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TISSUE CONCENTRATIONS AS THE DOSE METRIC TO ASSESS POTENTIAL TOXIC EFFECTS OF METALS IN FIELD-COLLECTED FISH: COPPER AND CADMIUM

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Abstract: The present study examined the available literature linking whole-body tissue concentrations with toxic effects in fish species for copper and cadmium. The variability in effect concentration for both copper and cadmium among species occurred within an order of magnitude for all responses, whereas the range for lethal toxicity based on water exposure spanned approximately 4 to 5 orders of magnitude. Fish tissue concentrations causing adverse effects were just above background concentrations, occurring between 1 μg/g and 10 μg/g for copper and 0.1 μg/g to 4 μg/g for cadmium. The results also show that salmonids are especially sensitive to cadmium, which appears to be a function of chemical potency. No studies were found that indicated adverse effects without increases in whole-body concentration of these metals. This narrow range for dose-response implies that a toxicological spillover point occurs when the detoxification capacity of various tissues within the animal are exceeded, and this likely occurs at a similar whole-body concentration for all naïvely exposed fish species. Elevated whole-body concentrations in fish from the field may be indicative of possible acclimation to metals that may or may not result in effects for target species. Acclimation concentrations may be useful in that they signal excessive metal concentrations in water, sediment, or prey species for a given site and indicate likely toxic effects for species unable to acclimate to excess metal exposure. Using tissue residues as the dose metric for these metals provides another line of evidence for assessing impaired ecosystems and greater confidence that hazard concentrations are protective for all fish species. *Environ Toxicol Chem* 2015;34:1309–1319. Published 2015 SETAC. This article is a US government work and, as such, is in the public domain in the United States of America.

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INTRODUCTION

Contaminant toxicity in the aquatic environmental is a function of chemical bioavailability, bioaccumulation, and potency. These processes are highly variable for metals, making toxicity assessment for this group of potential toxicants quite challenging. Bioavailability is influenced by physical–chemical parameters such as pH, organic carbon, redox state, and alkalinity. Bioaccumulation is governed by the uptake and elimination kinetics exhibited by each species, and these rates can be influenced by physical–chemical factors such as temperature, pH, hardness, and biological factors including animal health, life stage, organism weight, and others [1]. Toxic potency for a given chemical is another factor that varies among species and is also controlled by life stage, taxonomic position, acclimation, and several internal factors such as chemical competition for the receptor (e.g., antagonism or synergism) [1].

Toxicity metrics based on external exposure concentrations (e.g., water, sediment/soil, or diet) are a direct result of bioavailability, bioaccumulation, and toxic potency and are influenced by the variability each one exhibits. When toxicity is determined in terms of internal tissue concentrations, external contaminant bioavailability is not a relevant factor and variability in bioaccumulation among species for a given contaminant is far less important, especially for fish. This then allows the focus to center on potency as a function of the acquired tissue concentration, which often is similar among species. Thus, the tissue residue approach for toxicity assessment, or tissue residue-effects assessment (TRA), considers potency without the influence of external contaminant bioavailability and differential bioaccumulation among species [1]. TRA has been examined for a number of toxicants, and in many cases, the species-response curve is relatively steep and characterized by low variability among species [1]. This is the case for chlorophenols, organotins, methylmercury, organolead, and all organic compounds considered under the nonspecific mode of action [2,3].

For the TRA to be viable for a given toxicant, a correlation must exist between the whole-body concentration and the concentration at the receptor responsible for the toxic action [1]. For metals, this relationship frequently does not hold, which precludes a reliable way to associate the metabolically available metal causing toxicity with the total amount in whole body or individual organs [4]. This is very well studied for invertebrates but less so for fish when tissue residues are considered as the dose metric.

Fish can acclimate to elevated metal concentrations in the surrounding environment by reducing uptake, increasing elimination, or internally detoxifying excess metal. Elevated concentrations can occur in various organs without causing toxicity when metals are sequestered in tissue and rendered biologically inactive. Enhanced tolerance to elevated tissue concentrations can result from activation of sequestering ligands (e.g., metallothionein [MT]) [5], NaOH resistant granules [6], or complexation with other inorganic chemicals.

Whole-body tissue residue assessment for metals is a simplistic approach compared with others, such as the biotic ligand model (BLM) [7], the threshold model proposed by Campbell and Hare [8], a physiologically based pharmacokinetic model [9], and the biodynamic model of Luoma and

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Rainbow [10] to assess bioaccumulation. Far fewer environmental and organismal parameters are needed to conduct an assessment using the TRA and predict toxicity with whole-body tissue residues. Utilizing a combination of approaches such as those mentioned above in conjunction with the TRA may provide multiple lines of evidence to determine when organisms are impaired by exposure to metals.

It is important to note that any variability in toxicity metrics resulting from differential external conditions, kinetics, or acclimation will be far greater when those metrics are based on ambient exposure concentrations compared with the same metrics determined with tissue residues. Tissue-based toxicity metrics will always be less variable than those based on external concentrations because it is a better representation for toxicity at the receptor and less influenced by external factors [1]. Previous studies recommended against the use of tissue residues as the dose metric for elements because of the high variability observed among species [4], which is generally the case when all taxa are considered together. The present study focused only on fish species to demonstrate that variability for copper (Cu) and cadmium (Cd) toxicity metrics based on tissue residues would be far less when limited by taxa and that observed for external exposure.

METHODS

All available studies in which whole-body tissue concentrations for Cu and Cd were measured in fish and linked to a toxic effect were considered, regardless of the degree of response and metric. Sufficient data were available only for mortality, growth impairment, and development for these 2 metals. Lowest-observed-effect residues (LOERs) as well as lethal residues for all percentages (LRp) that were significantly different from the control response were included. These metrics are collectively known as critical body residues (CBRs) and are used to characterize adverse biologic responses [11]. The lowest reported value was always selected as the CBR. Any concentrations within a study causing no adverse responses was termed no-observed-effect residue (NOER) and was considered statistically invalid [12] and not relevant. Also, a common metric, such as the 50% lethal residue (LR50) or 20% effective residue (ER20), was not calculated because many of the studies did not contain enough information to perform regression analysis. Factors included few test concentrations (e.g., only 2 or 3), low but significant percentile responses, and inadequate matching of external and internal concentrations. Studies that reported no effects were excluded because of high uncertainty regarding the results. Most of these were bioaccumulation studies and were not designed to assess toxicity, even though they included statements highlighting the lack of mortality or sublethal effects.

