mg/L. The 7 d reproduction assay was found to be much more sensitive than a 48 h mortality assay (208.8 mg/L). The cations,  $\text{Pb}^{2+}$  and  $\text{PbOH}^+$ , were found to be 8.7 and 7 %, respectively, of total lead. These data led to a calculated log  $K_{\text{PbBL}}$  value of 7.62 (Table S1); this value increased to 7.75 if only  $\text{Pb}^{2+}$  causes toxicity. The  $(f_c)_{\text{M}^{50\%}}$  value from Keithly et al. (2004) for *C. dubia* was also used with the toxicity data from this study.

Schubauer-Berigan et al. (1993) also measured the toxicity of copper to the two freshwater crustaceans *C. dubia* and *H. azteca* as a function of pH. Toxicity data were only available for *C. dubia* at the lowest pH studied, whereas for lead, no data were reported for *H. azteca*. The  $\text{LC}_{50}$  value determined for *C. dubia*, taking account of the fraction of lead present as  $\text{Pb}^{2+}$  and  $\text{PbOH}^+$ , was used to calculate the log  $K_{\text{PbBL}}$  value (7.23). This value is listed in Table S1.

The study of Diamond et al. (1997) examined the toxicity of lead to *C. dubia* in both laboratory water and Lehigh River water sampled at different times of year. The geometric average of the  $\text{LC}_{50}$  data for the laboratory water was used in the present analysis and gave a concentration of 50 mg/L. The use of this toxicity concentration, together with the copper speciation ( $\text{Pb}^{2+}$  is 21.5 % and  $\text{PbOH}^+$  is 14.4 % of total lead) and the ameliorative cation concentrations resulted in a log  $K_{\text{PbBL}}$  value of 7.28 (Table S1). If only  $\text{Pb}^{2+}$  accounted for toxicity, the log  $K_{\text{PbBL}}$  value would increase to marginally to 7.40.

Nys et al. (2016b) also studied the toxicity of lead to *C. dubia* and found an average  $\text{EC}_{50}$  lead concentration of 12.2 nmol/L. With lead, it was necessary to combine the measured  $\text{EC}_{50}$  together with the  $(f_c)_{\text{Pb}^{50\%}}$  value derived from the study of Keithly et al. (2004) to determine the values of log  $K_{\text{MBL}}$ . As such, the obtained log  $K_{\text{PbBL}}$  value (corrected for pH and the concentrations of  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) is 6.65 (Table S1).

#### S7.4. *Daphnia Species*

There are seven data points for *Daphnia* species that have been used to derive log  $K_{\text{PbBL}}$  values. These values range from 6.39 to 6.96, with an average value of  $6.75 \pm 0.17$ . The average value is consistent with that found for *C. dubia*.

The toxic effect of lead to *D. magna* was studied by Okamoto et al. (2015) at a water hardness of 72 mg/L (as  $\text{CaCO}_3$ ) using a 48 h acute immobilisation test. Unlike the other metals studied by Okamoto et al. (2015), only their value was available for lead with *D. magna*. From the toxicity concentration determined in the study, a log  $K_{\text{PbBL}}$  value of 6.39 was determined by

correcting for the concentrations of  $H^+$ ,  $Na^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$  as well as for the speciation of lead. The obtained value is listed in Table S1.

Araujo et al. (2019) studied the toxicity of lead to two *Daphnia* species, *D. magna* and *D. similis*, in hard water and a relatively high pH (8.4). They determined 48 h  $EC_{50}$  values of 460 and 340 mg/L, respectively. Speciation of the test solutions indicated that 2.5 % of total lead occurred as  $Pb^{2+}$  and a further 17 % as  $PbOH^+$ . Utilising the  $EC_{50}$  data, and the lead speciation, together with the ameliorative cation concentrations, and adjusting both sets of data using the  $(f_c)_{M^{50\%}}$  value derived from the data of De Schamphalaere et al. (2007) for *D. magna*, leads to log  $K_{PbBL}$  values of 6.69 and 6.82, respectively. If only  $Pb^{2+}$  is responsible for toxicity, the log  $K_{PbBL}$  increases to 7.33 and 7.46.

Kim et al. (2017) studied the toxicity of lead to *D. magna* in hard water. An  $LC_{50}$  concentration of 695 mg/L was determined. The lead ion ( $Pb^{2+}$ ) was 3.0 % of lead in the test solutions and  $PbOH^+$  a further 4.2 %. Based on the  $LC_{50}$ , the lead speciation, the concentrations of the ameliorative cations and the  $(f_c)_{M^{50\%}}$  value determined by Van Regenmortel et al. (2017) for the same species, a log  $K_{PbBL}$  value of 6.79 was determined. This value is listed in Table S1.

The toxicity of lead to *D. carinata* was also studied by Cooper et al. (2009) using a 48 h mortality assay and they found an  $EC_{50}$  concentration of 444 mg/L. Speciation calculations indicated that the cations,  $Pb^{2+}$  and  $PbOH^+$ , were 8.1 and 6.5 %, respectively, of total lead. These data led to a calculated log  $K_{PbBL}$  value of 6.97 (Table S1); this value increases to 7.10 if only  $Pb^{2+}$  causes toxicity.

Khoa et al. (2020) also studied the toxicity of lead to *D. carinata* at a neutral pH. The  $Pb^{2+}$  ion was a relatively large proportion (37.8 %) of total lead, with  $PbOH^+$  accounting for a further 10.4 %. The 48 h  $LC_{50}$  determined in the study was 122 mg/L. Using this value and the lead speciation, together with the ameliorative cation concentrations, led to a log  $K_{PbBL}$  value of 6.84. The value is listed in Table S1.

### **S7.5. Other Crustacean Species**

Besser et al. (2005) studied the toxicity of lead to *H. azteca* using 28 to 42 d mortality assays. They found the same  $LC_{50}$  concentration across the range of the duration of 24 mg/L. Based on the data provided in the study, a log  $K_{PbBL}$  of 6.94 was determined (Table S1).

### **S7.6. Microalgae Species**

De Schamphalaere et al. (2014) studied the toxicity of lead to *R. subcapitata* at a variable pH (6.0 to 7.6) and concentrations of fulvic acid (4 to 29.1 mg/L), calcium (4.9 to 120 mg/L), magnesium (2.9 to 24.3 mg/L) and sodium (33 to 169 mg/L) using a 72 h growth assay. Toxicity in both synthetic and natural waters was tested. The study provided  $EC_{50}$  concentrations (as  $Pb^{2+}$ ) for various concentrations of fulvic acid, from which a log  $K_{PbBL}$  value of 6.93 was determined (from  $EC_{50}$  concentration for a projected zero fulvic acid concentration). This value is listed in Table S1.

Alho et al. (2018) also studied the toxicity of lead to *R. subcapitata*. The study determined an  $EC_{50}$  of 161 mg/L. The  $Pb^{2+}$  ion accounted for 16.1 % of total lead and  $PbOH^+$  a further 10.2 %. Taking account of the  $EC_{50}$  and the lead speciation, and adjusting for the ameliorative cation concentrations, leads to a log  $K_{PbBL}$  value of 7.00 (Table S1). This value increases to 7.12 if only  $Pb^{2+}$  accounts for lead toxicity.

### **S7.7. Mollusc Species**

Wang et al. (2010b) studied the toxicity of metals, including lead, to the early life stages of freshwater mussels using various mortality assays (1 to 28 days). For juvenile fatmucket, *L. siliquioidea*, the 96 h assay was used for the  $LC_{50}$  concentration (142 mg/L and 298 mg/L); this endpoint was also used for zinc and cadmium. Free lead (0.34 %) and  $PbOH^+$  (0.59 %) were only small proportions of total lead and correcting for the effect of the ameliorative cations led to a log  $K_{PbBL}$  value of 6.73 (Table S1). If only the free ion is responsible for toxicity, then the log  $K_{PbBL}$  value increases to 7.13. The same assay was also used for *L. rafinesqueana* and the  $LC_{50}$

determined was 188 mg/L. Correcting this value for speciation and the effect of the ameliorative cations led to a binding constant ( $\log K_{\text{PbBL}}$ ) of 6.80. This value is listed in Table S1.

Markich (2017) used mortality assays (24 to 72 hours) of six freshwater mussels (glochidia: *A. profuga*, *C. novaehollandiae*, *H. australis*, *H. depressa*, *H. drapeta* and *V. ambiguus*) to study the sensitivity of the bivalves to exposures of metals, including lead. The study found an average value for  $\log K_{\text{PbBL}}$  of 6.38, with a range of 6.24 to 6.53 (using the 48 h mortality data). The free lead ion (50.3 %) was the dominant species in the solution. Reanalysis of the toxicity data in the present study led to marginally larger  $\log K_{\text{PbBL}}$  values, ranging between 6.40 and 6.61. The six values are listed in Table S1.

### S7.8. Macrophyte Species

The toxicity of lead to *L. minor* was studied by Antunes and Kreager (2014) using a 7 d growth assay. The data provided in the study enabled the calculation of the concentrations of  $\text{Pb}^{2+}$  and  $\text{PbOH}^+$  at the  $\text{EC}_{50}$ . Using these data led to a  $\log K_{\text{PbBL}}$  value of 7.19 (Table S1); if only  $\text{Pb}^{2+}$  caused the toxicity, the  $\log K_{\text{PbBL}}$  value increased to 7.53.

### S7.9. Rotifer Species

The toxicity of lead to the rotifer, *B. calyciflorus*, was studied by Grossell et al. (2006b) in moderately hard water. A 48 h  $\text{EC}_{50}$  of 204 mg/L was determined in the study. The  $\text{Pb}^{2+}$  was 4.9 % of total lead, with  $\text{PbOH}^+$  a further 19.6 %. Using the  $\text{EC}_{50}$  and the lead speciation with the ameliorative cation concentrations led to a  $\log K_{\text{PbBL}}$  value of 7.35 (Table S1). This value increases to 7.83 if only  $\text{Pb}^{2+}$  accounts for toxicity.

Hernández-Flores and Rico-Martínez (2006) studied the toxicity of lead to *Lecane quadridentata*. Both  $\text{Pb}^{2+}$  and  $\text{PbOH}^+$  were between 19 and 20 % of total lead. The 5 d  $\text{EC}_{50}$  determined in the study was 56 mg/L. Using the  $\text{EC}_{50}$  and the lead speciation, together with the ameliorative cation concentrations, led to a  $\log K_{\text{PbBL}}$  value of 7.48. This value is listed in Table S1.

### S7.10. Insect Species

The insect species *T. dissimilis*, was studied by Anderson et al. (1980) in relation to the toxicity of lead. The species  $\text{Pb}^{2+}$  and  $\text{PbOH}^+$  were 9.0 and 7.6 % of total lead at the determined  $\text{LC}_{50}$  (258 mg/L). Using the speciation and the  $\text{LC}_{50}$ , and adjusting for the ameliorative cation concentrations, led to a  $\log K_{\text{PbBL}}$  value of 6.58. This value is listed in Table S1.

## S8. Evaluation of Binding Constants for the Ameliorative Cations

### S8.1. Protons ( $\text{H}^+$ )

De Schamphalaere and Janssen (2004a) studied the toxicity of zinc to juvenile rainbow trout (*O. mykiss*) with a varying pH (5.68 to 7.87) and concentrations of  $\text{Ca}^{2+}$  (8.4 to 159 mg/L) and  $\text{Mg}^{2+}$  (1.4 to 76 mg/L) using a 96 h mortality test. A  $\log K_{\text{HBL}}$  of 5.94 (Table S2) was derived from the data given in the study with respect to the change in the  $\text{LC}_{50}$  as a function of the  $\text{H}^+$  concentration.

Heijerick et al. (2005a) studied the toxicity of zinc to *D. magna* using chronic 21 d reproduction assays. The study used synthetic water with a varying pH (5.5 to 8.0) and concentrations of  $\text{Na}^+$  (48 to 290 mg/L),  $\text{Ca}^{2+}$  (10 to 120 mg/L) and  $\text{Mg}^{2+}$  (6.0 to 97 mg/L). The study reported a  $\log K_{\text{HBL}}$  value of 5.77 (Table S2).

De Schamphalaere and Janssen (2002) conducted a study of the toxicity of copper to *D. magna* using a 48 h immobilisation assay at a variable pH (5.98 to 7.92) and concentrations of calcium (10 to 160 mg/L), magnesium (6.1 to 122 mg/L), sodium (1.8 to 347 mg/L) and potassium (3.0 to 78 mg/L). The study reported a  $\log K_{\text{HBL}}$  value of about 5.4, which was assumed to be approximately the same as for fish species. From the dependence of the  $\text{EC}_{50}$  concentration with respect to the proton ( $\text{H}^+$ ) concentration reported in the study, the present study has determined a  $\log K_{\text{HBL}}$  value of 5.70 (Table S2).

The toxicity of zinc to *D. magna* was also conducted by Van Regenmortel et al. (2017) using a 21 d reproduction assay. The study examined toxicity in two synthetic waters and four

European natural waters. A log  $K_{HBL}$  value of 5.77 was used in the study, the same value as obtained by Heijerick et al. (2005a) for the same species (see the discussion in the Zinc section). This value is listed in Table S2.

Crémazy et al. (2017) studied the toxicity of copper to *O. mykiss* using both 96 h and 30 d mortality assays. Toxicity in both series of tests was studied at varying pH, DOC, calcium and magnesium concentrations; in the 30 d tests, the ranges used were as follows: pH (5.1 to 8.6), DOC (0.42 to 11 mg/L), Ca (2.4 to 124 mg/L) and Mg (0.97 to 75 mg/L). From the relationship between the  $LC_{50}$  and the  $H^+$  concentration, a log  $K_{HBL}$  value of 5.50 was determined. This value is listed in Table S2.

Copper toxicity to *O. mykiss* was studied by Cusimano et al. (1986) using 96 h mortality tests at a variable pH (4.7 to 7.0). The  $LC_{50}$  values determined were linearly dependent on the  $H^+$  concentration. Based on the relationship between  $LC_{50}$  and the  $H^+$  concentration, a log  $K_{HBL}$  value of 5.89 was determined (Table S2).

Bradley and Sprague (1985) studied the toxicity of zinc to *O. mykiss* at a varying pH (5.56 to 7.04). The 96 h  $LC_{50}$  values acquired were found to vary with respect to the  $H^+$  concentration. From this relationship, a log  $K_{HBL}$  value of 5.95 was determined. This value is included in Table S2.

Deleebeeck et al. (2007a) studied nickel toxicity to *O. mykiss*. The study determined the median 17-day lethal nickel concentrations to juvenile rainbow trout. The effect of  $Ca^{2+}$  (3.64 to 110 mg/L),  $Mg^{2+}$  (2.85 to 72 mg/L) and  $H^+$  (pH 5.48 to 8.47) on nickel toxicity was also studied. Based on the relationship between the  $LC_{50}$  values and the  $H^+$  concentrations, a log  $K_{HBL}$  value of 5.93 was determined if all the toxicity data with variable  $H^+$  concentrations are used. However, examination of these data show (Figure S1) that there is a distinct break point in the relationship between the  $LC_{50}$  concentrations and the  $H^+$  concentration. If only the first two data (highest  $H^+$  concentrations) are used to derive the log  $K_{HBL}$  value, then a lower value of 5.68 is derived. This value is listed in Table S2. The relationship of the remaining  $H^+$  concentration data with the  $LC_{50}$  concentrations leads to a much larger value of log  $K_{HBL}$ , namely 6.83. This larger value may suggest a differing binding site at the cell surface as the pH increases (but see the discussion in the main text).

The toxicity of nickel to *R. subcapitata* was studied by Deleebeeck et al. (2009) in synthetic water with a varying pH (6.01 to 7.95) and concentrations of  $Mg^{2+}$  (2.76 to 115 mg/L) and  $Ca^{2+}$  (2.97 to 144 mg/L) and eight natural waters using a 72 h growth assay. The toxicity data at varying  $H^+$  concentrations were re-evaluated in the present study to determine a log  $K_{HBL}$  value of 5.78, where all the  $H^+$  concentration data were used. As with the previous study (Deleebeeck et al., 2007a), there is a clear break point in the relationship between the  $EC_{50}$  concentrations and  $H^+$  concentrations (Figure S2). The two largest  $H^+$  concentration values lead to a log  $K_{HBL}$  value of 5.67. This value is listed in Table S2. The subsequent data again lead to a larger log  $K_{HBL}$  value of 6.66, in good agreement with the value determined for *O. mykiss* from the data of Deleebeeck et al. (2007a).

The toxicity of zinc to *R. subcapitata* was studied by Heijerick et al. (2002b) using a 72 h growth inhibition assay in synthetic waters. Toxicity was studied by varying major ions ( $Na^+$  (62 to 166 mg/L),  $K^+$  (3.1 to 44 mg/L),  $Ca^{2+}$  (4.8 to 80 mg/L) and  $Mg^{2+}$  (2.9 to 61 mg/L)) and pH (5.6 to 7.8). Analysis of the toxicity data at elevated  $H^+$  concentrations (pH 5.6 to 6.8) led to a log  $K_{HBL}$  value of 5.82 (Table S2). However, there is a clear break point in the relationship between the  $EC_{50}$  concentrations and  $H^+$  concentrations. The data at a higher pH (Figure S4) lead to a much higher log  $K_{HBL}$  value of 8.27. This value will be seen to be consistent with other data where the toxicity of zinc has been studied, but not with similar data obtained from nickel toxicity studies (see main text).