Tissue concentrations were used only when determined for the same time period used to characterize the response metric. Toxicity results based on egg concentrations were not included; however, studies that exposed eggs to Cu or Cd and evaluated toxicity for later life stages were included if tissue concentrations were determined concurrent with the response. Studies that used injection as a means for dosing also were excluded, as were those that reported tissue concentrations as ash weight because of difficulty of converting to dry or wet weight values. Field studies were not considered because of the possibility that toxic responses resulted from multiple contaminants. Studies that examined only one dose were not considered, except for Vergauwen et al. [13] who tested Cd under 4 different temperatures for 28 d, one of which resulted in an LR50 value. A large number of studies were examined, and several were eliminated from consideration because they failed one or more of the above criteria.

The wet to dry weight ratio for early-life stage (ELS) rainbow trout was obtained from Marr et al. [14] and determined to be 6.4, which was used to convert ELS salmonid concentrations from dry to wet weight when necessary. A factor of 10 was used for all other studies with larval fish, which was obtained from Fontagne et al. [15], Flik et al. [16], and Wuenschel et al. [17]. All 3 studies agreed that larval fish were approximately 90% moisture. For all juvenile and adult fish, a conversion factor of 4.5 was used [18]. All tissue concentrations in the present study expressed as wet weight.

The TRA considers only that portion of a potential toxicant that is assimilated by the animal [1]. Whole-body tissue concentrations can be influenced by the lack of removal of stomach contents, especially for metals. Some studies that exposed fish to dietary metals did not report if gut contents were removed; hence, these whole-body concentrations could be biased high compared with those experiments where water was the exposure route. Another important aspect concerns internal bioavailability and that proportion of the chemical that is biologically effective [11], which for metals is termed the "biologically active metal" [4]. This analysis focused on whole-body concentrations as the dose surrogate because few data were available that considered toxicity in terms of internal bioavailability.

Copper median lethal concentrations (LC50s) were obtained from the US Environmental Protection Agency (USEPA) [19], and multiple values for a species were averaged and presented as species mean acute values for comparison against CBRs. Many of these individual values are comprised of data from many experiments—sometimes dozens. All freshwater values for Cu were BLM normalized, meaning the raw LC50 was normalized to constant water chemistry consisting of the following values: pH, 7.5; temperature, 20 °C; dissolved organic carbon (DOC), 0.5 mg/L; alkalinity, 65 mg/L; and an approximate water hardness, 25 mg/L (as $CaCO_3$) [19]. Marine LC50s were not normalized and are actual values. Biotic ligand model normalization reduces the variability among species and tests because the aforementioned variables have a large effect on Cu bioavailability [20]. The LC50 values for 41 species were available for Cu—23 for freshwater and 18 for marine species, with only 1 duplicate (coho salmon, *Oncorhynchus kisutch,* for fresh and marine water).

Cadmium LC50 values were obtained from the USEPA [21] and were hardness corrected for freshwater species (to 50 mg/L as $CaCO_3$) and uncorrected for marine species values. For Cd, 97 values for 31 species—8 marine and 21 freshwater species, including 2 (coho and striped bass, *Morone saxatilis*)—tested in both media were plotted for comparison with tissue-residue values. In this case, several values for many of the species are shown to highlight the variability expected under different environmental conditions.

In addition to concentrations of these metals in control fish from the studies examined here, data from the US Geological Survey (USGS) National Water Quality Assessment (NAWQA) database [22] also were examined. The USGS data were compiled for these elements from a number of fish species collected from reference areas at several US locations between 1992 and 1999 (23 values for 9 species: brook trout, catfish, dace, and several cottids). Values were converted from dry to wet weight.

Table 1. Lethal and sublethal copper responses[a]

Species	Common name[b]	CBR (μg/g)	SD[c]	N[c]	CBR time	Source[d]	Media[e]	LCp or LOEC (ng/mL)[f]	Time	Ref
Growth										
Oreochromis niloticus	Nile tilapia (J)	3.7			14 d	A	Fw	150	14 d	[61]
Oncorhynchus mykiss	Rainbow trout (L,J)	1.2	0.3	4	14–56 d	A	Fw	4.5–55	14–56 d	[14,62,63]
Paralichthys olivaceur	Olive flounder (J)	3.5			84 d	D	Sw	304 μg/g	84 d	[37]
Pelteobagrus fulvidraco	Yellow catfish (J)	8.4			49 d	D	Fw	12.2 μg/g	49 d	[64]
Salmo salar	Atlantic salmon (J)	3.5			84 d	D	Fw	700 μg/g	84 d	[42]
Mortality										
Cyprinus carpio	Common carp (L)	2.8	3.1	2	≈ 170 h	A	Fw	19–50	≈ 170 h	[16[g],65]
Mugil cephalus	Striped mullet (L)	2.5			168 h	A	Fw	1800	168 h	[66]
O. mykiss	Rainbow trout (L,J,A)	2.1	0.8	6	8 h–78 d	A & D	Fw	35–570 A; 830 μg/g D	2–78 d	[52,62,63,67,68,69]
P. olivaceur	Olive flounder (J)	3.5			84 d	D	Sw	304 μg/g	84 d	[37]
Tilapia zilli	Red belly tilapia (L)	4.5			24 h	A	Fw	10 000	24 h	[66]
O. gorbuscha	Pink salmon (L)	6.8			96 h	A	Fw	55	96 h	[70]

[a]Critical body residue (CBR) values are lethal residue values for all percentages (LRp) and lowest-observed-effect residue (LOER) values. The proportion responding (p) is the 50th percentile (± 10%; e.g., LR40 to LR60) unless noted. Whole-body concentrations are μg/g wet weight. The LOER or lowest-observed-effect concentration (LOEC) is the value considered by the author to be statistically significant from the control.
[b]The life stage is shown next to common name (larval [L], juvenile [J], or adult [A]).
[c]For multiple studies, mean, standard deviation (SD), and number of studies (N) shown.
[d]Source was dietary (D) or aqueous (A) exposure.
[e]Media was either seawater (Sw) or freshwater (Fw).
[f]The LCp and LOEC are expressed as ng/mL, except for dietary exposures, which are μg/g dry weight (dw).
[g]20% mortality.
R = residue (tissue concentration); C = water or dietary concentration .

Tissue residue toxicity data were plotted and species sensitivity distributions (SSDs) were calculated with SSD Master [23]. In general, a SSD is based on a small subset of all related species and may be viewed as representing the variation expected for a larger group of species at the ecosystem or community level [24]. The SSD is characterized by an empirical or statistical distribution and commonly used to calculate a hazard concentration representing a low percentile (e.g., 5th percentile of species toxicity values) that can be used for setting environmental quality standards. One of the 5 models examined (Normal, Logistic, Gompertz, Weibull, and Fisher-Tippett) was selected based on the mean square error, distribution of residuals, and how well the data fit the model. A hazard concentration for the 5th percentile (HC5) was determined for each SSD, along with the 95% confidence limits for that value. The Cd CBRs represent a classic SSD; however, the SSDs for Cu CBRs and Cd LOERs were generated from a mix of endpoints and 2 species were represented twice.

For the data presented here, standard deviations (SDs) were reported to show the range in the data and the standard error of the mean (SE, a statistic of the mean) was reported when comparisons of means were intended.

RESULTS

Copper

The whole-body tissue concentrations for Cu causing adverse effects in fish exhibited low variability among species (Table 1 and Figure 1). The 11 values for 9 species from 6 families are shown in Table 1, indicating fairly broad coverage for this taxon. All values for growth and mortality were plotted together because they overlapped as a result of low overall variability. Because the toxicity metrics were not consistent (e.g., LRp or LOER at various percentiles), additional variability was expected. Life stage of the tested species is also shown in Table 1, and fish weight ranged from a few milligrams to 140 g. There is no obvious relationship between life stage and the CBR. The SSD for all Cu data was best represented by a logistic model. The HC5 and 95% confidence interval (CI) was 1.6 (1.3–1.9) μg/g, which is equivalent to 25.2 (20.5–30.0) nmol/g wet weight. All whole-body values for fish above this metric should be considered capable of causing adverse effects.

Cadmium

Cadmium data were analyzed separately by endpoint (lethal and sublethal) and whole-body tissue concentrations for Cd resulting in mortality are based on data for 14 species from 9 families (Figure 2, Table 2). Life stage of the tested species is

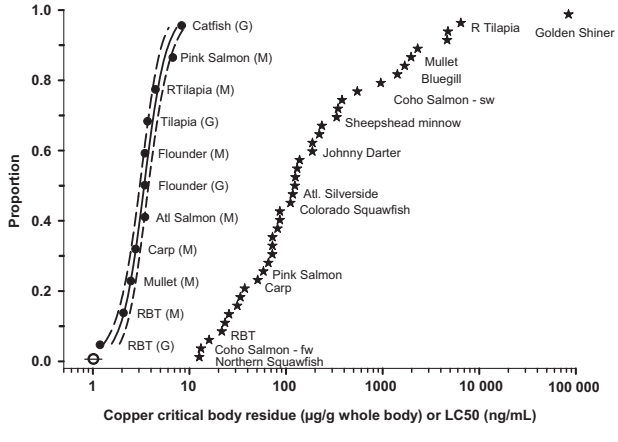

Figure 1. Copper toxicity metrics. Plot of critical body residue (CBR) values, which include lethal residues and effect residues for various percentiles, and lowest-observed-effect residues. Solid circles are whole-body copper concentrations associated with mortality (M) or growth impairment (G). Stars are aqueous median lethal concentration (LC50) values. All values are means for each species shown. The open circle shows the mean (SE) whole-body background concentration (0.99 μg/g [0.18 μg/g]). Species sensitivity distribution (SSD) regression with upper and lower 95% confidence interval (CI) is plotted for CBR values. Hazard concentration for the 5th percentile and 95% CI for the combined CBR value = 1.6 μg/g (1.3–1.9 μg/g). All tissue concentrations are expressed as wet weight. See text for additional details and tables for species names.

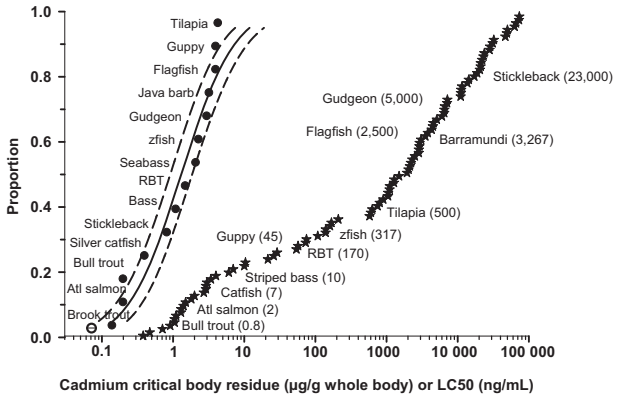

Figure 2. Cadmium toxicity metrics. Plot of whole-body lethal concentrations. Solid circles are species mean whole-body cadmium lethal residues for various percentiles or lowest-observed-effect-residues for mortality. Stars are aqueous median lethal concentration (LC50) values for 31 species under various environmental conditions ($n = 97$ values). Select LC50 values shown in parentheses. The open circle shows the mean (SE) whole-body background concentration (0.06 μg/g [0.02 μg/g]). Species sensitivity distribution (SSD) regression with upper and lower 95% CI is plotted for critical body residues (CBRs). Hazard concentration for the 5th percentile and 95% confidence interval for lethal residue values = 0.14 μg/g (0.09–0.23 μg/g). All tissue concentrations are expressed as wet weight. See text for additional details and tables for species names.

also shown in Table 2 and Table 3, and fish weight ranged from a few mg to approximately 50 g. There is no obvious relationship between life stage and the Cd CBR. The SSD for the Cd mortality data was best represented by a Normal model. The HC5 and 95% CI was 0.14 μg/g (0.09–0.23 μg/g), which is equivalent to 1.2 nmol/g (0.80–2.0 nmol/g) wet weight. The sublethal data were best represented by a Fisher-Tippett model and consisted of 6 species from 3 families of fish (Table 3). The HC5 and 95% CI was 0.07 μg/g (0.04–0.11 μg/g) or 0.62 nmol/g (0.36–0.98 nmol/g) wet weight. As with Cu, values above this metric should be consider capable of causing adverse effects in fish. It is noteworthy that the lower 95% CI for both Cu and Cd are very close to their respective background concentration (Figures 1 and 2).