Van Regenmortel et al. (2017) also studied the toxicity of zinc to *R. subcapitata* using the same 72 h growth inhibition assay as used by Heijerick et al. (2002b). The study of Van Regenmortel et al. (2017) found that the  $EC_{50}$  concentrations were dependent on the  $H^+$  concentration across the full range of pH values studied. Based on the analysis presented, a log  $K_{HBL}$  value of 7.85 was determined, in good agreement with that determined from the data of Heijerick et al. (2002b) for the same species.

The toxicity of copper to *R. subcapitata* was studied by Pascual et al. (2022) as a function of pH (6 to 8). The measured EC<sub>50</sub> concentrations were found to be linearly dependent on the H<sup>+</sup> concentration. Based on the relationship between the EC<sub>50</sub> values and the H<sup>+</sup> concentrations, a log  $K_{HBL}$  value of 5.56 was determined (Table S2).

Mager et al. (2011) studied the toxicity of lead to *P. promelas* at a varying pH (5.64 to 8.22) and Ca concentrations (6.4 to 118 mg/L). From the variation of the toxicity as a function of pH, a value of log  $K_{HBL}$  of 5.76 has been determined. This value is listed in Table S2.

The toxicity of copper to *P. promelas* was studied by Playle et al. (1993) using 2 to 3 h uptake assays. The study determined a log  $K_{HBL}$  value of 5.4. This value is listed in Table S2.

The toxicity of uranium to *Chlorella* sp. was studied by Franklin et al. (2000) using a 72 h growth assay and a variable pH (5.7 and 6.5). Based on the EC<sub>50</sub> data obtained in the study as a function of pH and the speciation of uranium, a log  $K_{HBL}$  value of 5.73 was determined (Table S2).

Wilde et al. (2006) measured zinc uptake in, and toxicity to, *Chlorella* sp. as a function of pH using a 48 h growth assay. The data provided in the study in relation to the variation in the EC<sub>50</sub> concentrations as a function of the H<sup>+</sup> concentration showed a distinct break point. At larger H<sup>+</sup> concentrations (see Figure S5), the relationship between the EC<sub>50</sub> and H<sup>+</sup> concentrations led to a log  $K_{HBL}$  value of 5.79 (Table S2; in excellent agreement with the value determined from the study of Franklin et al. (2000) for the same species); however, as the H<sup>+</sup> concentration decreased, a second log  $K_{HBL}$  value was derived (Figure S5) of 8.32. This latter value is in good agreement with the same value determined from the studies of Heijerick et al. (2002b) and Van Regenmortel et al. (2017), where the toxicity of zinc was also studied (see main text).

The toxicity of copper to *C. dubia* was also conducted by Hyne et al. (2005), where a 48 h immobilisation assay was utilised. The effect of pH (5.5 to 7.5) was studied, from which a log  $K_{HBL}$  of 5.62 was determined. This value is listed in Table S2.

Uranium toxicity to the unicellular alga *C. reinhardtii* was studied by Fortin et al. (2007) using 30 min uptake assays. The study examined the effect of uptake competition between the uranyl ion and competing cations (H<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>). A value for log  $K_{HBL}$  of 6.15 was reported in the study (Table S2).

Clifford and McGeer (2010) studied the acute toxicity of cadmium to *D. pulex* using a 48 h immobilisation assay. The toxicity was studied with a varying pH (6.10 to 8.02) and concentrations of Ca<sup>2+</sup> (1.2 to 64.5 mg/L), Mg<sup>2+</sup> (0.24 to 34 mg/L) and Na<sup>+</sup> (12.4 to 37.9 mg/L). From the study data, a log  $K_{HBL}$  value of 5.74 was derived (Table S2).

The toxic response of *H. azteca* to cadmium was studied by Schroeder (2008) in synthetic waters with varying pH (7.1 to 8.9) and Ca<sup>2+</sup> (11 to 300 mg/L) concentrations. The response of *H. azteca* to changes in pH was variable, but the use of the full range of pH data led to both a reasonable value for log  $K_{CDBL}$  and log  $K_{HBL}$  (5.95). The variation in the EC<sub>50</sub> concentrations for nickel with respect to pH (6.4 to 8.9) led to a log  $K_{HBL}$  value of 5.75. The latter log  $K_{HBL}$  value is listed in Table S2. However, it is clear that there is a break point in the relationship between the EC<sub>50</sub> concentrations and the H<sup>+</sup> concentrations at high pH (Figure S3). The variation in the EC<sub>50</sub> concentrations with respect to the H<sup>+</sup> concentrations at a high pH leads to a log  $K_{HBL}$  value of 6.93, assuming a slightly lower ( $f_C$ )<sub>Ni</sub><sup>50%</sup> value of 0.020 (compared to 0.070 for the other data; but again, see the main text). The value of log  $K_{HBL}$  derived from this study is in good agreement with those derived from the studies of Deleebeeck et al. (2007a, 2009) that were also for the toxicity of nickel.

Trenfield et al. (2011) studied the toxicity of uranium to *M. mogurnda* using a 96 h mortality assay. The average pH used in the study was 6.23. The toxicity of uranium to this species was also studied by Markich and Camilleri (1997), Cheng et al. (2010) and Pease et al. (2021) using the same assay, but at pH values of 6, 6.7 and 6.6, respectively. Using all these pH data and the toxicities determined led to a log  $K_{HBL}$  value of 5.83 (Table S2).

## S8.2. Calcium

De Schamphalaere and Janssen (2004a) studied the toxicity of zinc to juvenile rainbow trout (*O. mykiss*) with a varying pH (5.68 to 7.87) and concentrations of  $\text{Ca}^{2+}$  (8.4 to 159 mg/L) and  $\text{Mg}^{2+}$  (1.4 to 76 mg/L) using a 96 h mortality test. From the variation in the  $\text{LC}_{50}$  concentrations with respect to change in the calcium concentration, a log  $K_{\text{CaBL}}$  value of 3.34 (Table S2) was derived. From their data in another study (De Schamphalaere and Janssen, 2004b) and with respect to the toxicity of zinc to *O. mykiss*, where  $\text{Ca}^{2+}$  was varied from 6.6 to 93 mg/L, a log  $K_{\text{CaBL}}$  value of 3.49 was determined. This value is also listed in Table S2.

Niyogi et al. (2004, 2008) conducted two studies examining the toxicity of cadmium to *O. mykiss* using 3 h uptake assays. In the earlier uptake study, Niyogi et al. (2004) determined a log  $K_{\text{CaBL}}$  value of 3.69 (Table S2). They found a similar binding constant for yellow perch *P. flavescens*, namely 3.71. From the data given in their later study (Niyogi et al., 2008), a log  $K_{\text{CaBL}}$  value of 3.59 was determined. The stability constants for both fish species are listed in Table S2.

The toxicity of copper to *O. mykiss* was studied by Crémazy et al. (2017) using both 96 h and 30 d mortality assays. Toxicity in both series of tests was studied at a varying pH, DOC, calcium and magnesium concentrations; in the 30 d tests, the ranges used were as follows: pH (5.1 to 8.6), DOC (0.42 to 11 mg/L), Ca (2.4 to 124 mg/L) and Mg (0.97 to 75 mg/L). From the relationship between the  $\text{LC}_{50}$  and the  $\text{Ca}^{2+}$  concentration, a log  $K_{\text{CaBL}}$  value of 3.71 was determined (Table S2).

Deleebeeck et al. (2007a) studied nickel toxicity to *O. mykiss*. The study determined the median 17-day lethal nickel concentrations to juvenile rainbow trout. The effect of  $\text{Ca}^{2+}$  (3.64 to 110 mg/L),  $\text{Mg}^{2+}$  (2.85 to 72 mg/L) and  $\text{H}^+$  (pH 5.48 to 8.47) on nickel toxicity was also studied. Based on the relationship between the  $\text{LC}_{50}$  values and the  $\text{Ca}^{2+}$  concentrations, a log  $K_{\text{CaBL}}$  value of 3.11 was determined (Table S2).

Naddy et al. (2002) studied the toxicity of copper to several freshwater species. For *O. mykiss*, they studied the toxicity as a function of calcium concentration (10 to 68 mg/L). From the relationship between the measured  $\text{LC}_{50}$  data and the calcium concentration, a log  $K_{\text{CaBL}}$  value of 3.32 was derived (Table S2).

Heijerick et al. (2005a) studied the toxicity of zinc to *D. magna* using chronic 21 d reproduction assays. The study used synthetic water with varying pH (5.5 to 8.0) and concentrations of  $\text{Na}^+$  (48 to 290 mg/L),  $\text{Ca}^{2+}$  (10 to 120 mg/L) and  $\text{Mg}^{2+}$  (6.0 to 97 mg/L). The study reported a log  $K_{\text{CaBL}}$  value of 3.22 (Table S2). In an earlier study (Heijerick et al., 2002a), the toxicity of zinc to *D. magna* at a varying pH (6.0 to 8.0) and concentrations of  $\text{Na}^+$  (1.8 to 345 mg/L),  $\text{K}^+$  (3.0 to 78 mg/L),  $\text{Ca}^{2+}$  (5.0 to 320 mg/L) and  $\text{Mg}^{2+}$  (5.1 to 194 mg/L) was examined in synthetic water using acute 48 h immobilisation assays. This study reported a log  $K_{\text{CaBL}}$  value of 3.34, which is listed in Table S2.

Deleebeeck et al. (2008) studied the acute toxicity of nickel to *D. magna*, which included the amelioration effect of increases in the concentration of  $\text{H}^+$  (pH 5.95 to 8.13),  $\text{Na}^+$  (1.79 to 322 mg/L),  $\text{Ca}^{2+}$  (6.63 to 181 mg/L) and  $\text{Mg}^{2+}$  (5.18 to 111 mg/L) in synthetic water and also studied the toxicity in eight natural waters (in Europe) using 48 h immobilisation assays. Analysis of the data for the variation in the  $\text{EC}_{50}$  concentrations as a function of the change in Ca concentration led to a log  $K_{\text{CaBL}}$  value of 3.15 (Table S2).

De Schamphalaere and Janssen (2002) conducted a study of the toxicity of copper to *D. magna* using a 48 h immobilisation assay at a variable pH (5.98 to 7.92) and concentrations of calcium (10 to 160 mg/L), magnesium (6.1 to 122 mg/L), sodium (1.8 to 347 mg/L) and potassium (3.0 to 78 mg/L). The study reported a log  $K_{\text{CaBL}}$  value of 3.47. From the dependence of the  $\text{EC}_{50}$  concentration with respect to the  $\text{Ca}^{2+}$  concentration reported in the study, the present study has determined a log  $K_{\text{CaBL}}$  value of 3.21 (Table S2).

The toxicity of zinc to *D. magna* was also tested by Van Regenmortel et al. (2017) using a 21 d reproduction assay. The study examined toxicity in two synthetic waters and four European natural waters. A log  $K_{\text{CaBL}}$  value of 3.22 was used in the study, the same value as that obtained by Heijerick et al. (2005a) for the same species (see discussion in Zinc section). This value is listed in Table S2.

Mager et al. (2011) studied the toxicity of lead to *P. promelas* at a varying pH (5.64 to 8.22) and Ca concentrations (6.4 to 118 mg/L). From the variation in the toxicity as a function of



calcium concentration, a value of log  $K_{CaBL}$  value of 3.34 has been determined. This value is listed in Table S2.

The toxicity of copper to *P. promelas* was studied by Playle et al. (1993) using 2 to 3 h uptake assays. The study determined a log  $K_{CaBL}$  value of 3.4. This value is listed in Table S2.

Meyer et al. (1999) also studied the toxicity of nickel to *P. promelas* at varying calcium concentrations (0 to 361 mg/L) using a 96 h mortality test. The data were evaluated in the present study to determine a value for log  $K_{CaBL}$  of 3.02 (Table S2).

Di Toro et al. (2001) assessed the earlier work of Erickson et al. (1996). From the data provided, a log  $K_{CaBL}$  value of 3.12 was derived. This value is listed in Table S2.

Nys et al. (2014) studied the toxicity of lead to *C. dubia* at various calcium concentrations (9.6 to 72 mg/L) and pH levels (6.35 to 8.14) using a 7 d reproduction assay. From the correlation of the EC<sub>50</sub> concentration (as Pb<sup>2+</sup>) provided in the study with respect to the calcium concentration, a log  $K_{CaBL}$  value of 3.05 was obtained. This value is listed in Table S2.

Schwartz and Vigneault (2007) studied the toxicity of copper to *C. dubia* in varying concentrations of calcium (7.7 to 108 mg/L), magnesium (3.1 to 54.1 mg/L) and sodium (12.9 to 265 mg/L). They only reported data for EC<sub>25</sub> concentrations and, as such, EC<sub>50</sub> concentrations could not be derived. From the EC<sub>25</sub> concentration's variation with respect to the Ca concentration, a log  $K_{CaBL}$  value of 3.27 was determined (Table S2).

The effect of varying pH (6.3 to 8.3) and Ca<sup>2+</sup> (0.8 to 59 mg/L), Mg<sup>2+</sup> (0.24 to 35 mg/L), Na<sup>+</sup> (4.6 to 23 mg/L) and K<sup>+</sup> (1.1 to 31 mg/L) concentrations on the toxicity of nickel to *D. pulex* was studied by Kozlova et al. (2009) using a 48 h immobilisation test. Only Ca<sup>2+</sup> and Mg<sup>2+</sup> were found to ameliorate the nickel toxicity. Based on the relationship between the EC<sub>50</sub> values and the Ca concentration, a log  $K_{CaBL}$  value of 3.54 was derived and is listed in Table S2.

Clifford and McGeer (2010) studied the acute toxicity of cadmium to *D. pulex* using a 48 h immobilisation assay. The toxicity was studied with a varying pH (6.10 to 8.02) and concentrations of Ca<sup>2+</sup> (1.2 to 64.5 mg/L), Mg<sup>2+</sup> (0.24 to 34 mg/L) and Na<sup>+</sup> (12.4 to 37.9 mg/L). Table S2 contains the derived log  $K_{CaBL}$  value of 3.52.

The toxic response of *H. azteca* to cadmium was studied by Schroeder (2008) in synthetic waters with varying pH (7.1 to 8.9) and Ca<sup>2+</sup> (11 to 300 mg/L) concentrations. The response of *H. azteca* to changes in calcium concentration led to a value for log  $K_{CaBL}$  of 3.30. The same variation with respect to nickel toxicity led to almost the same log  $K_{CaBL}$  (3.29). The average log  $K_{CaBL}$  value is listed in Table S2.

Jackson et al. (2000) studied the toxicity of cadmium to *H. azteca* using a 96 h mortality assay with varying calcium (2 to 150 mg/L) and magnesium (1.2 to 83.2 mg/L) concentrations. Variation in the LC<sub>50</sub> concentrations with respect to the calcium concentration led to the log  $K_{CaBL}$  value of 3.41 (Table S2).

The toxicity of nickel to *H. azteca* was studied by Chan (2013) in test waters of varying calcium concentration (0.09 to 2.19 mmol/L). Toxicity was found to decrease (i.e., increasing LC<sub>50</sub> concentrations) as the calcium concentration increased. From the relationship between the LC<sub>50</sub> and Ca<sup>2+</sup> concentrations, a log  $K_{CaBL}$  value of 3.46 was determined (Table S2).

A 7 d growth assay was used by Markich (2013) to study the toxicity of uranium to *C. demersum* at varying hardness. The relationship between the EC<sub>50</sub> concentrations and the Ca concentration (which could be determined independently of the Mg concentration) was used to calculate a log  $K_{CaBL}$  value of 3.17 (Table S2).

Tan et al. (2017) studied the uptake (1 h) of samarium by *C. reinhardtii* in varying concentrations of calcium (0.04 to 40 mg/L) or magnesium (0 to 24 mg/L). The uptake data presented in the study as a function of Ca concentration were analysed in the present work to determine a log  $K_{CaBL}$  value of 3.72, which is listed in Table S2.

Deleebeeck et al. (2007b) studied the toxicity of nickel to 10 species of cladocerans collected from soft and hard water lakes in Sweden. The study used both chronic and acute toxicity assays. For acute toxicity (48 h immobilisation), they reported a value for log  $K_{CaBL}$  of 3.2. This value is listed in Table S2.

The toxicity of copper to *C. fluminea* was studied by Liao et al. (2007) using up-to-5 h valve closure assays. The study reported a value for log  $K_{CaBL}$  of 3.53. This value is listed in Table S2.

Clifford (2009) studied the toxicity of cadmium to *H. attenuata* using a 96 h growth assay in variable concentrations of  $\text{Ca}^{2+}$  (8.0 to 48.5 mg/L) and  $\text{Mg}^{2+}$  (4.4 to 15 mg/L). The data obtained in the study were recalculated to determine the effect of the hardness cations on toxicity, from which a log  $K_{\text{CaBL}}$  value of 3.65 was calculated. This value is listed in Table S2.

Heijerick et al. (2002b) studied the toxicity of zinc to *R. subcapitata* using a 72 h growth inhibition assay in synthetic waters. Toxicity was studied by varying major ion concentrations ( $\text{Na}^+$  (62 to 166 mg/L),  $\text{K}^+$  (3.1 to 44 mg/L),  $\text{Ca}^{2+}$  (4.8 to 80 mg/L) and  $\text{Mg}^{2+}$  (2.9 to 61 mg/L)) and pH (5.6 to 7.8). Analysis of the toxicity data related to changes in the  $\text{Ca}^{2+}$  concentration led to a log  $K_{\text{CaBL}}$  value of 3.05 (Table S2).

Chang et al. (1998) studied the toxicity of cadmium to *O. mossambicus*. They also studied how cadmium affected the uptake of calcium. They determined a  $K_m$  for calcium of 0.29 mmol/L. From this value, a log  $K_{\text{CaBL}}$  of 3.54 is derived. This value is listed in Table S2.

### S8.3. Magnesium

Heijerick et al. (2005a) studied the toxicity of zinc to *D. magna* using chronic 21 d reproduction assays. The study used synthetic water with a varying pH (5.5 to 8.0) and concentrations of  $\text{Na}^+$  (48 to 290 mg/L),  $\text{Ca}^{2+}$  (10 to 120 mg/L) and  $\text{Mg}^{2+}$  (6.0 to 97 mg/L). The study reported a log  $K_{\text{MgBL}}$  value of 2.69 (Table S2). In an earlier study (Heijerick et al., 2002a), the toxicity of zinc to *D. magna* at a varying pH (6.0 to 8.0) and concentrations of  $\text{Na}^+$  (1.8 to 345 mg/L),  $\text{K}^+$  (3.0 to 78 mg/L),  $\text{Ca}^{2+}$  (5.0 to 320 mg/L) and  $\text{Mg}^{2+}$  (5.1 to 194 mg/L) was examined in synthetic water using acute 48 h immobilisation assays. This study reported a log  $K_{\text{MgBL}}$  value of 3.12, which is also listed in Table S2.

Deleebeeck et al. (2008) studied the acute toxicity of nickel to *D. magna*, including the amelioration effect of increases in the concentration of  $\text{H}^+$  (pH 5.95 to 8.13),  $\text{Na}^+$  (1.79 to 322 mg/L),  $\text{Ca}^{2+}$  (6.63 to 181 mg/L) and  $\text{Mg}^{2+}$  (5.18 to 111 mg/L) in synthetic water, and also studied the toxicity in eight natural waters (in Europe) using 48 h immobilisation assays. Analysis of the data for the variation in the  $\text{EC}_{50}$  concentrations as a function of the change in Mg concentration led to a log  $K_{\text{MgBL}}$  value of 2.61 (Table S2).

De Schamphalaere and Janssen (2002) conducted a study of the toxicity of copper to *D. magna* using a 48 h immobilisation assay at a variable pH (5.98 to 7.92) and concentrations of calcium (10 to 160 mg/L), magnesium (6.1 to 122 mg/L), sodium (1.8 to 347 mg/L) and potassium (3.0 to 78 mg/L). The study reported a log  $K_{\text{MgBL}}$  value of 3.58. From the dependence of the  $\text{EC}_{50}$  concentration with respect to the  $\text{Mg}^{2+}$  concentration reported in the study, the present study has determined a log  $K_{\text{MgBL}}$  value of 2.92 (Table S2).

The toxicity of zinc to *D. magna* was also conducted by Van Regenmortel et al. (2017) using a 21 d reproduction assay. The study examined toxicity in two synthetic waters and four European natural waters. A log  $K_{\text{MgBL}}$  value of 2.69 was used in the study, the same value as that obtained by Heijerick et al. (2005a) for the same species (see discussion in Zinc section). This value is listed in Table S2.

The effect of varying pH (6.3 to 8.3) and  $\text{Ca}^{2+}$  (0.8 to 59 mg/L),  $\text{Mg}^{2+}$  (0.24 to 35 mg/L),  $\text{Na}^+$  (4.6 to 23 mg/L) and  $\text{K}^+$  (1.1 to 31 mg/L) concentrations on the toxicity of nickel to *D. pulex* was studied by Kozlova et al. (2009) using a 48 h immobilisation test. Only  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  were found to ameliorate the nickel toxicity. Based on the relationship between the  $\text{EC}_{50}$  values and the Mg concentration, a log  $K_{\text{MgBL}}$  value of 2.93 was derived and is listed in Table S2.

Clifford and McGeer (2010) studied the acute toxicity of cadmium to *D. pulex* using a 48 h immobilisation assay. The toxicity was studied with a varying pH (6.10 to 8.02) and concentrations of  $\text{Ca}^{2+}$  (1.2 to 64.5 mg/L),  $\text{Mg}^{2+}$  (0.24 to 34 mg/L) and  $\text{Na}^+$  (12.4 to 37.9 mg/L). Table S2 contains the derived log  $K_{\text{MgBL}}$  value of 2.96.

De Schamphalaere and Janssen (2004a) studied the toxicity of zinc to juvenile rainbow trout (*O. mykiss*) with a varying pH (5.68 to 7.87), and concentrations of  $\text{Ca}^{2+}$  (8.4 to 159 mg/L) and  $\text{Mg}^{2+}$  (1.4 to 76 mg/L) using a 96 h mortality test. From the variation in the  $\text{LC}_{50}$  concentrations with respect to change in the magnesium concentration, a log  $K_{\text{MgBL}}$  value of 2.99 (Table S2) was derived.



The toxicity of copper to *O. mykiss* was studied by Crémazy et al. (2017) using both 96 h and 30 d mortality assays. Toxicity in both series of tests was studied at a varying pH, DOC, calcium and magnesium concentrations; in the 30 d tests, the ranges used were as follows: pH (5.1 to 8.6), DOC (0.42 to 11 mg/L), Ca (2.4 to 124 mg/L) and Mg (0.97 to 75 mg/L). From the relationship between the  $LC_{50}$  and the  $Mg^{2+}$  concentration, a  $\log K_{MgBL}$  value of 2.97 was determined (Table S2).

Heijerick et al. (2002b) studied the toxicity of zinc to *R. subcapitata* using a 72 h growth inhibition assay in synthetic waters. Toxicity was studied by varying major ions ( $Na^+$  (62 to 166 mg/L),  $K^+$  (3.1 to 44 mg/L),  $Ca^{2+}$  (4.8 to 80 mg/L) and  $Mg^{2+}$  (2.9 to 61 mg/L)) and pH (5.6 to 7.8)). Analysis of the toxicity data related to changes in  $Mg^{2+}$  concentration led to a  $\log K_{MgBL}$  value of 2.99 (Table S2).

The toxicity of nickel to *R. subcapitata* was studied by Deleebeeck et al. (2009) in synthetic water with a varying pH (6.01 to 7.95) and concentrations of  $Mg^{2+}$  (2.76 to 115 mg/L) and  $Ca^{2+}$  (2.97 to 144 mg/L) and in eight natural waters using a 72 h growth assay. The toxicity data at varying  $Mg^{2+}$  concentrations were re-evaluated in the present study to determine a  $\log K_{MgBL}$  value of 2.76. This value is listed in Table S2.

Markich (2013) used a 7 d growth assay to study the toxicity of uranium to *C. demersum* at a varying hardness. The relationship between the  $EC_{50}$  concentrations and the Mg concentration (which was determined independently of the Ca concentration) was used to calculate a  $\log K_{MgBL}$  value of 2.73 (Table S2).

Schwartz and Vigneault (2007) studied the toxicity of copper to *C. dubia* in varying concentrations of calcium (7.7 to 108 mg/L), magnesium (3.1 to 54.1 mg/L) and sodium (12.9 to 265 mg/L). They only reported data for  $EC_{25}$  concentrations and, as such,  $EC_{50}$  concentrations could not be derived. From the  $EC_{25}$  concentration's variation with respect to the Mg concentration, a  $\log K_{MgBL}$  value of 2.60 was determined (Table S2).

The uptake (1 h) of samarium by *C. reinhardtii* was studied by Tan et al. (2017) in varying concentrations of calcium (0.04 to 40 mg/L) or magnesium (0 to 24 mg/L). The uptake data presented in the study as a function of Ca concentration were analysed in the present work to determine a  $\log K_{MgBL}$  value of 2.77, which is listed in Table S2.

Deleebeeck et al. (2007b) studied the toxicity of nickel to 10 species of cladocerans collected from soft and hard water lakes in Sweden. The study used both chronic and acute toxicity assays. For acute toxicity (48 h immobilisation), they reported a value for  $\log K_{MgBL}$  of 2.60. This value is listed in Table S2.

Schroeder (2008) studied the toxic response of *H. azteca* to nickel in synthetic waters with a varying pH (7.1 to 8.9) and  $Mg^{2+}$  (6.9 to 47 mg/L) concentrations. The response of *H. azteca* to changes in magnesium concentration led to a value for  $\log K_{MgBL}$  of 2.91. The  $\log K_{MgBL}$  value is listed in Table S2.

Jackson et al. (2000) studied the toxicity of cadmium to *H. azteca* using a 96 h mortality assay with varying calcium (2 to 150 mg/L) and magnesium (1.2 to 83.2 mg/L) concentrations. Variation in the  $LC_{50}$  concentrations with respect to the magnesium concentration led to the  $\log K_{MgBL}$  value of 2.73 (Table S2).

Clifford (2009) studied the toxicity of cadmium to *H. attenuata* using a 96 h growth assay in variable concentrations of  $Ca^{2+}$  (8.0 to 48.5 mg/L) and  $Mg^{2+}$  (4.4 to 15 mg/L). The data obtained in the study were recalculated to determine the effect of the hardness cations on toxicity, from which a  $\log K_{MgBL}$  value of 2.75 was calculated. This value is listed in Table S2.

Chen et al. (2016) studied the toxicity of zinc to the fish species, *C. idellus*, using 96 h acute and 28 d chronic endpoints. For both endpoints, they studied the toxicity as a function of magnesium concentration. From the data reported in the study, a  $\log K_{MgBL}$  value was determined for both endpoints, which were in excellent agreement. The average value is 2.77 (this value is listed in Table S2).

Kleinhenz et al. (2019) studied the toxicity of magnesium to *V. angasi* using 24 h mortality assays. The derivation of  $\log K_{MgBL}$  in this study differs from the other studies whereby in this study, magnesium toxicity is studied, whereas in the other studies, the amelioration of metal toxicity results from increases in the concentration of magnesium. However, when the

speciation of magnesium is considered, together with the concentrations of calcium and sodium as well as pH, a log  $K_{MgBL}$  value of 2.66 is determined and is in excellent agreement with all other values derived. The value is listed in Table S2.

Okamoto et al. (2015) also studied the toxicity of magnesium, but to *D. magna*, using 24 h mortality assays. The derivation of log  $K_{MgBL}$  is the same as in the previous study where magnesium toxicity is studied. Again, when the speciation of magnesium is considered, together with the concentration of calcium, a log  $K_{MgBL}$  value of 2.82 is determined in excellent agreement with all other values derived for *D. magna*. The value is listed in Table S2.

#### S8.4. Sodium

Heijerick et al. (2005a) studied the toxicity of zinc to *D. magna* using chronic 21 d reproduction assays. The study used synthetic water with a varying pH (5.5 to 8.0) and concentrations of  $Na^+$  (48 to 290 mg/L),  $Ca^{2+}$  (10 to 120 mg/L) and  $Mg^{2+}$  (6.0 to 97 mg/L). The study reported a log  $K_{NaBL}$  value of 1.90 (Table S2). In an earlier study (Heijerick et al., 2002a), the toxicity of zinc to *D. magna* at a varying pH (6.0 to 8.0) and concentrations of  $Na^+$  (1.8 to 345 mg/L),  $K^+$  (3.0 to 78 mg/L),  $Ca^{2+}$  (5.0 to 320 mg/L) and  $Mg^{2+}$  (5.1 to 194 mg/L) was examined in synthetic water using acute 48 h immobilisation assays. This study reported a log  $K_{NaBL}$  value of 2.37, which is listed in Table S2.

De Schamphalaere and Janssen (2002) conducted a study of the toxicity of copper to *D. magna* using a 48 h immobilisation assay at a variable pH (5.98 to 7.92) and concentrations of calcium (10 to 160 mg/L), magnesium (6.1 to 122 mg/L), sodium (1.8 to 347 mg/L) and potassium (3.0 to 78 mg/L). The study reported a log  $K_{NaBL}$  value of 3.19. From the dependence of the  $EC_{50}$  concentration with respect to the  $Na^+$  concentration reported in the study, the present study has determined a log  $K_{NaBL}$  value of 2.28 (Table S2).

The toxicity of zinc to *D. magna* was also conducted by Van Regenmortel et al. (2017) using a 21 d reproduction assay. The study examined toxicity in two synthetic waters and four European natural waters. A log  $K_{MgBL}$  value of 1.90 was used in the study, the same value as that obtained by Heijerick et al. (2005a) for the same species (see discussion in Zinc section). This value is listed in Table S2.

The toxicity of copper to cladoceran species was studied by De Schamphalaere et al. (2007) using a 48 h immobilisation assay with varying sodium concentrations (1.8 to 230 mg/L) and included three *Ceriodaphnia* sp., *C. dubia*, *C. reticulata* and *C. pulchella*, three *Daphnia* sp., *D. magna*, *D. galeata* and *D. longispina* and two *Sinocephalus* sp., *S. vetulus* and *S. exspinosus*. The toxicity of copper to the species was always found to increase as the concentration of sodium in the test solutions decreased. The study carried out toxicity tests using various natural European waters and one synthetic water. Toxicity tests on some species were carried out in more than one of the waters used in the study. Where this occurred, all the data for a single species were combined into a single toxicity dataset for the species. Based on the  $EC_{50}$  concentration data measured as a function of the Na concentration for each species, log  $K_{CuBL}$  and log  $K_{NaBL}$  values were determined for each species. These values were initially determined without any correction for  $(f_c)_{Cu^{50\%}}$ ; the corrected values for log  $K_{CuBL}$  have already been provided above. However, when the uncorrected log  $K_{CuBL}$  and log  $K_{NaBL}$  values derived were plotted against one another, it was clear that a significant relationship existed between the two parameters (Figure S6). The study also examined the toxicity of copper to another species, *Acroperus elongatus*, and the uncorrected log  $K_{CuBL}$  and log  $K_{NaBL}$  data for this species are also shown in Figure S6 (but these latter values are not used to derive the parameters of the regression shown in the figure). The relationship found between log  $K_{CuBL}$  and log  $K_{NaBL}$  allows for the determination of the  $(f_c)_{Cu^{50\%}}$  value for each species; however, it is clear from the relationship that not only must this value be necessary to explain copper toxicity, but it is also required to explain the effect that sodium has on the amelioration of the toxicity. Therefore, both the log  $K_{CuBL}$  and log  $K_{NaBL}$  values have been corrected by the same amount to determine values quoted in the present study. Those for log  $K_{CuBL}$  have already been presented; those for log  $K_{NaBL}$  are 2.14 (*C. reticulata*), 2.43 (*S. vetulus*), 2.04 (*S. exspinosus*), 2.42 (*C. pulchella*), 2.43 (*C. dubia*), 2.24 (*D. galeata*), 2.50 (*D. magna*) and 2.06 (*D. longispina*). These data are presented in

Table S2. Even though the data for *A. elongatus* are consistent with the relationship between  $\log K_{\text{CuBL}}$  and  $\log K_{\text{NaBL}}$ , no data are provided for this species because the analysis would require  $(f_{\text{c}})_{\text{Cu}}^{50\%}$  to be greater than 0.5, which is not permitted. However, the fact that the data for this species are fully consistent with the relationship implies that this species has a mechanism for protecting surface sites from occupation by metals in solution (in this case, copper). The binding capacity of the metal at the cell surface is still the same, but there is a mechanism preventing the metal or ameliorative cations getting to the surface sites. The data for *A. elongatus* are not provided in Table S2, but calculations may suggest that values for this species might be a  $\log K_{\text{CuBL}}$  of 7.81 and  $\log K_{\text{NaBL}}$  of 2.12. Two *Chydonas* sp., *C. sphaericus* and *C. ovalis*, were also studied. The binding constants derived for both these species were not consistent with those found for all other species. For both species, the derived  $\log K_{\text{NaBL}}$  value was much higher than could be explained based on the derived  $\log K_{\text{CuBL}}$  values. These effects need further examination.

Schwartz and Vigneault (2007) studied the toxicity of copper to *C. dubia* in varying concentrations of calcium (7.7 to 108 mg/L), magnesium (3.1 to 54.1 mg/L) and sodium (12.9 to 265 mg/L). They only reported data for  $\text{EC}_{25}$  concentrations and, as such,  $\text{EC}_{50}$  concentrations could not be derived. From the  $\text{EC}_{25}$  concentration's variation with respect to the Na concentration, a  $\log K_{\text{NaBL}}$  value of 2.26 was determined (Table S2).