The results from the present study indicate that species in the family Salmonidae are especially sensitive to Cd, which was not observed for Cu. For those studies that examined mortality for salmonids (bull trout, brook trout, rainbow trout, and Atlantic salmon) the mean and SD lethal tissue Cd concentration was 0.55 μg/g (0.8 μg/g) wet weight ($n = 4$ species). This is 5 times lower than the mean LRp for all other species (2.5 μg/g wet wt) excluding the salmonid values ($p = 0.01$, t test). The same pattern was also observed for the salmonid LOER for growth, which was 6.6 times lower (mean [SD] of 0.19 μg/g [0.09 μg/g] for 3 species vs 1.25 μg/g [1.8 μg/g] for all other species). In terms of external exposure, salmonids are generally considered relatively sensitive to environmental contaminants, which is likely a function of their high rates of ventilation and dietary uptake [25]. For both Cu and Cd, salmonids generally exhibit relatively low exposure-based toxicity metrics compared with other fish species [19,21]. These are the first data to support the contention of increased sensitivity for salmonids as a function of toxicant potency. An alternate explanation would be that induction of MT in salmonids is weaker than found in other species, which would imply less of the total metal is sequestered from sensitive biomolecules and organelles. Numerous studies, however, show strong increases of MT in tissues of salmonids after exposure to Cd [26–28].

Even though the response concentrations among species based on tissue concentrations exhibited relatively low variability, some variation was expected. This is likely attributable to a number of factors such as dry to wet weight conversion, analytical error, response variability (e.g., LR10, LR50, or LOER), duration of exposure, number of replicates, spacing of doses, husbandry conditions, and overshoot of tissue concentrations in relation to toxic effect.

The difference between toxicity and control concentrations within studies was very low for Cu. The mean (SD) ratio between paired effect and control concentrations was 3.5 (2.5) for all Cu data in this analysis ($n = 15$). The values for Cd were

Table 2. Lethal cadmium responses[a]

Species	Common name[b]	CBR (μg/g)	SD[c]	N[c]	CBR time	LCp (ng/mL)	LCp time	Media[d]	Ref
Salmo salar	Atlantic salmon (L)	0.20	0.06	2	34–44 d	2–8.2	34–44 d	Fw	[71[e],72]
Rhamdia quelen	Silver catfish (L)	0.40			21 d	7.2	21 d	Fw	[73]
Gasterosteus aculeatus	Three-spined Stickleback (A)	0.83	0.11	2	7–16 d	1000–23 000	96 h	Fw	[74[e],75]
Gobio gobio	Gudgeon (A)	3			120 h	9000	120 h	Fw	[34][f]
Morone saxatilis	Striped bass (L)	1.1	0.4	2	96–120 h	10	51–96 h	Fw	[55,76]
Danio rerio	Zebrafish (A)	2.3			28 d	317	28 d	Fw	[13]
Oncorhynchus mykiss	Rainbow trout (L,J)	1.1	1.2	2	96–320 h	170–2300	96–320 h	Fw	[77,78]
Puntius gonionotus	Java barb (L)	3.2			96 h	2920	96 h	Fw	[79]
Jordanella floridae	Flagfish (J)	4.0			100 d	2500	96 h	Fw	[80]
Lates calcarifer	Seabass (Barramundi) (L)	2.1			16 d	3267	12 d	Sw	[81][g]
Poecilia reticulata	Guppy (L)	4			30 d	45	30 d	Fw	[82]
Salvelinus confluentus	Bull trout (J)	0.2			55 d	0.80	55 d	Fw	[83]
Tilapia zilli	Red belly Tilapia (L)	4.8			428 h	500	428 h	Fw	[66]
Salvelinus fontinalis	Brook trout (J)	0.14			30 d	3.6	30 d	Fw	[26][h]

[a]The effect is mortality and represents the lethal residue for all percentages (LRp), lowest-observed-effect residue (LOER), or lethal concentration for all percentages (LCp). The proportion responding (p) is the 50th percentile (± 10%; e.g. LR40 to LR60) unless noted. Whole-body concentrations are μg/g wet weight.
[b]The life stage is shown next to common name (larval [L], juvenile [J], or adult [A]).
[c]For multiple studies, mean, standard deviation (SD), and number of studies (N) shown.
[d]Media was either seawater (Sw) or freshwater (Fw).
[e]80% mortality.
[f]100% mortality.
[g]10% mortality.
[h]20% mortality.
R = residue (tissue concentration); C = water concentration.

Table 3. Sublethal cadmium responses[a]

Species	Common name[b]	Effect	LOER μg/g	LOER time	LOER Source[c]	LOEC (ng/mL)	LOEC[d] time	Media[e]	Ref
Cyprinodon variegatus	Sheepshead minnow (L)	Dev	0.50	7.5 d	A	5.8	7.5 d	Sw	[84]
Jordanella floridae	American flagfish (J)	Gro	4.0	100 d	A	16	100 d	Fw	[80]
Salvelinus fontinalis	Brook trout (J)	Gro	0.28	84 d	A	3.4	84 d	Fw	[85]
Salvelinus confluentus	Bull trout (J)	Gro	0.10	20 d	A	0.8	55 d	Fw	[83]
Poecilia reticulata	Guppy (L)	Gro	0.4	20 d	D	125 μg/g[f]	5 d	Fw	[82]
Cyprinodon variegatus	Sheepshead minnow (L)	Gro	0.1	7.5 d	A	5.8	7.5 d	Sw[g]	[84]
Salmo salar	Atlantic salmon (L)	Gro	0.19[h]	30–44 d	A	1.7–0.78	30–46 d	Fw	[71,72]
Jordanella floridae	American flagfish (J)	Repro	2.0	100 d	A	8.1	100 d	Fw	[80]

[a]Lowest-observed-effect residue (LOER) is the value considered by author to be statistically significant from the control. Effects are growth (Gro), development (Dev), or reproduction (Repro). Whole-body concentrations are expressed as μg/g wet weight.
[b]The life stage is shown next to common name (larval [L], juvenile [J], or adult [A]).
[c]The source was dietary (D) or aqueous (A) exposure.
[d]Lowest-observed-effect concentration (LOEC).
[e]Media was either seawater (Sw) or freshwater (Fw).
[f]Diet expressed as μg/g dry weight.
[g]5% salinity.
[h]Mean and standard deviation (SD) for *Salmo salar* (0.19 μg/g [0.05 μg/g] for 2 studies).

somewhat higher and more variable with the mean (SD) ratio for mortality at 21 (19) ($n = 11$) and for sublethal responses at 9.4 (4.6) ($n = 4$). There were only a few data points that could be used to determine a lethal to sublethal ratio (LSR) based on toxicity metrics for a given species, which is similar to the acute to chronic ratio but based on response, not duration. For Cu, the mean (SD) LSR was 1.4 (0.5) for 2 species (rainbow trout and olive flounder). For Cd, the mean (SD) LSR for 5 species (flagfish, brook and bull trout, guppy, and Atlantic salmon) was 3.1 (3.9), which was highly variable, ranging from 0.5 to 10. These low and variable LSR values fall within the range of variability observed among species and endpoints; hence, the differences between lethal and sublethal values are essentially indistinguishable over the larger dataset.

Aqueous LC50s

For both metals, the range in LC50 values based on water exposure spans approximately 4 orders of magnitude (Figures 1 and 2), whereas the range for lethal concentrations based on tissue residues is within 1 order of magnitude for many of the same species. As shown by Sorensen [29], the 48-h LC50 for one species (*O. mykiss*) exposed to Cd as a function of hardness can vary almost 50 fold. Even though the freshwater Cu LC50 values in Figure 1 were adjusted with the BLM to common water parameters such as pH, DOC, and hardness, variability among fish species was substantial. This observation of high variability after BLM normalization indicates substantial differences in uptake and elimination kinetics for these species of fish is likely. Several LC50 values from Tables 1 and 2 were highlighted in the figures to show their approximate ranking. Chronic external-exposure toxicity metrics for Cu or Cd were not included in the figures as they would add minimally to the observed variability among species.

Background tissue concentrations

The mean (SE) whole-body Cu concentration for the NAWQA data was 0.99 μg/g (0.24 μg/g) wet weight ($n = 23$) [22], which was identical to the value determined from the laboratory studies examined in the present study. When the NAWQA data and laboratory studies were combined, the overall mean (SE) background whole-body tissue concentration for Cu was 0.99 μg/g (0.18 μg/g) wet weight ($n = 34$). Salmonids in general exhibited a lower background whole-body tissue concentration with low variability (mean [SE] = 0.86 μg/g [0.14 μg/g]). Some species (e.g., tilapia) appear to have higher background concentrations, which should be considered when relating tissue concentrations to effects. Additional data include an older dataset from 1984 showing the geometric mean for 315 composite samples from 112 stations across the United States (0.65 μg/g wet wt) [30]. This study reported the national 85th percentile in the United States for Cu in whole body fish to be 1 μg/g wet weight at that time [30]. Based on the available data for fish, a reference whole-body tissue concentration of 0.99 μg/g or lower likely represents normal physiologic levels and would indicate very low or no exposure to concentrations of Cu expected to cause adverse effects.

The mean (SE) whole-body Cd concentration from the NAWQA database for fish from reference sites was 0.04 μg/g (0.01 μg/g) wet weight ($n = 23$) [22], which was slightly lower than that compiled from the laboratory studies examined in the present analysis (mean [SE] = 0.06 μg/g [0.02 μg/g]; $n = 14$). The same dataset described above from Schmitt and Brumbaugh [30] reported a geometric mean of 0.03 μg/g for whole-body Cd from 321 composite samples. Additional data from Schmitt [31] reported whole-body Cd concentrations <0.03 μg/g in reference fish. Murphy et al. [32] also found the mean (SD) whole-body concentration for 9 species of fish sampled in an uncontaminated lake basin to be 0.03 μg/g (0.009 μg/g). Taken together, these data indicate that a whole-body tissue concentration of approximately 0.04 μg/g or lower would likely not result in adverse effects and would be a useful indicator of background tissue concentrations.

DISCUSSION

These data indicate that the TRA is viable for some metals as determined in whole-body fish. A recent review on tissue-residue toxicity for metals found high variability for a given metal among species, and the authors concluded that this approach was generally not useful for characterizing toxicity or setting environmental quality standards [4]. This is generally true for invertebrates that are well-known regulators of internal metals [33] and can sequester excess concentrations creating a large pool of biologically inactive metal [4]. Adams et al. [4] showed a difference of 4 orders of magnitude in whole-body Cd

LOER values when fish and invertebrates were considered together. Concentrations of Cu in invertebrate tissue can also vary by orders of magnitude as a result of sequestration in granules and therefore are not appropriate for tissue-based toxicity metrics [4,33]. Fish can sequester metals but not to the degree observed for invertebrates. As noted by Kamunde [6] and Campbell and Hare [8], metals sequestered in granules (NaOH resistant fraction) is an important detoxification mechanism for invertebrates and much less so for fish. The heat stable protein fraction containing MTs is the major pool of detoxified metals for fish. This difference may explain why the dose-response characteristics for fish containing these metals internally are so different from that observed for invertebrates, which may have very high internal concentrations and not exhibit toxic effects [33].

Although the BLM is useful for refining aqueous concentrations expected to be toxic to biota, tissue concentrations for fish are less ambiguous and do not require normalization to site conditions before interpreting the results. In addition, freshwater and marine species can be combined to determine effect concentrations because external factors are generally unimportant [1]. As seen in the data presented in the present study, both freshwater and marine species are represented, and they exhibited no differences in CBRs. Also, because lethal and sublethal values are relatively close for Cu and Cd and potentially indistinguishable, as seen with the narrow response curves, these metrics may be combined to arrive at protective values.

Tissue concentrations integrate exposure over space and time and are advantageous for assessing environmental contamination compared with sediment, prey, or water concentrations that can be extremely heterogeneous. Tissue concentrations can also give an instantaneous snapshot of potential contamination for a system and require less monitoring of environmental concentrations. The use of whole-body tissue concentrations in forensic evaluations is also important. Unusual fish kills should be investigated by analyzing tissue concentrations to determine whether excess concentrations of metals or organics are the proximate cause, because they are likely to persist longer than water concentrations after such an event.

Concentrations of metals in specific organs, such as liver, gill, kidney, or intestine, are generally highly variable and lead to different interpretations regarding bioaccumulation and toxicity; however, whole-body concentrations linked to toxic effects are relatively consistent over species, exposure conditions, and duration. Given the low variability in CBRs compared with ambient-dose toxicity metrics, these data are more representative for all species and not just the lower 5th percentile. Whereas the HC5 for exposure-based metrics usually occurs far below the values for most species, tissue CBRs ensure that a very high percentage of species are being protected given the narrow range between high and low percentiles.

Species sensitivity distributions were calculated primarily to highlight the low variability among species when whole-body tissue concentrations were used as the dose metric compared with aqueous exposure. As an extrapolation method, the SSD provides a good representation expected for all species [24], especially when based on a random selection of species from several families of fish. Given the low variability seen in the tissue-residue SSDs (Figures 1 and 2) it is unlikely that far more extreme values will occur for naïvely exposed fish on the upper end or for the lower values, which are essentially bounded by background concentrations.