Clifford and McGeer (2010) studied the acute toxicity of cadmium to *D. pulex* using a 48 h immobilisation assay. The toxicity was studied with a varying pH (6.10 to 8.02) and concentrations of  $\text{Ca}^{2+}$  (1.2 to 64.5 mg/L),  $\text{Mg}^{2+}$  (0.24 to 34 mg/L) and  $\text{Na}^{+}$  (12.4 to 37.9 mg/L). Table S2 contains the derived  $\log K_{\text{NaBL}}$  value of 2.05.

De Schamphalaere and Janssen (2004a) studied the toxicity of zinc to juvenile rainbow trout (*O. mykiss*) with a varying pH (5.68 to 7.87) and concentrations of  $\text{Ca}^{2+}$  (8.4 to 159 mg/L) and  $\text{Mg}^{2+}$  (1.4 to 76 mg/L) using a 96 h mortality test. From the variation in the  $\text{LC}_{50}$  concentrations with respect to change in the sodium concentration, a  $\log K_{\text{NaBL}}$  value of 2.43 (Table S2) was derived.

Heijerick et al. (2002b) studied the toxicity of zinc to *R. subcapitata* using a 72 h growth inhibition assay in synthetic waters. Toxicity was studied by varying major ions ( $\text{Na}^{+}$  (62 to 166 mg/L),  $\text{K}^{+}$  (3.1 to 44 mg/L),  $\text{Ca}^{2+}$  (4.8 to 80 mg/L) and  $\text{Mg}^{2+}$  (2.9 to 61 mg/L)) and pH (5.6 to 7.8). Analysis of the toxicity data related to changes in  $\text{Na}^{+}$  concentration led to a  $\log K_{\text{NaBL}}$  value of 2.17 (Table S2).

## S9. Binding Constants of Organisms in Other Aquatic Systems

Reanalysis of the data provided by Lock et al. (2007) for the toxicity of nickel to barley (*Hordeum vulgare*) led to a  $\log K_{\text{NiBL}}$  of 5.11 as a function of changes in the concentrations of  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Na}^{+}$ ,  $\text{K}^{+}$  and  $\text{H}^{+}$ . Only the  $\text{Mg}^{2+}$  concentration was shown to influence toxicity. Phillips et al. (2003) studied the toxicity of nickel to the marine purple sea urchin (*Strongylocentrotus purpuratus*), from which a  $\log K_{\text{NiBL}}$  value of 5.24 was determined. Both these values are within the range of  $\log K_{\text{NiBL}}$  values found for freshwater aquatic organisms.

Phillips et al. (2003) also examined the toxicity of zinc to *S. purpuratus*, from which a  $\log K_{\text{ZnBL}}$  of 5.82 was determined. In addition, Nadella et al. (2013) studied the toxicity of zinc to several marine organisms (*S. purpuratus*, *Mytilus galloprovincialis* and *Mytilus trossolus*), from which an average  $\log K_{\text{ZnBL}}$  value of 5.61 was determined. Both  $\log K_{\text{ZnBL}}$  values determined in these studies are within the range of values found in the present study.

Lock et al. (2006) studied the toxicity of cobalt to the potworm (*Enchytraeus albidus*) as a function of pH and the concentrations of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Na}^{+}$ . Based on the change in toxicity as a function of the concentration of the ameliorative cations, the present study determined a  $\log K_{\text{CoBL}}$  value of 5.20. Again, this value is in good agreement with the range of values found for  $\log K_{\text{CoBL}}$  in the present study.

Qu et al. (2013) studied the toxicity of cadmium to the marine bacterium, *Photobacterium phosphoreum*, as a function of water chemistry (variable  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{H}^{+}$  concentrations). Based on the cadmium toxicity as a function of the changes in these concentrations, the present

study determined a log  $K_{CdBL}$  value of 6.69. This value is in excellent agreement with the range of values determined for log  $K_{CdML}$  found in the present study.

Vijver et al. (2011) studied the toxicity of copper to lettuce (*Lactuca sativa*). The study determined a log  $K_{CuBL}$  value of 7.72 when 50 % of the biological membrane surface sites are occupied. Again, the binding constant determined is virtually the same as the average value found in the present study (Table 1).

Nadella et al. (2013) also studied the toxicity of lead to the same marine organisms as they did for zinc. The toxicity results from the study lead to a log  $K_{PbBL}$  of 6.89 when the speciation of lead in seawater is considered. This binding constant is also in very good agreement with the average value determined in the present study (Table 1).

Saenen et al. (2015) studied the toxicity of uranium to the plant mouse-ear cress (*Arabidopsis thaliana*). An  $EC_{50}$  of 70.24 mM (as U) was found using a 3 d root growth assay. The pH was 7.5 and the free uranyl ion and  $UO_2OH^+$  were found to be only a small proportion of total uranyl, which led to a log  $K_{UO_2BL}$  value of 7.86. This value is within the range of binding constants found for the uranyl ion in the present study.

From the studies of Lock et al. (2006) and Qu et al. (2013), binding constants for calcium could also be determined. The values obtained for log  $K_{CaBL}$  were 3.23 and 3.50, respectively. In addition, Li et al. (2008) studied the toxicity of cadmium to the earthworm, *Eisenia fetida*. In their study, they reported a value for log  $K_{CaBL}$  of 3.35. The average of the three binding constants (log  $K_{CaBL}$ ) is 3.36, in excellent agreement with the average binding constant found in this study.

Again, the studies of Lock et al. (2006) and Qu et al. (2013) were analysed, from which log  $K_{MgBL}$  values of 2.95 and 3.26 were determined. A binding constant was also determined from the later study of Lock et al. (2007), namely a log  $K_{MgBL}$  of 3.09. Li et al. (2008) also reported a value for log  $K_{MgBL}$  of 2.82. Finally, Wu et al. (2017) studied the toxicity of copper, cadmium and their mixtures to wheat (*Triticum aestivum*). From their study, a log  $K_{MgBL}$  value of 2.88 was determined. The average of these five binding constants (log  $K_{MgBL}$ ) is 3.00, in very good agreement with the average value found in the present study.

From four of the studies already cited (Lock et al., 2006; Li et al., 2008; Vijver et al., 2011; Wu et al., 2017), log  $K_{HBL}$  values of 6.07, 5.41, 6.21 and 5.93, respectively, were either reported or determined in the present study, with an average value of 5.91. Again, this latter value is in good agreement with the average value for log  $K_{HBL}$  found in this study.

From the study of Lock et al. (2006), a log  $K_{NaBL}$  value of 1.58 was determined, and Li et al. (2008) reported an almost identical value of 1.57. Both these values are lower than the range of values found for log  $K_{NaBL}$  in the present study. Nevertheless, they are still considered to be consistent with the values reported in this study.

Overall, it would appear that the log  $K_{MBL}$  values determined in the present study are also applicable for other aquatic environments, including soils (e.g., aquatic environments of plants and soil organisms) as well as seawater (marine organisms). This includes both the binding constants of all seven metals assessed in the present study ( $Ni^{2+}$ ,  $Zn^{2+}$ ,  $Co^{2+}$ ,  $Cd^{2+}$ ,  $Cu^{2+}$ ,  $Pb^{2+}$  and  $UO_2^{2+}$ ) as well as those for the ameliorative cations ( $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Na^+$  and  $H^+$ ).

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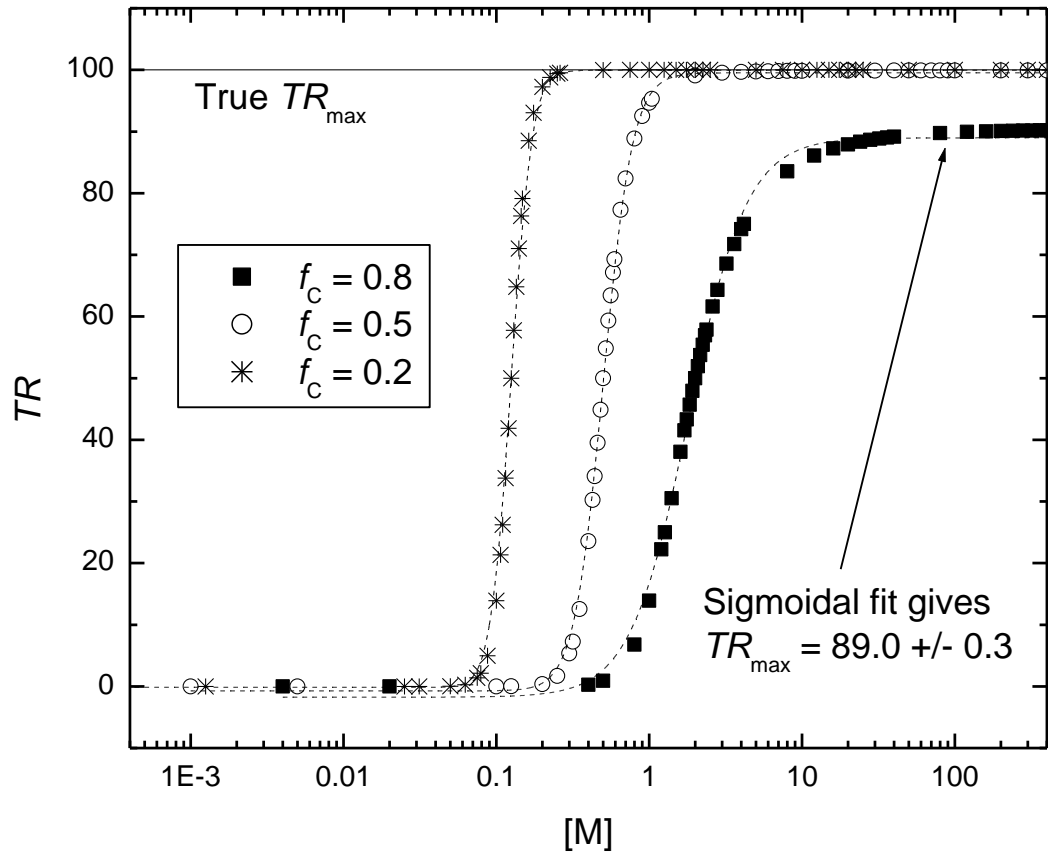
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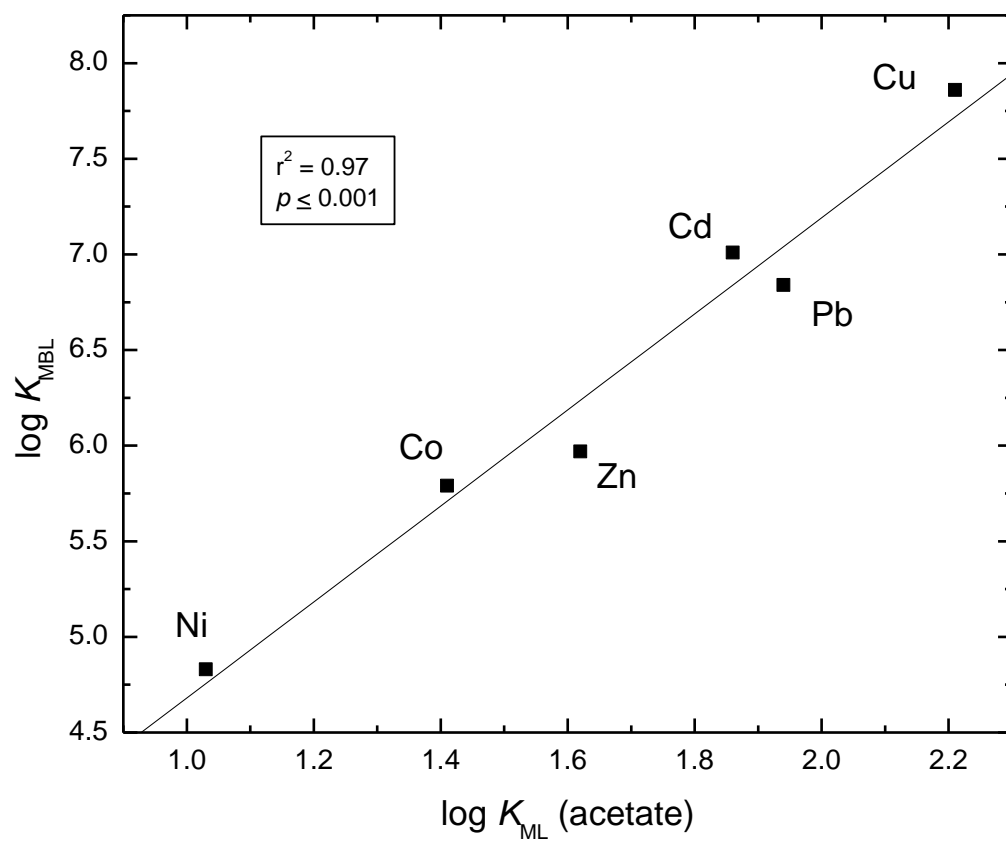
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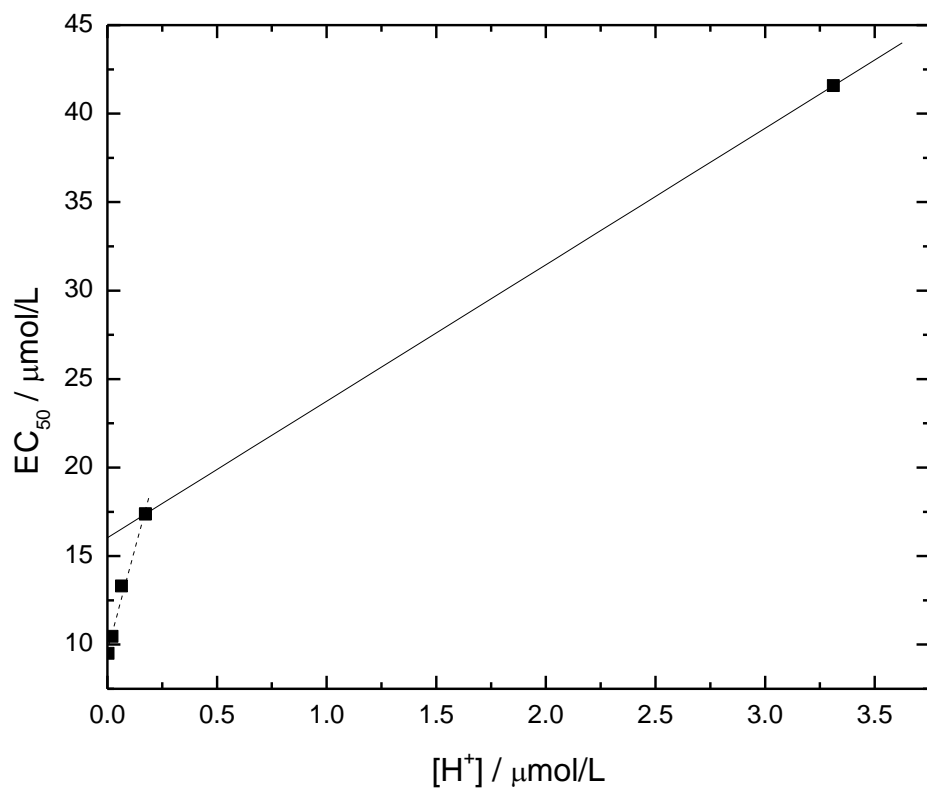




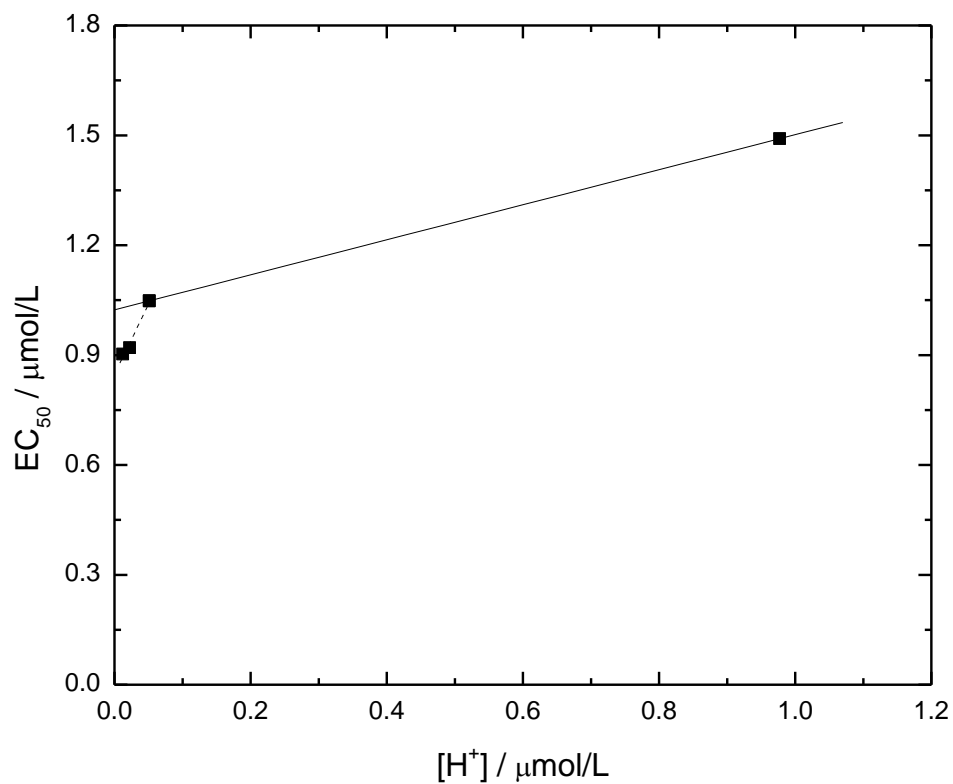
**Figure S1.** Calculated concentration-response curves when  $f_c = 0.2$  (less than 50 % of receptor sites are occupied at the  $TR_{50}$ ), 0.5 (50 % of receptor sites are occupied at the  $TR_{50}$ ) and 0.8 (more than 50 % of receptor sites are occupied at the  $TR_{50}$ ). Since the true  $TR_{max}$  is not reached when  $f_c > 0.5$ , such scenarios are invalid.