The rate of uptake for metals has also been noted as an important factor determining toxicity [4,10], which is information not available from the studies used in this analysis. This observation is well noted for invertebrates and may be more important for this group because they can sequester high concentrations of excess metals in granules. One study examined this for fish taken from contaminated and clean environments and found no difference in their rates of uptake or lethal body burden when exposed to high aqueous concentrations of Cd [34].

The prevailing literature indicates that organismal and physiologic toxic effects do not occur until whole-body concentrations of Cd or Cu increase to detectable concentrations above background. All of the studies examined in the present study assessed organismal responses (mortality, growth, and reproductive impairment); however other studies also demonstrated physiological responses with increases in whole-body concentration. One study that examined sublethal effects reported alterations in antipredator behavior and plasma cortisol levels in conjunction with increased whole-body concentrations [35]. Fish from the Warm Springs area of the Clark Fork River exhibited significant increased lipid peroxidation products and lower concentrations of Ca^{+2} in blood at a whole-body Cu concentration of approximately 1.6 μg/g wet weight, an increase of 2× over control fish [36]. Mohseni et al. [37] observed reduced lipid content, reduced feed conversion efficiency, protein efficiency ratio, and whole-body protein content at concentrations between 1.5 μg/g and their growth impairment concentration (3.5 μg/g) after 84 d of dietary exposure, which were all above background concentrations.

Based on the data examined, the current hypothesis is that adverse biological effects are not likely to occur in fish without elevated whole-body concentrations of Cu or Cd. This is supported by the available laboratory studies with fish that were not pre-exposed to these metals; however, it is unknown if this is also true for fish in the field. One noteworthy exception may be found in recent studies on fish sensory systems showing effects in organisms after just a few hours of exposure; however, none have reported whole-body tissue concentrations. One such example is McIntyre et al. [38], who showed alterations in coho salmon behavior after a 3-h exposure to low aqueous concentrations of Cu.

Organ versus whole-body concentrations

Whole-body concentrations are preferred for assessing potentially toxic concentrations of Cu and Cd in fish compared with specific tissues such as liver, muscle, or gill primarily because of the induction of MT that occurs in these tissues. Several studies have demonstrated that MT increased substantially in various organs after exposure to Cu and Cd [26,39,40], with some tissues showing much stronger responses than others. When considered on a whole-body basis, induction of MT has not been well studied. A few authors have demonstrated only modest or no changes in whole-body concentrations of MT [14,41] when exposed to metals. Organ concentrations are likely the preferred choice for assessing bioaccumulation and excess exposure to metals because of substantial accumulations with increasing ambient concentrations allowing more definitive separation from control concentrations [26,42]. Defining toxic concentrations with specific tissue concentrations among species is more difficult because of detoxification within the organ as a consequence of increasing MT and metal concentrations that may not result in toxic effects. Once MT capacity is exceeded, concentrations of labile metal would increase, causing potentially adverse effects.

Cellular partitioning within an organ [8] makes it difficult to determine the threshold concentration for effects based on organ concentrations and it would likely be species- and time-dependent and a function of exposure history. Using whole-body concentrations appears to minimize the influence of many of those variables as evidenced by the consistent response profiles for numerous species listed in the present study.

One drawback for the TRA is fish size. Whole-body tissue residue analysis is amenable for small-body fish and juveniles and less practical for large individuals. Laboratory and field evaluation would likely be conducted with small fish and extrapolated to larger species. There are no data suggesting that the tissue residue response for these metals would vary as a function of fish weight or life stage, as seen in the tables.

Spillover

One explanation for the low variability observed for toxic effects at similar whole-body concentrations among species can be found in the spillover hypothesis [5]. The observation of relatively narrow variability in tissue toxicity metrics shown in the present study may be attributable to an exceedance in complexing capacity by MT in fish such that it occurs at similar whole-body concentrations leading to toxic effects. The implication is that the proportion of labile metal in relation to total whole-body concentration available to interact with receptors and cause toxic effects is relatively consistent among species.

It is also important to note that spillover can be considered in terms of bioaccumulation or toxicity. The amount of metal in various labile and detoxified cellular compartments can be quantified without consideration of toxic effects. The point at which metals exceed complexing capacity and become available to sensitive cellular components is detectable but not necessarily toxic, at least by standard organismal and physiological measures. The spillover hypothesis was reviewed by Kamunde [6], who noted that this topic is relatively complex for fish. As highlighted by Kamunde [6], some authors observed spillover from sequestered forms to labile forms and others did not in laboratory and field studies. In some cases, all elevated external exposure concentrations for a given metal resulted in excess metal in subcellular compartments that are considered sensitive [8], which may or may not result in toxic effects. Most of these studies were conducted with invertebrates and a limited number with fish. Few, if any, considered the role of whole-body concentrations for fish in relation to subcellular compartmentalization and spillover.

In Cope et al. [42], non-thionein cytosolic Cd concentrations increased with increasing liver concentrations in bluegill (*Lepomis macrochirus*), which was highly correlated with increasing whole-body concentrations during aqueous exposure (7 doses) over 28 d. There were no effects on survival or growth up to a whole-body maximum of 1.3 μg/g wet weight, which is lower than effect concentrations observed for many fish species (Tables 2 and 3). Because the free or labile concentration of metal increased greatly in the cytosol, it is crucial to determine if adverse effects are possible. There are very few studies with a complete dataset, such as the study by Cope et al. [43], which examined whole-body, organ, and subcellular concentrations for these metals and considered toxic effects. It is clear that concentrations can increase in metal-sensitive subcellular compartments; however, the concentration of labile (unbound) metal needed in organs or whole body to elicit a toxic effect is unknown.

Thus, the observed similarity in toxicity metrics among fish may be a function of metal detoxification. Vijver et al. [44] hypothesized that differences in species' CBRs may be more a function of internal detoxification and that the toxic fraction of the total metal (biologically effective dose) may be similar among species. As indicated by Kamunde [6], the vast majority of tissue Cd resides in metal sensitive tissue compartments or those containing MT. Because MT is the main detoxification system for fish, its induction and capacity may be similar among species such that once capacity has been attained, spillover occurs at relatively similar concentrations with little of the excessive metal sequestered by other mechanisms, such as granules. At that spillover point, labile metal would rapidly increase causing adverse effects at similar whole-body concentrations. Support for this comes from Bervoets et al. [45], who found less than a 2-fold difference in MT capacity among 3 species of fish (*Gobio gobio*, *Perca fluviatilis*, and *Rutilus rutilus*). Another comparative study of MT in various organs of 3 species of fish (*Oncorhynchus mykiss*, *Carassius auratus gibelio*, and *Cyprinus carpio*) found a difference in MT content of approximately 2- to 3-fold among species for gill, liver, kidney, and muscle [46] after sublethal aqueous exposure to Cu (63 ng/mL) for 168 h. Both studies indicate relatively low differences among species regarding MT content that would fall within the variability observed among species tissue toxicity metrics.

Acclimation

Increased tolerance to metals in organisms results from acclimation or adaptation, the former mechanism allows change within the organism's inherent physiological limits, and the later requires genetic change. Adaptation was not considered in the present study because of the difficulty in demonstrating genetic change in fish exposed to metals [47]. It is well known that fish can acclimate to elevated metal concentrations, causing media-based (water, sediment, and prey) toxicity metrics to increase [48,49]. The mechanism for this increased tolerance may result from a reduction in the rate of uptake, enhanced elimination, or tolerance to higher tissue concentrations through inactivation and sequestration of excess metal. Up or down regulation of the genes coding for molecular targets is also a possibility, which may alter toxic potency [11].

The rate of uptake during high Cu exposure was reduced in killifish (*Heterandria formosa*) that were pre-exposed to Cu [41] indicating resistance to elevated external exposure concentrations. These authors found an elevated whole-body concentration of Cu in acclimated individuals that were challenged with a high water concentration for 8 h; however, the difference was not statistically significant compared with nonacclimated fish (5 μg/g vs 7 μg/g wet wt). Another study pre-exposed minnows to sublethal concentrations of Cd and observed a decrease in uptake by the gill and also noted no increase in MT for various tissues [50]. Grosell et al. [51] demonstrated increased excretion of Cu in Cu-acclimated rainbow trout. Similarly, Dang et al. [40] found large differences in Cu retention between fish acclimated to Cu and those not acclimated when depurated for several days following dietary ingestion. Dixon and Sprague [52] reported increased lethal tissue concentrations for rainbow trout pre-exposed to Cu for 21 d. When challenged with a high concentration of Cu ($1.7 \times$ LC50) for 6 d the pre-exposed fish exhibited a 30% mortality rate for a whole-body tissue concentration of 7 μg/g wet weight compared with 100% mortality at 1.6 μg/g wet weight for the control fish that were not pre-exposed. Dixon and

Sprague [49] noted that fish lost acclimation tolerance rapidly after 7 d and completely after 3 wk in control water, indicating that fish in the field may acclimate to elevated concentrations but can lose this ability if they move to a less contaminated location within the ecosystem. Consequently, they may be re-exposed after this time and respond to elevated concentrations as a naïve fish would.

An important factor to consider is the pre-exposure concentration that leads to acclimation. Some studies indicate that low aqueous concentrations do not increase tissue or MT concentrations substantially in fish that are pre-exposed, resulting in similar toxicity metrics when challenged with toxic concentrations. Dixon and Sprague [49] found that tolerance to Cu in rainbow trout did not occur until water concentrations were greater than 0.18× the incipient (steady state) LC50, indicating that fairly high concentrations were needed to invoke this response. Szebedinszky et al. [53] observed no increase in the waterborne Cd 96-h LC50 for rainbow trout pre-exposed to an aqueous concentration approximately 0.1× the LC50 for 30 d. Fish in that study did exhibit an aqueous 96-h LC50 that was approximately 2× that over the control when pre-exposed to 800 μg/g or 1500 μg/g dietary Cd for 30 d. The lower dietary dose was approximately 0.5× the dietary LC50 and the high dose was almost equal to the dietary LC50 (43% mortality). Another study [54] observed increased tolerance in *Fundulus heteroclitus* to subsequent challenges of Cd when the aqueous pre-exposure concentration was 10 μg/mL Cd, but not at 1 μg/mL. Based on the existing literature, it appears that a relatively high pre-exposure concentration is needed to induce levels of MT that are protective of Cd and Cu toxicity, which may or may not lead to a higher CBR.

Interestingly, some of the data in Tables 1 and 2 were long-term exposures; yet, the tissue concentrations associated with toxicity are not substantially higher than those for the short-term experiments. Either acclimation to higher body burdens did not occur in these species, or any increased tolerance to elevated tissue concentrations of the metal was slight and within the variability observed among species and experiments.

Even though metal-acclimated fish may contain concentrations higher than naïve fish expected to exhibit adverse effects, these elevated concentrations may prove useful. Field-collected fish with concentrations exceeding expected toxic levels may indicate that the environment where they resided is highly contaminated. Based on the data in Tables 1 and 2 for previously unexposed fish, it is clear that for most naïvely exposed species, whole-body concentrations exceeding 10 μg/g for Cu and 4 μg/g for Cd should be lethal. If fish from the field are observed with higher concentrations, it is possible they have adjusted physiologically, indicating that the system where they reside likely contains elevated concentrations.

Field values

A number of studies reported concentrations in whole-body fish collected from various reference and contaminated sites. Wright [55] found that whole-body Cd concentrations in field-collected striped bass larvae were mostly below the sublethal HC5 value of 0.07 μg/g; however, 34% were above this concentration (maximum of 1.2 μg/g) before 23 May but only 5% after this date. The same seasonal pattern was observed by Wright [55] for Cu, with 42% of the larvae exceeding 1 μg/g (maximum 6 μg/g wet wt) before 23 May but only 20% above this concentration after 23 May. Seasonality for metal toxicity has been noted before and is a function of environmental conditions such as pH and DOC levels [56], which are at minimal values before the spring phytoplankton bloom. This is an important aspect that will determine the percentage of fish exhibiting potentially toxic whole-body concentrations of these metals. Based on the data presented, a relatively high percentage of striped bass larvae exceeded the Cd and Cu HC5 thresholds presented here, indicating that the areas sampled likely contain elevated ambient concentrations of Cd and Cu at levels expected to be toxic for this species and others.