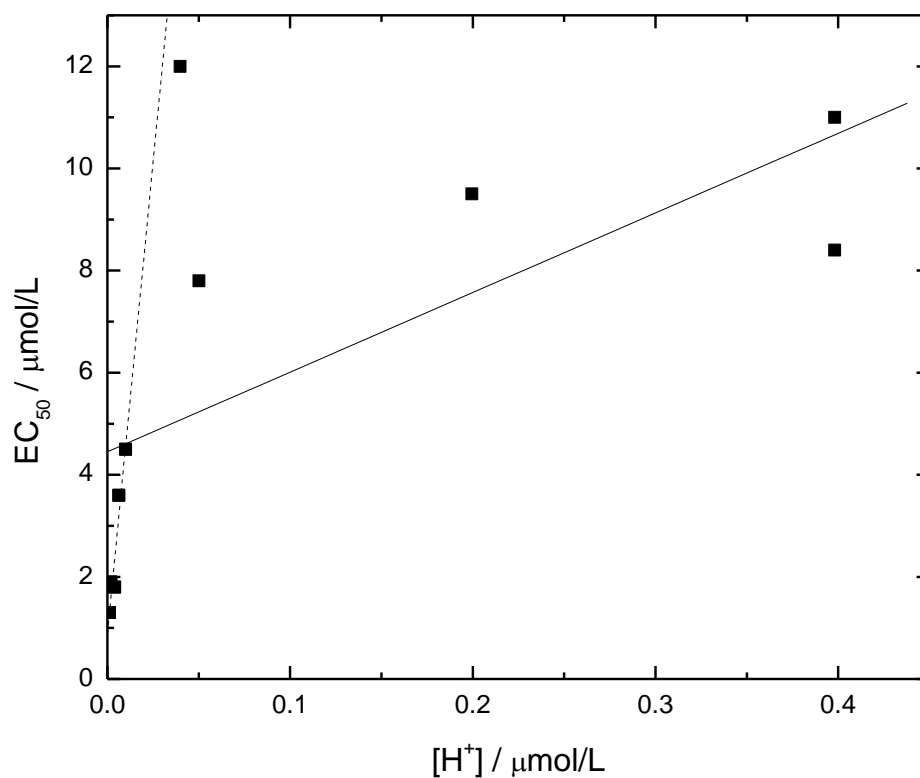
**Figure S2.** The linear regression between metal binding constants ( $\log K_{\text{MBL}}$ ) with cell surface receptors and metal stability constants ( $\log K_{\text{ML}}$ ) with acetate (a simple carboxylate ligand).



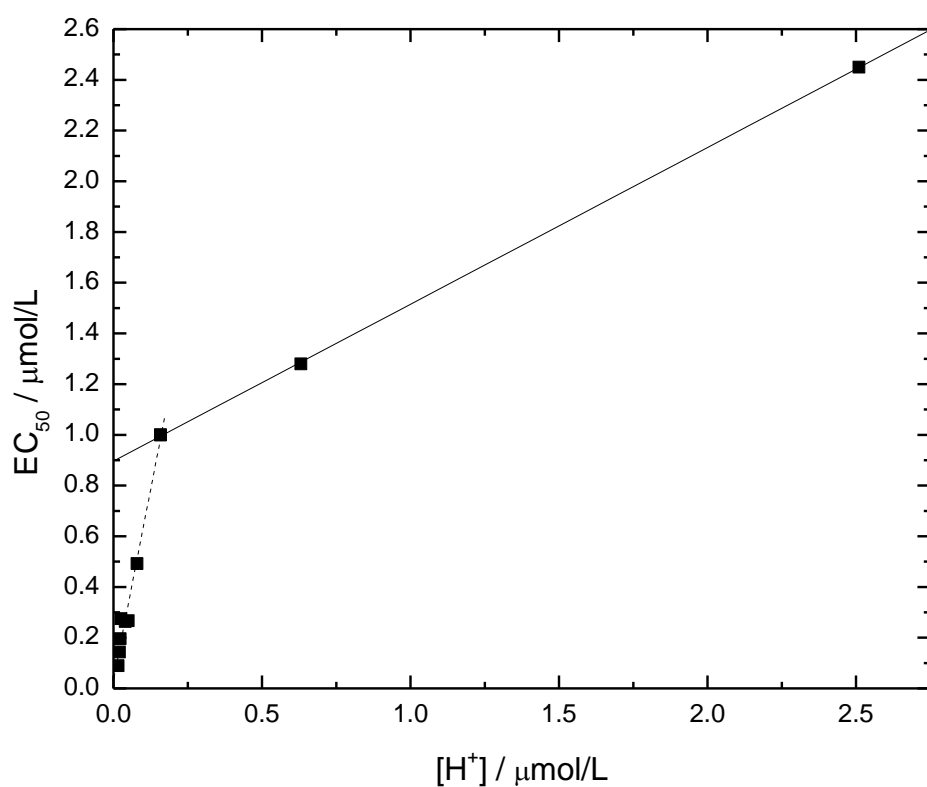
**Figure S3.** Linear regression between nickel toxicity ( $EC_{50}$ ) in *Oncorhynchus mykiss* (fish) and pH (as proton ( $H^+$ ) concentration)—from Deleebeeck et al. (2007a).



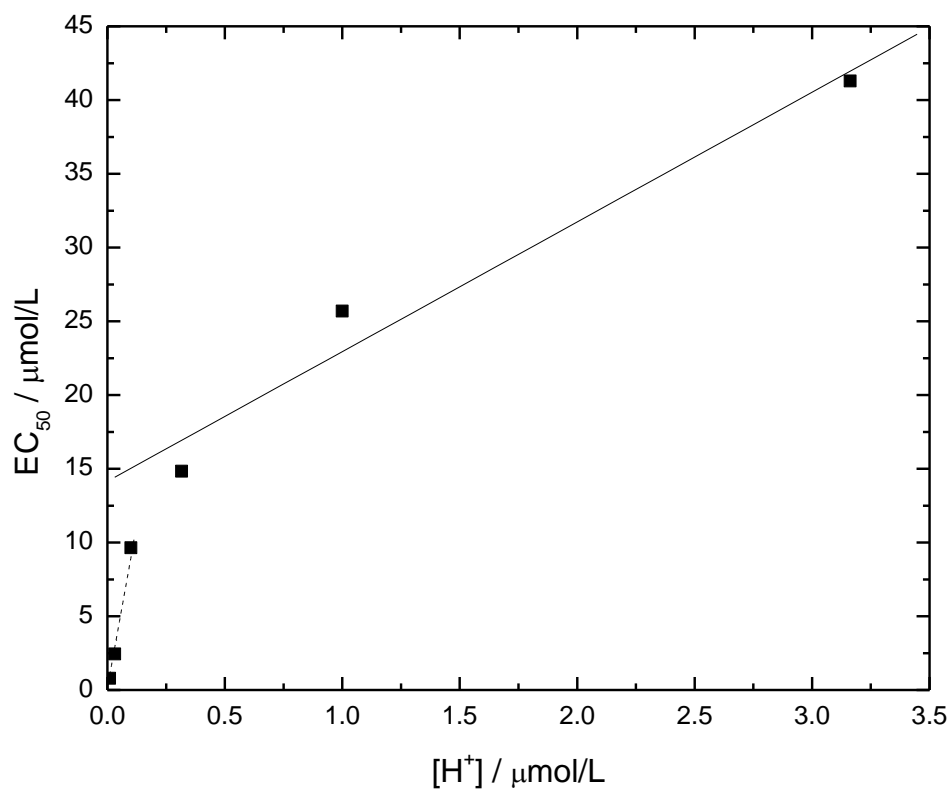
**Figure S4.** Linear regression between nickel toxicity ( $EC_{50}$ ) in *Raphidocelis subcapitata* (microalgae) and pH (as proton ( $H^+$ ) concentration)—from Deleebeeck et al. (2009).



**Figure S5.** Linear regression between nickel toxicity ( $EC_{50}$ ) in *Hyallela azteca* (crustacean) and pH (as proton ( $H^+$ ) concentration)—from Schroeder (2008).

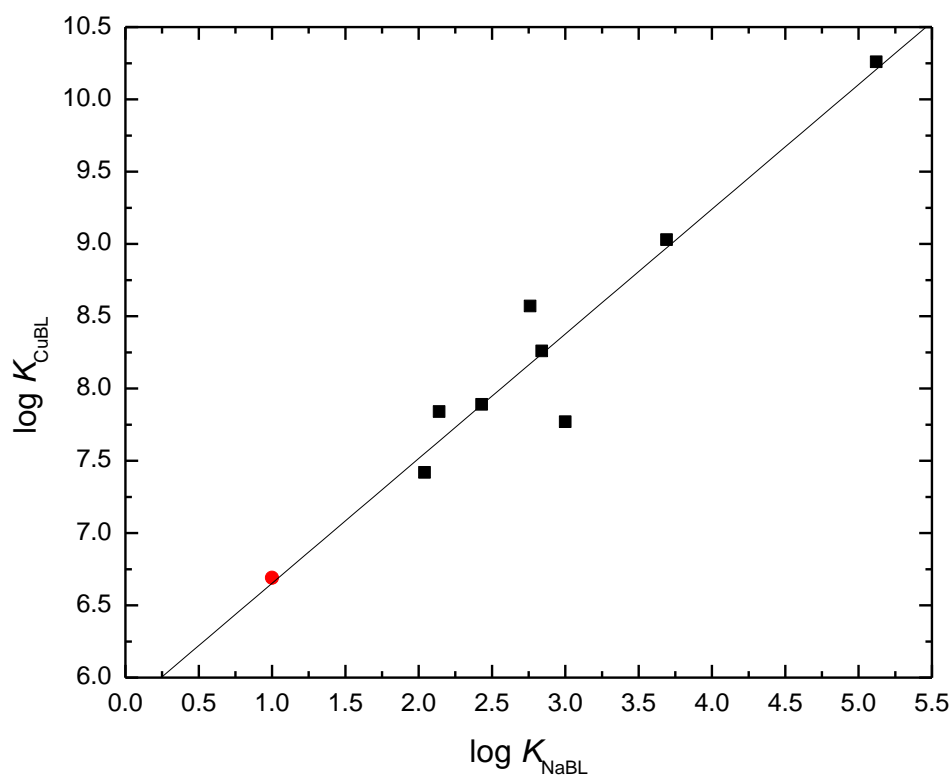


**Figure S6.** Linear regression between zinc toxicity ( $EC_{50}$ ) in *Raphidocelis subcapitata* (microalgae) and pH (as proton ( $H^+$ ) concentration)—from Heijerick et al. (2002b).



**Figure S7.** Linear regression between zinc toxicity ( $EC_{50}$ ) in *Chlorella* sp. (microalgae) and pH (as proton ( $H^+$ ) concentration)—from Wilde et al. (2006).





**Figure S8.** The linear regression between sodium ( $\log K_{\text{NaBL}}$ ) and copper ( $\log K_{\text{CuBL}}$ ) binding constants for cladocerans (crustaceans)—from De Schamphalaere et al. (2007). The red data point is for *A. elongatus* and was not used in the linear regression because it required an  $f_c$  factor greater than 0.5 (nevertheless, it is consistent with the linear relationship).

**Table S1.** The binding constants (log  $K_{\text{MBL}}$ ) of zinc (Zn), nickel (Ni), cadmium (Cd), cobalt (Co), copper (Cu), uranium (UO<sub>2</sub>) and lead (Pb) at cell surface receptor sites (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log $K_{\text{MBL}}$						Reference
			Zn	Ni	Cd	Co	Cu	UO <sub>2</sub>	
Fish	<i>Acipenser transmontanus</i>	96 h mortality					8.29*		Ivey et al. (2019)
		96 h mortality	6.11*		7.32*		8.14*	7.26*	Vardy et al. (2014)
	<i>Cottus bairdi</i>	7 d mortality	5.97*						Brinkman and Woodling (2005)
		96 h mortality	5.78*						Brinkman and Johnston (2012)
		96 h mortality	6.45*		7.18*		8.13*		Besser et al. (2007)
	<i>Ctenopharyngodon idellus</i>	96 h mortality	5.69*						Chen et al. (2016)
	<i>Danio rerio</i>	48 h growth					7.70*		Abdel-monein et al. (2015)
	<i>Mogurnda mogurnda</i>	96 h mortality						7.38*	Trenfield et al. (2011) <sup>a</sup>
		7 d growth					7.34*		Trenfield et al. (2022)
		7 d growth	6.13*						Trenfield et al. (2023)
	<i>Neogobius melanostomus</i>	96 h mortality/96 h uptake		4.72*					Leonard et al. (2014)
	<i>Oncorhynchus clarkii</i>	96 h mortality	5.77*						Brinkman and Johnston (2012)
		96 h mortality					7.79*		Chakoumakos et al. (1979)
	<i>Oncorhynchus kisutch</i>	96 h mortality					7.82*		Porter et al. (2023)
	<i>Oncorhynchus mykiss</i>	81 d biomass				5.27*			Stubblefield et al. (2020)
		3 h uptake	5.60			5.10			Alsop and Wood (2000)
		96 h mortality	5.93**						De Schamphalaere and Janssen (2004a)
		30 d mortality	5.76**						De Schamphalaere and Janssen (2004b)
		96 h mortality	6.06*				7.77*		Cusimano et al. (1986)
		14 d mortality				5.42*	7.97*		Marr et al. (1998)
		30 d mortality					8.18*		Crémazy et al. (2017)
		96 h mortality					7.63*		Ng et al. (2010)
		96 h mortality					7.66*		Morris et al. (2019)
		7 d mortality					7.92*		Welsh et al. (2008)
		2 h uptake					8.02*		Grosell and Wood (2002)
		96 h mortality	5.85*						Bradley and Sprague (1985)
		3 h uptake			7.34				Niyogi et al. (2004)

**Table S1 Continued.** The binding constants (log  $K_{MBL}$ ) of zinc (Zn), nickel (Ni), cadmium (Cd), cobalt (Co), copper (Cu), uranium (UO<sub>2</sub>) and lead (Pb) at cell surface receptor sites (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log $K_{MBL}$							Reference
			Zn	Ni	Cd	Co	Cu	UO <sub>2</sub>	Pb	
Fish	<i>Oncorhynchus mykiss</i>	<a href="#">3 h uptake</a>			7.33				7.05	Birceanu et al. (2008)
		<a href="#">3 h uptake</a>			7.37					Hollis et al. (2000)
		<a href="#">3 h uptake</a>			7.52					Niyogi et al. (2008)
		<a href="#">96 h uptake</a>			7.35**					Liao et al. (2010)
		<a href="#">96 h mortality</a>			7.46*					Davies et al. (1993)
		<a href="#">96 h mortality</a>			7.40*					Spehar and Carlson (1984)
		<a href="#">96 h mortality</a>	6.23*		7.59*		7.79*			Naddy et al. (2015)
		<a href="#">96 h mortality</a>	5.92*		7.48*		8.09*			Calfee et al. (2014)
		<a href="#">96 h mortality</a>	6.06*		7.50*		8.07*			Besser et al. (2007)
		<a href="#">96 h mortality</a>	6.06*		7.44*		7.79*			Chapman (1978)
		<a href="#">96 h mortality</a>	5.90*		7.70*				6.92*	Mebane et al. (2008)
		<a href="#">120 h mortality</a>	5.93*		7.59*					Hansen et al. (2002)
		<a href="#">96 h mortality</a>							7.38*	Alsop et al. (2016)
		<a href="#">28 d uptake</a>	5.98*							Hogstrand et al. (1998)
		<a href="#">72 h uptake</a>		4.71*						Pane et al. (2006)
		<a href="#">96 h mortality</a>		4.47*						Brix et al. (2004)
		<a href="#">17 d mortality</a>		4.89*						Deleebeeck et al. (2007a)
		<a href="#">96 h mortality/96 h uptake</a>		4.33*						Leonard et al. (2014)
		<a href="#">96 h mortality</a>	5.97*							Mebane et al. (2021)
		<a href="#">96 h mortality</a>					8.28*			Naddy et al. (2002)
		<a href="#">96 h mortality/30 d renewal</a>						7.34*		Goulet et al. (2015)
	<i>Oncorhynchus nerka</i>	<a href="#">96 h mortality</a>					7.66*			Porter et al. (2023)
	<i>Oncorhynchus tshawytscha</i>	<a href="#">96 h mortality</a>	6.07*		7.57*		7.82*			Chapman (1978)
		<a href="#">96 h mortality</a>					7.53*			Porter et al. (2023)
	<i>Oreochromis mossambicus</i>	<a href="#">96 h mortality</a>			7.00*					Chang et al. (1998)
	<i>Perca flavescens</i>	<a href="#">3 h uptake</a>			7.20					Niyogi et al. (2004)
	<i>Pimephales promelas</i>	2 to <a href="#">3 h uptake</a>					7.40			Playle et al. (1993)