Another field study noted increases in whole-body concentrations of Cu in brown trout from contaminated areas of the Clark Fork River in Montana, USA [36]. Other metals and metalloids (Cd, arsenic, and lead) exhibited increased organ concentrations but not whole-body concentrations. A comprehensive analysis of metals and organics in whole-body fish from a large national biomonitoring program covering the years 1995 to 2004 reported a geometric mean of 0.03 μg/g and an 85th percentile Cd concentration of 0.09 μg/g ($n = 180$), with a maximum of 0.5 μg/g indicating a number of sites with fish containing elevated concentrations of Cd [57]. A similar finding for Cu was observed in that report [57], with a geometric mean of 0.8 μg/g and an 85th percentile of 1.3 μg/g, with a 3.92 μg/g maximum value ($n = 409$). In both cases, the mean values indicate levels similar to background concentrations for most samples, with a number of values occurring at suspected toxic concentrations. Another study quantified whole-body Cd concentrations for 11 species of fish sampled from a contaminated lake basin in Indiana and found species mean concentrations ranging from 0.01 μg/g to 1.76 μg/g (overall mean = 0.65 μg/g), with many values in the range shown to affect growth in other species (Table 3) [32]. A follow-up study at this site compared growth parameters in yellow perch from the contaminated basin with those collected from the uncontaminated site and found significant reductions in weight, length, and RNA/DNA ratios for contaminated fish, which contained a mean whole-body Cd concentration of 0.38 μg/g wet weight [58]. Whole-body concentrations in field-collected fish that are higher than the maximum reported effect concentrations for naïvely exposed laboratory fish are unusual but have been observed [30].

Mixtures

No comprehensive dataset exists that evaluates the toxicity of metal mixtures based on whole-body tissue concentrations. A recent series of papers [59] addressed the toxicity of metal mixtures based on media exposure and reported that additive and less-than-additive toxicity responses were the most common and that current modeling approaches were reliable when bioavailability was considered. Additivity is more common than interactive toxicity for organic compounds [2], and is a reasonable default assumption for any contaminant mixture. For dose additivity a common mechanism of action is necessary [2]; however, this is not required for response additivity. Response additivity is a reasonable assumption for complex mixtures containing chemicals with unknown mechanisms of action. Because bioavailability and toxicokinetics are greatly reduced as factors in toxicity determination, a tissue residue analysis provides a simple, defensible approach for assessing mixtures. Using tissue residues can improve the accuracy and efficiency of characterizing mixture effects for metals because the impact of confounding factors would be reduced. Additionally, reliable data should be obtainable with fewer dose combinations than is usually required for external concentrations. The best approach for this would be to add tissue threshold values for each metal or use a toxic unit approach that considers multiple toxicants [2].

One interesting aspect concerns tolerance to higher metal concentrations. Metallothionein is not metal-specific, and some metals can be displaced by increasing concentrations of other metals [5]. This would be an important aspect to consider when assessing metal mixtures in field exposures. Interestingly, Dixon and Sprague [52] found that Cu tolerance in *O. mykiss* resulted in sensitivity to zinc by a large margin (56% decline the steady-state LC50). This observation supports the contention that mixtures of metals may result in unexpected toxic responses.

Environmental quality standards

Most environmental criteria, standards, or guidelines for water, sediment, and tissue are determined with laboratory toxicity studies. Any acclimation by organisms in the field is ignored—and rightly so—which builds conservatism into these protective values. This phenomenon occurs with metals and organics and is usually not considered relevant for environmental protection because it is too variable [60]. Reasons for this include weak acclimation by some species, dose and metal-mixture dependent variability [52], and potential migration of previously unexposed individuals into contaminated areas. For Cu and Cd, any whole-body tissue concentrations observed in field collected fish that exceed the maximum laboratory-derived toxic levels reported in the present study are likely a result of acclimation. As such, adverse effects may not occur in these populations; however, elevated concentrations may indicate that the site contains excess levels of metal in water, sediment, and prey and would thus be considered contaminated.

Although an HC5 was calculated for these data, using tissue residues provides an opportunity for a more graded response that can be used to characterize no effects (background concentrations) to highly probable effects that can be categorized into several levels of potential harm based on observed tissue concentrations. Even with potential acclimation in the field, high tissue concentrations can indicate potentially toxic levels, especially for those species unable to acclimate or those that experience naïve exposure because of immigration into the contaminated system.

Tissue quality guidelines. Based on these data, tissue quality guidelines for both Cu and Cd would be very close to background concentrations. Because of the inherent variability for estimated background and effect concentrations, additional data should be collected to further refine the threshold limits for toxic responses. It is important to keep in mind that the HC5 presented here is an effect concentration; hence, if no adverse effects are desired, a safety factor or lower percentile should be employed. The interconversion between internal and external concentrations is often very predictable for organic compounds [2], whereas this frequently is not the case for metals [4], especially for invertebrates. Therefore, tissue residue toxicity values are preferred for establishing environmental quality standards values because of the difficulty in correlating metal residues to water, sediment, or prey concentrations; however, the utility of this should be examined for fish. Although a precise CBR for a given species may not exist due to variable ambient exposure conditions, as suggested by Adams et al. [4], the variability is low among species and this provides utility for establishing a tissue-based guideline for protection. Even though tissue-based toxicity metrics may exhibit variability as a function of exposure conditions, any comparable analysis based on external exposure concentrations would magnify such variability and result in greater uncertainty.

CONCLUSION

The results presented here share many of the characteristics delineated under the TRA for organic and organometallic compounds. The narrow range in toxicity values observed for numerous fish species appears to support the contention of similar toxic potency for these metals in tissue when expressed as whole-body concentrations. Specifically, the implication is for a relatively similar spillover point for both metals that allows labile metal to increase above MT sequestering levels to affect important physiologic parameters. Also, the TRA characteristic of time independence for the toxic response appears to be supported by the wide variability in exposure times utilized for these experiments characterizing CBRs in naïve fish. Once whole-body concentrations achieve the critical level, adverse effects occur, regardless of the time needed to achieve those levels. If the detoxification system in fish is limited essentially to MT and that capacity is relatively similar among fish species, then similar CBRs are expected.

The data highlighted in the present study support the development of tissue quality standards or guidelines for Cd and Cu that could serve as an additional line of evidence for environmental risk assessment. The sensitivity to Cd exhibited by salmonid species, which is an important consideration for ecological risk assessment, is noteworthy. Fish-specific environmental quality standard values cannot be used alone because invertebrates, plants, and algae may be more sensitive to external concentrations. The recommendation is not to supplant water and sediment guidelines or criteria but supplement with a tissue guideline that would add confidence to the goal of environmental protection.

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Data availability—All data, associated metadata, and calculation tools for this research are available publicly. All data are from existing documents cited in this paper.

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