**Table S1 continued.** The binding constants (log  $K_{MBL}$ ) of zinc (Zn), nickel (Ni), cadmium (Cd), cobalt (Co), copper (Cu), uranium (UO<sub>2</sub>) and lead (Pb) at cell surface receptor sites (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log $K_{MBL}$						Reference
			Zn	Ni	Cd	Co	Cu	UO <sub>2</sub>	
Fish	<i>Pimephales promelas</i>	<a href="#">96 h</a> mortality					7.78*		Di Toro et al. (2001)
		<a href="#">96 h</a> mortality					7.53*		Naddy et al. (2002)
		<a href="#">96 h</a> mortality					7.74*		Sciera et al. (2004)
		<a href="#">96 h</a> mortality					7.65*		Erickson et al. (1996)
		<a href="#">96 h</a> mortality		4.48*			7.44*		Meyer et al. (1999)
		<a href="#">96 h</a> mortality					7.62*		Welsh et al. (1996)
		<a href="#">96 h</a> mortality					7.52*		Morris et al. (2019)
		<a href="#">96 h</a> mortality					7.59*		Nimmo et al. (2006)
		<a href="#">96 h</a> mortality			7.09*				Spehar and Carlson (1984)
		<a href="#">96 h</a> mortality						6.90*	Grosell et al. (2006a)
		<a href="#">96 h</a> mortality		5.14*					Hoang et al. (2004)
		<a href="#">96 h</a> mortality						6.63*	Mager et al. (2011)
		<a href="#">96 h</a> mortality						6.98*	Esbaugh et al. (2011)
		<a href="#">48 h</a> mortality	5.86*		6.99*		7.55*	6.62*	Diamond et al. (1997)
		<a href="#">96 h</a> mortality	5.56*	4.52*	7.17*		7.67*	6.76*	Schubauer-Berigan et al. (1993)
		<a href="#">96 h</a> mortality	5.54*						Bringolf et al. (2006)
		<a href="#">34 d</a> mortality				5.68*			Stubblefield et al. (2020)
		<a href="#">96 h</a> mortality/7 d growth						7.38*	Goulet et al. (2015)
	<i>Prosopium williamsoni</i>	<a href="#">96 h</a> mortality	5.46*						Brinkman and Johnston (2012)
	<i>Salmo trutta</i>	<a href="#">96 h</a> mortality			7.62*				Spehar and Carlson (1984)
	<i>Salvelinus conflentus</i>	<a href="#">120 h</a> mortality	5.74*		7.19*				Hansen et al. (2002)
Crustaceans	<i>Acantholebris curvirostris</i>	<a href="#">48 h</a> immobilisation					7.75*		Bossuyt and Janssen (2005)
	<i>Acroperus elongatus</i>	<a href="#">48 h</a> immobilisation					8.22*		Bossuyt and Janssen (2005)
	<i>Acroperus harpae</i>	<a href="#">48 h</a> immobilisation					8.32*		Bossuyt and Janssen (2005)
	<i>Alona affinis</i>	<a href="#">48 h</a> reproduction		4.66*					Deleebeeck et al. (2007b)
	<i>Alona quadrangularis</i>	<a href="#">48 h</a> immobilisation					7.98*		Bossuyt and Janssen (2005)
	<i>Alona</i> sp.	<a href="#">48 h</a> immobilisation					8.08*		Bossuyt and Janssen (2005)

**Table S1 continued.** The binding constants (log  $K_{MBL}$ ) of zinc (Zn), nickel (Ni), cadmium (Cd), cobalt (Co), copper (Cu), uranium (UO<sub>2</sub>) and lead (Pb) at cell surface receptor sites (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log $K_{MBL}$							Reference
			Zn	Ni	Cd	Co	Cu	UO <sub>2</sub>	Pb	
Crustaceans	<i>Bosmina coregoni</i>	48 h reproduction		4.98*						Deleebeeck et al. (2007b)
	<i>Bosmina longirostris</i>	48 h immobilisation					7.87*			Bossuyt and Janssen (2005)
	<i>Bosmina longirostris</i>	48 h mortality					7.94*			Koivisto et al. (1992)
	<i>Camptocercus lilljeborgi</i>	48 h reproduction		4.90*						Deleebeeck et al. (2007b)
	<i>Ceriodaphnia carnuta</i>	24 h immobilisation					7.92*			Choueri et al. (2009)
	<i>Ceriodaphnia dubia</i>	48 h immobilisation					8.02*			Markich et al. (2005)
		48 h immobilisation	5.86*				7.76*			Hyne et al. (2005)
		48 h immobilisation					7.77*			De Schamphalaere et al. (2007)
		48 h immobilisation					7.81*			Harmon et al. (2003)
		7 d reproduction	5.63*				7.47*		7.62*	Cooper et al. (2009)
		48 h mortality	5.84*	5.00*	6.60*		7.87*		7.23*	Schubauer-Berigan et al. (1993)
		48 h mortality					7.37*			Ivey et al. (2019)
		48 h mortality					8.25*			Naddy et al. (2002)
		48 h immobilisation					8.07*			Gensemer et al. (2002)
		48 h immobilisation					8.36*			Bossuyt and Janssen (2005)
		7 d mortality	6.28*		7.35*		7.97*			Naddy et al. (2015)
		7 d reproduction	5.97*				7.98*			Stauber et al. (2023)
		48 h mortality					8.23*			Wang et al. (2011b)
		48 h mortality					8.22*			Nimmo et al. (2006)
		48 h mortality	5.55*							Mebane et al. (2021)
		7 d reproduction							6.98*	Nys et al. (2014)
		7 d reproduction	6.10*	5.39*					6.65*	Nys et al. (2016b)
		48 h mortality							6.63*	Mager et al. (2011)
		48 h mortality							6.53*	Esbaugh et al. (2011)
		48 h mortality		5.48*						Peters et al. (2018)
		48 h mortality		4.85*						Keithly et al. (2004)
		48 h mortality	5.98*				8.30*		7.28*	Diamond et al. (1997)

**Table S1 continued.** The binding constants (log  $K_{MBL}$ ) of zinc (Zn), nickel (Ni), cadmium (Cd), cobalt (Co), copper (Cu), uranium (UO<sub>2</sub>) and lead (Pb) at cell surface receptor sites (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log $K_{MBL}$						Reference
			Zn	Ni	Cd	Co	Cu	UO <sub>2</sub>	
Crustaceans	<i>Ceriodaphnia dubia</i>	<u>7 d</u> mortality/reproduction						7.36*	Goulet et al. (2015)
		<u>7 d</u> reproduction				5.48*			Stubblefield et al. (2020)
		<u>7 d</u> reproduction and mortality			7.10*				Sofyan et al. (2007)
	<i>Ceriodaphnia pulchella</i>	<u>48 h</u> immobilisation					7.56*		De Schamphalaere et al. (2007)
		<u>48 h</u> immobilisation					7.78*		Bossuyt and Janssen (2005)
		<u>48 h</u> reproduction		4.81*					Deleebeeck et al. (2007b)
	<i>Ceriodaphnia quadrangula</i>	<u>48 h</u> reproduction		4.82*					Deleebeeck et al. (2007b)
	<i>Ceriodaphnia reticulata</i>	<u>48 h</u> immobilisation					7.84*		De Schamphalaere et al. (2007)
		<u>48 h</u> immobilisation					8.24*		Bossuyt and Janssen (2005)
		<u>48 h</u> mortality	6.19*		6.50*		8.12*		Mount and Norberg (1984)
	<i>Ceriodaphnia rigaudi</i>	<u>48 h</u> immobilisation			7.02*				Raymundo et al. (2024)
	<i>Ceriodaphnia silvestri</i>	<u>48 h</u> immobilisation			6.91*				Raymundo et al. (2024)
	<i>Chydorus ovalis</i>	<u>48 h</u> reproduction		4.71*					Deleebeeck et al. (2007b)
		<u>48 h</u> immobilisation					7.91*		Bossuyt and Janssen (2005)
	<i>Chydorus sphearicus</i>	<u>48 h</u> mortality					8.23*		Koivisto et al. (1992)
		<u>48 h</u> immobilisation					8.05*		Bossuyt and Janssen (2005)
	<i>Cypridopsis vidua</i>	<u>96 h</u> mortality						7.80*	Chen et al. (2022)
	<i>Daphnia ambigua</i>	<u>48 h</u> immobilisation					7.88*		Harmon et al. (2003)
	<i>Daphnia carinata</i>	<u>48 h</u> mortality	5.70*				7.42*	6.97*	Cooper et al. (2009)
		<u>48 h</u> mortality						6.84*	Khoa et al. (2020)
	<i>Daphnia exilis</i>	<u>48 h</u> mortality					7.84*		Hernandez-Zamora et al. (2023)
	<i>Daphnia galeata</i>	<u>48 h</u> immobilisation					7.66*		De Schamphalaere et al. (2007)
		<u>48 h</u> immobilisation					7.48*		Bossuyt and Janssen (2005)
		<u>48 h</u> mortality					7.64*		Koivisto et al. (1992)
	<i>Daphnia longispina</i>	<u>48 h</u> immobilisation					7.87*		De Schamphalaere et al. (2007)
		<u>48 h</u> reproduction		4.89*					Deleebeeck et al. (2007b)
		<u>48 h</u> immobilisation					7.70*		Bossuyt and Janssen (2005)

**Table S1 continued.** The binding constants (log  $K_{MBL}$ ) of zinc (Zn), nickel (Ni), cadmium (Cd), cobalt (Co), copper (Cu), uranium (UO<sub>2</sub>) and lead (Pb) at cell surface receptor sites (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log $K_{MBL}$							Reference
			Zn	Ni	Cd	Co	Cu	UO <sub>2</sub>	Pb	
Crustaceans	<i>Daphnia magna</i>	<a href="#">48 h</a> immobilisation	5.31							Heijerick et al. (2002a)
		<a href="#">48 h</a> immobilisation	5.88*	4.98*	7.14*	5.11*	7.93*		6.39*	Okamoto et al. (2015)
		<a href="#">21 d</a> reproduction	5.31							Van Regenmortel et al. (2017)
		<a href="#">21 d</a> reproduction	6.14**							Heijerick et al. (2005a)
		<a href="#">48 h</a> immobilisation	5.67*							Muyssen and Janssen (2005)
		<a href="#">72 h</a> mortality	6.09*							Paulauskis and Winner (1988)
		<a href="#">96 h</a> mortality	5.88*							Paylar et al. (2022)
		<a href="#">48 h</a> mortality	6.24*		6.25*		7.59*			Mount and Norberg (1984)
		<a href="#">48 h</a> immobilisation					7.90**			De Schamphalaere and Janssen (2002)
		<a href="#">48 h</a> immobilisation					7.27*			De Schamphalaere et al. (2007)
		<a href="#">48 h</a> mortality					7.85*			Long et al. (2004)
		<a href="#">48 h</a> immobilisation					7.75*			Kramer et al. (2004)
		<a href="#">48 h</a> mortality					7.86*			Naddy et al. (2002)
		<a href="#">48 h</a> mortality					7.77*			Ryan et al. (2009)
		<a href="#">48 h</a> immobilisation					8.05*			De Schamphalaere et al. (2002)
		<a href="#">48 h</a> mortality					7.95*			Villavicencio et al. (2005)
		<a href="#">48 h</a> immobilisation					7.86*			Bossuyt and Janssen (2005)
		<a href="#">48 h</a> mortality					7.42*			Koivisto et al. (1992)
		<a href="#">48 h</a> mortality	5.95*				7.88*		6.72*	LeBlanc (1982)
		<a href="#">48 h</a> immobilisation		4.67**						Deleebeeck et al. (2008)
		<a href="#">48 h</a> immobilisation		5.01**						Mano and Shinohara (2020)
		<a href="#">21 d</a> reproduction		4.76*						He et al. (2023)
		<a href="#">21 d</a> reproduction		4.54*						Nys et al. (2016a)
		<a href="#">21 d</a> reproduction		5.18*						Pavlaki et al. (2011)
		<a href="#">48 h</a> immobilisation			6.97*					Tan and Wang (2011)
		<a href="#">48 h</a> mortality			6.89*				6.79*	Kim et al. (2017)
		<a href="#">48 h</a> immobilisation							6.69*	Araujo et al. (2019)



**Table S1 continued.** The binding constants (log  $K_{MBL}$ ) of zinc (Zn), nickel (Ni), cadmium (Cd), cobalt (Co), copper (Cu), uranium (UO<sub>2</sub>) and lead (Pb) at cell surface receptor sites (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log $K_{MBL}$						Reference
			Zn	Ni	Cd	Co	Cu	UO <sub>2</sub>	
Crustaceans	<i>Daphnia magna</i>	<a href="#">48 h</a> immobilisation			7.22*				Lee et al. (2009)
		<a href="#">72 h</a> mortality			6.79*				Liu and Wang (2015)
		<a href="#">48 h</a> mortality			6.74*				Schuytema et al. (1984)
		<a href="#">21 d</a> reproduction				5.67*			Stubblefield et al. (2020)
		<a href="#">48 h</a> mortality						8.04*	Zeman et al. (2008)
	<i>Daphnia obtusa</i>	<a href="#">48 h</a> mortality					7.84*		Villavicencio et al. (2005)
	<i>Daphnia pulex</i>	<a href="#">48 h</a> mortality/uptake		4.77*					Leonard and Wood (2013)
		<a href="#">48 h</a> immobilisation		4.62**					Kozlova et al. (2009)
		<a href="#">48 h</a> immobilisation			6.79*				Clifford and McGeer (2010)
		<a href="#">72 h</a> mortality					8.02*		Winner (1985)
		<a href="#">48 h</a> mortality					8.14*		Villavicencio et al. (2005)
		<a href="#">48 h</a> mortality					7.87*		Koivisto et al. (1992)
		<a href="#">48 h</a> mortality	6.05*		6.49*		7.60*		Mount and Norberg (1984)
		<a href="#">48 h</a> immobilisation	5.70**						Clifford and McGeer (2009)
	<i>Daphnia similis</i>	<a href="#">48 h</a> immobilisation						6.82*	Araujo et al. (2019)
	<i>Daphnia thomsoni</i>	<a href="#">21 d</a> reproduction	6.27*						Stauber et al. (2023)
	<i>Disparalona rostrata</i>	<a href="#">48 h</a> immobilisation					7.68*		Bossuyt and Janssen (2005)
	<i>Eurycercus lamellatus</i>	<a href="#">48 h</a> immobilisation					8.05*		Bossuyt and Janssen (2005)
	<i>Gammarus</i> sp.	<a href="#">96 h</a> mortality					7.48*		Naddy et al. (2002)
	<i>Gammarus pseudolimnaeus</i>	<a href="#">96 h</a> mortality			6.49*				Spehar and Carlson (1984)
	<i>Gammarus pulex</i>	<a href="#">7 d</a> uptake		4.70					Lebrun et al. (2011)
	<i>Hyaella azteca</i>	<a href="#">28 d</a> mortality		5.34*	7.16*				Schroeder (2008)
		<a href="#">96 h</a> mortality	5.86*	5.03*			7.76*		Schubauer-Berigan et al. (1993)
		<a href="#">96 h</a> mortality		4.75*					Keithly et al. (2004)
		<a href="#">96 h</a> mortality		5.19*					Chan (2013)
		<a href="#">14 d</a> mortality						7.42*	Goulet et al. (2015)
		<a href="#">96 h</a> mortality			6.83*				Jackson et al. (2000)

**Table S1 continued.** The binding constants (log  $K_{MBL}$ ) of zinc (Zn), nickel (Ni), cadmium (Cd), cobalt (Co), copper (Cu), uranium (UO<sub>2</sub>) and lead (Pb) at cell surface receptor sites (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log $K_{MBL}$							Reference
			Zn	Ni	Cd	Co	Cu	UO <sub>2</sub>	Pb	
Crustaceans	<i>Hyalella azteca</i>	<a href="#">96 h</a> mortality	5.83*							Poynton et al. (2019)
		<a href="#">28 d</a> growth				5.83*				Stubblefield et al. (2020)
		<a href="#">28 d/42 d</a> mortality							6.94*	Besser et al. (2005)
	<i>Macrobrachium rosenbergii</i>	<a href="#">48 h</a> mortality	5.58*							Satapornvanit et al. (2009)
	<i>Moina affinis</i>	<a href="#">48 h</a> mortality	6.18*		6.95*		8.02*			Zou and Bu (1994)
	<i>Moinodaphnia macleayi</i>	<a href="#">48 h</a> immobilisation / mortality						8.17*		Semaan et al. (2001)
		<a href="#">6 d</a> reproduction					7.72*			Trenfield et al. (2022)
		<a href="#">96 h</a> reproduction	6.26*							Trenfield et al. (2023)
	<i>Notodiaptomus iheringi</i>	<a href="#">48 h</a> mortality			7.43*		7.52*			Rocha et al. (2024)
	<i>Peracantha truncata</i>	<a href="#">48 h</a> reproduction		4.58*						Deleebeeck et al. (2007b)
	<i>Pleuroxus truncatus</i>	<a href="#">48 h</a> immobilisation					7.72*			Bossuyt and Janssen (2005)
	<i>Pseudosida ramosa</i>	<a href="#">48 h</a> mortality			7.24*					Freitas and Rocha (2011)
	<i>Pseudosida variabilis</i>	<a href="#">48 h</a> immobilisation					8.33*			Gutierrez et al. (2011)
	<i>Scapholebris microcephala</i>	<a href="#">48 h</a> immobilisation					8.23*			Bossuyt and Janssen (2005)
	<i>Scapholebris mucronata</i>	<a href="#">48 h</a> immobilisation					8.11*			Bossuyt and Janssen (2005)
	<i>Sinocephalus exspinosus</i>	<a href="#">48 h</a> immobilisation					7.42*			De Schamphalaere et al. (2007)
		<a href="#">48 h</a> immobilisation					8.15*			Bossuyt and Janssen (2005)
	<i>Sinocephalus serrulatus</i>	<a href="#">48 h</a> reproduction		4.99*						Deleebeeck et al. (2007b)
		<a href="#">48 h</a> mortality			6.94*					Spehar and Carlson (1984)
	<i>Sinocephalus vetulus</i>	<a href="#">48 h</a> immobilisation					7.89*			De Schamphalaere et al. (2007)
		<a href="#">48 h</a> reproduction		4.84*						Deleebeeck et al. (2007b)
		<a href="#">48 h</a> immobilisation					8.16*			Bossuyt and Janssen (2005)
		<a href="#">48 h</a> mortality			6.94*		7.57*			Mount and Norberg [1984]
Microalgae	<i>Chlorella</i> sp.	<a href="#">48 h</a> growth	5.80				7.69**			Wilde et al. (2006)
		<a href="#">48 h</a> growth					7.65*			Markich et al. (2005)
		<a href="#">48 h/72 h</a> growth	5.92*				7.82*			Franklin et al. (2002a)
		<a href="#">72 h</a> growth	5.83*				7.76*			Johnson et al. (2007)

**Table S1 continued.** The binding constants (log  $K_{MBL}$ ) of zinc (Zn), nickel (Ni), cadmium (Cd), cobalt (Co), copper (Cu), uranium (UO<sub>2</sub>) and lead (Pb) at cell surface receptor sites (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log $K_{MBL}$							Reference
			Zn	Ni	Cd	Co	Cu	UO <sub>2</sub>	Pb	
Microalgae	<i>Chlorella</i> sp.	<a href="#">72 h growth</a>					7.97*			Franklin et al. (2002b)
		<a href="#">72 h growth</a>					8.18*			Franklin et al. (2001)
		<a href="#">72 h growth</a>					8.10*			Angel et al. (2017)
		<a href="#">72 h growth</a>					7.95*			Trenfield et al. (2022)
		<a href="#">72 h growth</a>					7.49*			Macoustra et al. (2019)
		<a href="#">72 h growth</a>		5.12*			7.64*			McKnight et al. (2023)
		<a href="#">72 h growth</a>					7.93*	7.87*		Franklin et al. (2000)
		<a href="#">72 h growth</a>	5.77*							Price et al. (2023)
	<i>Raphidocelis subcapitata</i>	<a href="#">72 h growth</a>						7.77*		Hogan et al. (2005)
		<a href="#">72 h growth</a>						7.87*		Trenfield et al. (2011)
		<a href="#">72 h growth</a>						7.86*		Charles et al. (2002)
		<a href="#">72 h growth</a>		4.80*						Peters et al. (2018)
		<a href="#">72 h growth</a>	6.15*							Heijerick et al. (2002b)
		<a href="#">72 h growth</a>	6.09*							Franklin et al. (2007)
		<a href="#">72 h growth</a>	6.17*							Graff et al. (2003)
		<a href="#">72 h growth</a>	5.91*							Van Regenmortel et al. (2017)
		<a href="#">72 h growth</a>	6.08*		6.71*		8.02*			Alves et al. (2017)
		<a href="#">72 h growth</a>	6.33*		6.97*		7.65*			Franklin et al. (2001)
		<a href="#">96 h growth</a>	6.25*		6.49*		7.75*			Blaise et al. (1986)
		<a href="#">72 h growth</a>	5.99*							Stauber et al. (2023)
		<a href="#">72 h growth</a>			7.18*					Kallqvist (2009)
		<a href="#">96 h growth</a>			7.37*					Paquet et al. (2015)
		<a href="#">72 h growth</a>			7.64*				7.00*	Alho et al. (2019)
		<a href="#">72 h growth</a>					8.20*			Angel et al. (2017)
		<a href="#">72 h growth</a>		4.93*						Deleebeeck et al. (2009)
		<a href="#">96 h growth</a>		4.97*						He et al. (2023)
		<a href="#">96 h growth</a>		4.64*	6.61*					dos Reis et al. (2024a)

**Table S1 continued.** The binding constants (log  $K_{MBL}$ ) of zinc (Zn), nickel (Ni), cadmium (Cd), cobalt (Co), copper (Cu), uranium (UO<sub>2</sub>) and lead (Pb) at cell surface receptor sites (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log <i>K</i> <sub>MBL</sub>							Reference
			Zn	Ni	Cd	Co	Cu	UO <sub>2</sub>	Pb	
Microalgae	<i>Raphidocelis subcapitata</i>	<a href="#">96 h growth</a>		4.73*		5.65*				dos Reis et al. (2024b)
		<a href="#">96 h growth</a>	5.81*	4.42*			7.72*			Filova et al. (2021)
		<a href="#">72 h growth</a>		4.58*						Nys et al. (2016a)
		<a href="#">72 h growth</a>				5.68*				Stubblefield et al. (2020)
		<a href="#">72 h growth</a>					7.64*			Heijerick et al. (2005b)
		<a href="#">72 h growth</a>					7.76*			Pascual et al. (2022)
		<a href="#">72 h growth</a>					7.85*			Franklin et al. (2002b)
		<a href="#">72 h growth</a>						8.13*		Goulet et al. (2015)
		<a href="#">72 h growth</a>							6.93*	De Schamphalaere et al. (2014)
	<i>Chlamydomonas reinhardtii</i>	30 min uptake						7.70		Fortin et al. (2007)
		<a href="#">96 h growth</a>						7.68*		Lavoie et al. (2014)
	<i>Ankistrodesmus arcuatus</i>	<a href="#">72 h growth</a>		4.67*			8.05*			McKnight et al. (2023)
	<i>Euglena gracilis</i>	<a href="#">96 h growth</a>						7.67*		Trenfield et al. (2012)
Molluscs	<i>Alathyria profuga</i>	<a href="#">48 h mortality</a>	5.85**	4.80**	6.57**	5.93**	7.37**		6.41**	Markich (2017)
	<i>Amerianna cumingi</i>	<a href="#">96 h egg production</a>						8.07*		Hogan et al. (2010)
		<a href="#">96 h reproduction</a>					7.94*			Trenfield et al. (2022)
		<a href="#">6 d reproduction</a>	6.17*							Trenfield et al. (2023)
	<i>Corbicula fluminea</i>	<a href="#">5 h value closure</a>					7.70			Liao et al. (2007)
		<a href="#">5 h valve closure</a>						7.63*		Fournier et al. (2004)
	<i>Cucumerunio novaehollandiae</i>	<a href="#">48 h mortality</a>	6.04**	5.00**	6.77**	6.12**	7.66**		6.61**	Markich (2017)
	<i>Echyridella menziesii</i>	<a href="#">48 h mortality</a>	5.70*				8.06*			Clearwater et al. (2014)
	<i>Epioblasma rangiana</i>	<a href="#">48 h mortality</a>					7.93*			Gillis et al. (2008)
	<i>Epioblasma triquetra</i>	<a href="#">48 h mortality</a>					8.17*			Gillis et al. (2008)
	<i>Fluminicola</i> sp.	<a href="#">28 d mortality</a>					8.15*			Besser et al. (2016)
	<i>Fontigens aldrichi</i>	<a href="#">28 d mortality</a>					7.90*			Besser et al. (2016)
	<i>Hyridella australis</i>	<a href="#">48 h mortality</a>	6.03**	4.98**	6.75**	6.10**	7.62**		6.59**	Markich (2017)
	<i>Hyridella depressa</i>	<a href="#">48 h mortality</a>	5.95**	4.90**	6.68**	6.03**	7.51**		6.51**	Markich (2017)

**Table S1 continued.** The binding constants (log  $K_{MBL}$ ) of zinc (Zn), nickel (Ni), cadmium (Cd), cobalt (Co), copper (Cu), uranium (UO<sub>2</sub>) and lead (Pb) at cell surface receptor sites (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log $K_{MBL}$							Reference
			Zn	Ni	Cd	Co	Cu	UO <sub>2</sub>	Pb	
Molluscs	<i>Hyridella drapeta</i>	48 h mortality	5.94**	4.88**	6.61**	6.01**	7.49**		6.48**	Markich (2017)
	<i>Lampsilis abrupta</i>	48 h mortality					7.92*			Wang et al. (2007)
	<i>Lampsilis fasciola</i>	48 h mortality					8.19*			Wang et al. (2007)
		48 h mortality					7.76*			Gillis et al. (2008)
	<i>Lampsilis rafinesqueana</i>	48 h mortality	6.32*		7.37*				6.80*	Wang et al. (2010b)
		48 h mortality					7.75*			Wang et al. (2007)
	<i>Lampsilis siliquoidea</i>	48 h mortality	6.27*		7.47*				6.73*	Wang et al. (2010b)
		48 h mortality					7.69*			Wang et al. (2007)
		48 h mortality					7.52*			Gillis et al. (2008)
		28 d mortality					8.14*			Jorge et al. (2013)
	<i>Lymnaea stagnalis</i>	24 h uptake			6.60		8.18			Croteau and Luoma (2007)
		7 d reproduction			6.64*					Capela et al. (2024)
		28 d mortality					7.83*			Besser et al. (2016)
		96 h mortality					7.98*			Brix et al. (2011)
		96 h mortality					8.14*			Ng et al. (2011)
		40 d growth		4.58*						Schlekat et al. (2010)
		96 h mortality		5.21						Leonard and Wood (2013)
		3 h uptake		4.84*						Mattsson (2020)
		28 d growth				5.91*				Stubblefield et al. (2020)
	<i>Obovaria subrotunda</i>	48 h mortality					7.81*			Gillis et al. (2008)
	<i>Ortmanniana ligamentina</i>	48 h mortality					7.65*			Gillis et al. (2008)
		48 h mortality					7.60*			Wang et al. (2007)
	<i>Physa acuta</i>	96 h mortality					8.29*			Gao et al. (2017)
	<i>Physa gyrina</i>	28 d mortality					7.69*			Besser et al. (2016)
	<i>Planorbella pilsbryi</i>	72 h mortality					8.16*			Osborne et al. (2020)
	<i>Pomacea paludosa</i>	96 h mortality	5.81*							Hoang and Tong (2015)
	<i>Potamilus ohiensis</i>	48 h mortality					7.92*			Wang et al. (2007)

**Table S1 continued.** The binding constants (log  $K_{MBL}$ ) of zinc (Zn), nickel (Ni), cadmium (Cd), cobalt (Co), copper (Cu), uranium (UO<sub>2</sub>) and lead (Pb) at cell surface receptor sites (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log $K_{MBL}$						Reference
			Zn	Ni	Cd	Co	Cu	UO <sub>2</sub>	
Molluscs	<i>Potamopyrgus antipodarum</i>	<a href="#">28 d growth/reproduction</a>			7.32*				Ruppert et al. (2016)
	<i>Pyrgulopsis robusta</i>	<a href="#">28 d mortality</a>					8.02*		Besser et al. (2016)
	<i>Racesina luteola</i>	<a href="#">28 d growth</a>			6.46*				Das and Khangarot (2010)
		<a href="#">28 d growth</a>					8.22*		Das and Khangarot (2011)
	<i>Taylorconcha serpenticola</i>	<a href="#">28 d mortality</a>					8.09*		Besser et al. (2016)
	<i>Velesunio ambiguus</i>	<a href="#">48 h mortality</a>	5.84**	4.80**	6.56**	5.93**	7.37**		Markich (2017)
	<i>Velesunio angasi</i>	<a href="#">24 h mortality</a>	6.27*						Trenfield et al. (2023)
	<i>Velesunio</i> sp.	<a href="#">24 h mortality</a>					8.21*		Trenfield et al. (2022)
	<i>Venustaconcha ellipsiformis</i>	<a href="#">48 h mortality</a>					8.10*		Wang et al. (2007)
	<i>Paetulonio fabalis</i>	<a href="#">48 h mortality</a>					8.23*		Gillis et al. (2008)
	<i>Cambarunio iris</i>	<a href="#">28 d mortality</a>					8.24*		Wang et al. (2011b)
		<a href="#">48 h mortality</a>					7.69*		Wang et al. (2007)
		<a href="#">48 h mortality</a>					8.32*		Salerno et al. (2020)
Macrophytes	<i>Ceratophyllum demersum</i>	<a href="#">96 h growth</a>					7.19*		Gupta et al. (1981)
		<a href="#">7 d growth</a>							Markich et al. (2006)
	<i>Lemna aequinoctialis</i>	<a href="#">96 h growth</a>						7.65*	Markich (2013)
		<a href="#">96 h growth</a>						7.25*	Charles et al. (2006)
		<a href="#">96 h growth</a>						7.47*	Hogan et al. (2010)
		<a href="#">96 h growth</a>						7.58*	Pease et al. (2016)
		<a href="#">96 h growth</a>					7.73*		Trenfield et al. (2022)
	<i>Lemna minor</i>	<a href="#">7 d growth</a>						7.86*	Goulet et al. (2015)
		<a href="#">7 d growth</a>						7.63*	Horemans et al. (2016)
		<a href="#">7 d growth</a>				5.89*			Stubblefield et al. (2020)
		<a href="#">7 d growth</a>		4.52*					Schlekat et al. (2010)
		<a href="#">7 d growth</a>	6.22*	4.82*	7.07*	6.36*	7.42*		Naumann et al. (2007)
		<a href="#">7 d growth</a>		4.69*					Nys et al. (2016a)
		<a href="#">7 d growth</a>						7.19*	Antunes and Kreager (2014)
		<a href="#">7 d growth</a>							

**Table S1 continued.** The binding constants (log  $K_{MBL}$ ) of zinc (Zn), nickel (Ni), cadmium (Cd), cobalt (Co), copper (Cu), uranium (UO<sub>2</sub>) and lead (Pb) at cell surface receptor sites (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log $K_{MBL}$							Reference
			Zn	Ni	Cd	Co	Cu	UO <sub>2</sub>	Pb	
Cnidaria	<i>Hydra viridissima</i>	<a href="#">96 h growth</a>						7.43*		Trenfield et al. (2011)
		<a href="#">96 h growth</a>						7.19*		Markich and Camilleri (1997)
		<a href="#">96 h growth</a>					7.37*	7.15*		Reithmuller et al. (2000)
		<a href="#">96 h growth</a>					7.98*			Trenfield et al. (2022)
		<a href="#">96 h growth</a>	6.14*							Trenfield et al. (2023)
		<a href="#">96 h growth</a>		5.15*						Peters et al. (2018)
	<i>Hydra circumcincta</i>	<a href="#">96 h growth</a>			7.12*					Clifford (2009)
Rotifers	<i>Anuraeopsis fissa</i>	<a href="#">24 h mortality</a>	5.75*							Sarma et al. (2007)
	<i>Branchionus calyciflorus</i>	<a href="#">48 h reproduction</a>		4.60*						Nys et al. (2016a)
		<a href="#">48 h reproduction</a>							7.35*	Grosell et al. (2006b)
	<i>Branchionus rubens</i>	<a href="#">24 h mortality</a>	5.50*							Sarma et al. (2007)
	<i>Euchlanis dilatata</i>	<a href="#">24 h mortality</a> / <a href="#">5 d reproduction</a>	6.12*				7.94*			Hernandez-Flores et al. (2020)
	<i>Lecane inermis</i>	<a href="#">24 h mortality</a>	6.27*				7.99*			Klimek et al. (2013)
	<i>Lecane quadridentate</i>	<a href="#">48 h mortality</a>	6.18*							Guzman et al. (2010)
		<a href="#">5 d growth</a>							7.48*	Hernandez-Flores and Rico-Martínez (2006)
Annelids	<i>Aeolosoma</i> sp.	<a href="#">14 d growth</a>				5.36*				Stubblefield et al. (2020)
Bacteria	<i>Erwinnia</i> sp.	<a href="#">4 h growth</a>					7.66*			Markich et al. (2005)
Insects	<i>Chironomus tentans</i>	<a href="#">96 h immobilisation</a>					7.59*			Gauss et al. (1985)
	<i>Tanytarsus dissimilis</i>	<a href="#">10 d mortality</a>	6.09*		7.29*		7.53*		6.58*	Anderson et al. (1980)
Average			5.94	4.83	7.06	5.73	7.85	7.64	6.86	
95 % Confidence Interval			0.05	0.06	0.09	0.16	0.04	0.11	0.10	
Number of values			86	58	68	20	177	27	38	

<sup>a</sup> Reported value at pH 6.23 and combined with data from Markich and Camilleri (1997) [pH 6], Cheng et al. (2010) [pH 6.7] and Pease et al. (2021) [pH 6.6].

\* Calculated from data provided in the cited study.

\*\* Binding constant quoted in cited study, but recalculated in the present study.

No asterisk(s): reported in cited study.



**Table S2.** The binding constants (log  $K_{\text{MBL}}$ ) of the ameliorative cations (calcium (Ca), magnesium (Mg), sodium (Na) and protons ( $\text{H}^+$ )) at cell surface receptors (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log $K_{\text{MBL}}$				Reference
			Ca	Mg	Na	H	
Fish	<i>Ctenopharyngodon idella</i>	96 h mortality (Zn)		2.77*			Chen et al. (2016)
	<i>Mogurnda mogurnda</i>	96 h mortality (U)				5.83*	Trenfield et al. (2011) <sup>a</sup>
	<i>Oncorhynchus mykiss</i>	96 h mortality (Zn)	3.34*	2.99*	2.43*	5.94*	De Schamphalaere and Janssen (2004a)
		3 h uptake (Cd)	3.69				Niyogi et al. (2004)
		30 d mortality (Cu)	3.71*	2.97*		5.50*	Crémazy et al. (2017)
		3 h uptake (Cd)	3.59*				Niyogi et al. (2008)
		96 h mortality (Zn)	3.49*				De Schamphalaere and Janssen (2004b)
		96 h mortality (Cu)	3.32*				Naddy et al. (2002)
		96 h mortality (Zn)				5.95*	Bradley and Sprague (1985)
		17 d mortality (Ni)	3.11*			5.68*	Deleebeeck et al. (2007a)
		96 h mortality (Zn, Cu)				5.89*	Cusimano et al. (1986)
	<i>Oreochromis mossambicus</i>	96 h mortality (Cd)	3.54*				Chang et al. (1998)
	<i>Perca flavescens</i>	3 h uptake (Cd)	3.71				Niyogi et al. (2004)
	<i>Pimephales promelas</i>	96 h mortality (Pb)	3.34*			5.76*	Mager et al. (2011)
		2 to 3 h uptake (Cu)	3.40			5.40	Playle et al. (1993)
		96 h mortality (Cu)	3.02*				Meyer et al. (1999)
		96 h mortality (Cu)	3.12*				Di Toro et al. (2001)
Crustaceans	<i>Ceriodaphnia dubia</i>	7 d reproduction (Cu)	3.27*	2.60*	2.26*		Schwartz and Vigneault (2007)
		7 d reproduction (Pb)	3.05*				Nys et al. (2014)
		48 h immobilisation (Cu)				5.62*	Hyne et al. (2005)
		48 h immobilisation (Cu)			2.43*		De Schamphalaere et al. (2007)
	<i>Ceriodaphnia pulchella</i>	48 h immobilisation (Cu)			2.42*		De Schamphalaere et al. (2007)
	<i>Ceriodaphnia reticulata</i>	48 h immobilisation (Cu)			2.14*		De Schamphalaere et al. (2007)

**Table S2 continued.** The binding constants (log  $K_{\text{MBL}}$ ) of the ameliorative cations (calcium (Ca), magnesium (Mg), sodium (Na) and protons (H<sup>+</sup>)) at cell surface receptors (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	log $K_{\text{MBL}}$				Reference
			Ca	Mg	Na	H	
Crustaceans	<i>Daphnia magna</i>	21 d reproduction (Zn)	3.22	2.69	1.90	5.77	Heijerick et al. (2005a)
		48 h immobilisation (Cu)	3.21**	2.92**	2.28**	5.70**	De Schamphalaere and Janssen (2002)
		21 d reproduction (Zn)	3.22	2.69	1.90	5.77	Van Regenmortel et al. (2017)
		48 h immobilisation (Zn)	3.34	3.12	2.37		Heijerick et al. (2002a)
		48 h immobilisation (Ni)	3.15**	2.61**			Deleebeeck et al. (2008)
		48 h immobilisation		2.80*			Okamoto et al. (2015)
		48 h immobilisation (Cu)			2.50*		De Schamphalaere et al. (2007)
	<i>Daphnia galeata</i>	48 h immobilisation (Cu)			2.24*		De Schamphalaere et al. (2007)
	<i>Daphnia longispina</i>	48 h immobilisation (Cu)			2.06*		De Schamphalaere et al. (2007)
	<i>Daphnia pulex</i>	48 h immobilisation (Cd)	3.52**	2.96**	2.05*	5.74**	Clifford and McGeer (2010)
		48 h immobilisation (Ni)	3.54*	2.93*			Kozlova et al. (2009)
	<i>Hyalella azteca</i>	28 d mortality (Ni, Cd)	3.29*	2.91*		5.75*	Schroeder (2008)
		96 h mortality (Ni)	3.46*				Chan (2013)
		96 h mortality (Cd)	3.41*	2.73*			Jackson et al. (2000)
	<i>Sinocephalus exspinosus</i>	48 h immobilisation (Cu)			2.04*		De Schamphalaere et al. (2007)
	<i>Sinocephalus vetulus</i>	48 h immobilisation (Cu)			2.43*		De Schamphalaere et al. (2007)
	10 species of cladocerans	48 h immobilisation (Ni)	3.20	2.60			Deleebeeck et al. (2007b)
Microalgae	<i>Chlamydomonas reinhardtii</i>	1 h uptake (Sm)	3.72*	2.77*			Tan et al. (2017)
		30 min uptake (U)				6.15	Fortin et al. (2007)
	<i>Chlorella</i> sp.	48 h growth (Zn)				5.79*	Wilde et al. (2006)
		72 h growth (U)				5.73*	Franklin et al. (2000)
	<i>Raphidocelis subcapitata</i>	72 h growth (Zn)	3.05**	2.99**	2.17**	5.82**	Heijerick et al. (2002b)
		72 h growth (Cu)				5.56*	Pascual et al. (2022)
		72 h growth (Ni)		2.76**		5.67**	Deleebeeck et al. (2009)
Molluscs	<i>Corbicula fluminea</i>	5 h valve closure (Cu)	3.53				Liao et al. (2007)
	<i>Velesunio angasi</i>	24 h mortality		2.66*			Kleinhenz et al. (2019)

**Table S2 continued.** The binding constants ( $\log K_{\text{MBL}}$ ) of the ameliorative cations (calcium (Ca), magnesium (Mg), sodium (Na) and protons ( $\text{H}^+$ )) at cell surface receptors (biotic ligands) in freshwater organisms (including the toxic endpoint for each study).

Taxa	Species	End-point	$\log K_{\text{MBL}}$				Reference
			Ca	Mg	Na	H	
Macrophytes	<i>Ceratophyllum demersum</i>	7 d growth (U)	3.17*	2.73*			Markich (2013)
Cnidaria	<i>Hydra circumcincta</i>	96 h growth (Cd)	3.65*	2.75*			Clifford (2009)
Average			3.37	2.81	2.23	5.75	
95 % Confidence Interval			0.08	0.07	0.10	0.08	
Number of values			31	21	16	20	

<sup>a</sup> Reported value at pH 6.23 and combined with data from Markich and Camilleri (1997) [pH 6], Cheng et al. (2010) [pH 6.7] and Pease et al. (2021) [pH 6.6].

\* Calculated from data provided in the cited study.

\*\* Binding constant quoted in cited study, but recalculated in the present study.

No asterisk(s): reported in cited study.

**Table S3.** Comparison of metal binding constants (log  $K_{\text{MBL}}$ ) at cell surface receptors (biotic ligands) from uptake and toxicity experiments.

Metal	Species	Uptake			Toxicity	Overall Average <sup>a</sup>
		Values	Average	Metal Average	Average	
Zn	<i>O. mykiss</i>	5.60, 5.98	5.79 ± 0.19 <sup>b</sup>	5.79 ± 0.19 <sup>b</sup>	5.97 ± 0.09	5.97 ± 0.07
Ni	<i>O. mykiss</i>	4.71	4.71	4.71 ± 0.16	4.68 ± 0.21 <sup>b</sup>	4.84 ± 0.06
	<i>D. pulex</i>	4.59 <sup>c</sup>	4.59		4.79 ± 0.17 <sup>b,d</sup>	
	<i>G. pulex</i>	4.70	4.70		ND	
	<i>L. stagnalis</i>	4.84	4.84		4.90 ± 0.31 <sup>b</sup>	
Cd	<i>O. mykiss</i>	7.34, 7.33, 7.37, 7.52, 7.35	7.38 ± 0.10	7.24 ± 0.28	7.52 ± 0.08	7.01 ± 0.11
	<i>P. flavescens</i>	7.20	7.20		ND	
	<i>L. stagnalis</i>	6.60	6.60		6.64	
Co	<i>O. mykiss</i>	5.10	5.10	5.10	5.34 ± 0.07 <sup>b</sup>	5.79 ± 0.16
Cu	<i>O. mykiss</i>	8.02	8.02	7.87 ± 0.47 <sup>b</sup>	7.92 ± 0.14	7.85 ± 0.05
	<i>P. pimephales</i>	7.40	7.40		7.61 ± 0.07	
	<i>L. stagnalis</i>	8.18	8.18		7.98 ± 0.39	
U	<i>C. reinhardtii</i>	7.70	7.70	7.70	7.68	7.67 ± 0.15
Pb	<i>O. mykiss</i>	7.05	7.05	7.05	7.15 ± 0.23 <sup>b</sup>	6.84 ± 0.14

<sup>a</sup> Overall values as given in Table 1 for all species.

<sup>b</sup> Uncertainty quoted to cover the range in values (only two or three (Cu uptake) values available).

<sup>c</sup> Average of two uptake values quoted by Leonard and Wood (2013).

<sup>d</sup> Average includes value from Leonard and Wood (2013) that is the average from their toxicity tests.

**Table S4.** Values derived for  $(f_c)_M^{50\%}$  (for values < 0.5).

Taxa	Species	$(f_c)_M^{50\%}$	Metal	Derived	Comments	Number
Fish	<i>C. idellus</i>	0.435	Zn	N	Also derived in paper was value of 0.209 for 28 d exposures that leads to same log $K_{ZnBL}$	1
	<i>O. mykiss</i>	0.216	Cd	Y	Derived from difference between uptake and toxicity log $K_{CdBL}$ values	9
	<i>O. mykiss</i>	0.246	Zn	N		1
	<i>O. mykiss</i>	0.00676	Cd	Y	Value quoted in paper was 0.0128 (range: 0 to 0.0634)	1
	<i>O. mykiss</i>	0.221	Ni	Y	Value also used for Cu	1
	<i>P. promelas</i>	0.499	Ni	Y		1
	<i>S. trutta</i>	0.216	Cd	Y	Value used as derived for <i>O. mykiss</i> as similar species	1
	<i>S. confluentus</i>	0.216	Cd	Y	Value used as derived for <i>O. mykiss</i> as similar species	1
Crustaceans	<i>B. coregoni</i>	0.240	Ni	Y		1
	<i>B. longirostris</i>	0.200	Cu	Y		1
	<i>C. lilljeborgi</i>	0.240	Ni	Y	Value used as derived for <i>B. coregoni</i> from same study	1
	<i>C. dubia</i>	0.0521	Ni	Y	Values also used for Pb, U, Cu, Zn and Co	17
	<i>C. pulchella</i>	0.00199	Cu	Y		1
	<i>C. pulchella</i>	0.240	Ni	Y	Value used as derived for <i>B. coregoni</i> from same study	1
	<i>D. galeata</i>	0.201	Cu	Y		1
	<i>D. longispina</i>	0.166	Cu	Y		1
	<i>D. magna</i>	0.45	Zn	Y	Same value also derived for Ni and Cu – values range from 0.451 to 0.454	4
		0.117	Zn	N	Also used for Ni in one study	2
		0.240	Cu	Y	Also used for Pb in one study	4
		0.0279	Ni	Y		1
		0.417	Zn	N		1
	<i>D. pulex</i>	0.411	Ni	Y	Also used for Cu in one study	2
	<i>D. similis</i>	0.240	Pb	Y	Value used as derived for <i>D. magna</i> as similar species	1
	<i>H. azteca</i>	0.184	Ni	Y	Also used for Cd in same study as well as another study	3
	<i>S. vetulus</i>	0.240	Cu	Y	Value used as derived for <i>B. coregoni</i> from same study	1
Microalgae	<i>Chlorella</i> sp.	0.120	Cu	Y	Also used in another study for Cu and Ni. Algae from same location in both studies	3
	<i>M. arcuatum</i>	0.120	Cu	Y	Also used for Ni in same study. Same value as derived for <i>Chlorella</i> sp.	2
	<i>R. subcapitata</i>	0.114	Ni	Y	Also used for U in one study	6
		0.0177	Ni	Y		1

**Table S4 continued.** Values derived for  $(f_c)_M^{50\%}$  (for values < 0.5).

Taxa	Species	$(f_c)_M^{50\%}$	Metal	Derived	Comments	Number
Molluscs	<i>A. profuga</i>	0.0720	Ni	Y		1
	<i>C. novaehollandiae</i>	0.0720	Ni	Y	Value used as derived for <i>A. profuga</i> from same study	1
	<i>H. australis</i>	0.0720	Ni	Y	Value used as derived for <i>A. profuga</i> from same study	1
	<i>H. depressa</i>	0.0720	Ni	Y	Value used as derived for <i>A. profuga</i> from same study	1
	<i>H. drapeta</i>	0.0720	Ni	Y	Value used as derived for <i>A. profuga</i> from same study	1
	<i>L. stagnalis</i>	0.0225	Ni	Y		1
	<i>V. ambiguus</i>	0.0720	Ni	Y	Value used as derived for <i>A. profuga</i> from same study	1
Macrophytes	<i>C. demersum</i>	0.470	U	Y		1
	<i>L. minor</i>	0.0349	Ni	Y		1
		0.0225	Ni	Y		1
Rotifers	<i>B. calyciflorus</i>	0.146	Ni	Y		1
Insects	<i>T. dissimilis</i>	0.258	Zn	Y	Also used for Cd, Cu and Pb from the same study	4

Derived: “Y” indicates the value has been derived in the present study, whereas “N” indicates the value has been quoted as that reported.

Number: the number of times that the  $(f_c)_M^{50\%}$  value has been applied in the present study.

The total number of  $(f_c)_M^{50\%}$  values used is 88, which represents 16 % of the total number of log  $K_{MBL}$  values derived. A  $(f_c)_M^{50\%}$  value has been used at least on one occasion for all seven metals; a value has been used for Ni for the largest number of species.

**Table S5.** Coefficients of determination ( $r^2$  values—above the diagonal) and slopes (below the diagonal) for linear regressions of  $\log K_{\text{MBL}}$  values among freshwater organisms (see Table S1 for full species names).

Species	<i>O. mykiss</i>	<i>P. promelas</i>	<i>C. dubia</i>	<i>D. magna</i>	<i>H. azteca</i>	<i>R. subcapitata</i>	<i>L. minor</i>
<i>O. mykiss</i>		0.96 ***	0.97 ***	0.88 **	0.95 ***	0.90 **	0.86 **
<i>P. promelas</i>	$1.1 \pm 0.1$		0.95 ***	0.93 ***	0.99 ***	0.95 ***	0.93 ***
<i>C. dubia</i>	$1.2 \pm 0.1$	$1.0 \pm 0.1$		0.92 ***	0.97 ***	0.91 ***	0.82 **
<i>D. magna</i>	$1.0 \pm 0.2$	$0.9 \pm 0.1$	$0.8 \pm 0.1$		0.94 ***	0.98 ***	0.87 **
<i>H. azteca</i>	$1.2 \pm 0.1$	$1.09 \pm 0.05$	$1.1 \pm 0.1$	$1.2 \pm 0.1$		0.95 ***	0.90 **
<i>R. subcapitata</i>	$1.0 \pm 0.1$	$0.9 \pm 0.1$	$0.8 \pm 0.1$	$1.0 \pm 0.1$	$0.8 \pm 0.1$		0.94 ***
<i>L. minor</i>	$1.1 \pm 0.2$	$1.0 \pm 0.1$	$0.9 \pm 0.2$	$1.1 \pm 0.2$	$0.9 \pm 0.1$	$1.1 \pm 0.1$	

\*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$ .