July 28, 2017

Paula Wilson
Idaho Department of Environmental Quality
1410 N. Hilton, Boise, ID 83706

Re: Negotiated Rulemaking - Water Quality Standards/Copper Criteria, Docket No. 58-0102-1502

Dear Ms. Wilson,

Thank you for the opportunity to provide comments on the Idaho Department of Environmental Quality's (IDEQ) July 18, 2017 negotiated rulemaking presentation regarding updates to the state's copper aquatic life criteria. GEI Consultants and Windward Environmental, along with our client, the Copper Development Association (CDA), would like to comment on the issue of selecting an appropriate pH value for deriving BLM-based criteria at a site.

The draft *Implementation Guidance for the Idaho Copper Criteria for Aquatic Life* (IDEQ 2017) notes that BLM "users should consider using continuous pH data to capture the daily variability of pH at a given site" and that "[w]hen continuous data are available the minimum daily pH value should be used to generate BLM criteria."

The issue is whether short-term increases in bioavailable copper concentrations, during times of the lowest pH levels during a diel cycle (typically at night), are sufficient to result in increased copper toxicity and warrant lowering of BLM-based copper criteria (relative to copper criteria derived based on a daily average pH or single pH measurement during the day that may be more representative of an "average" pH between diurnal minima and maxima). We believe this issue can be empirically evaluated in at least two ways. The first is to review whether there are copper toxicity studies that included diurnal fluctuations in pH and whether BLM-based copper criteria using average pH would have been protective of toxicity. The second is to review whether there are copper toxicity studies that compared toxicity based on fluctuating dissolved copper concentrations and constant dissolved copper concentrations. The latter type of study can be considered a surrogate for evaluating the potential influence of diurnal fluctuations in pH on bioavailable copper concentrations.

Copper Toxicity vs. BLM-based Criteria in Experiments with Diurnal Fluctuations in pH

Empirical data are limited for specifically evaluating how diurnal fluctuations in pH may influence copper toxicity. In laboratory tests with relatively clean waters these diurnal fluctuations may be minimal, although it's possible that single-species laboratory tests conducted in natural waters may contain some algae that could contribute to diurnal pH patterns. Unfortunately, in most laboratory tests, it is common to only monitor pH once per day and we are not aware of any single-species copper toxicity studies that have documented diurnal fluctuations in pH during exposures. On the other hand, mesocosm studies, which include communities of aquatic biota, including algae, would account for diurnal variation in both water chemistry (e.g., pH) and organism physiology (e.g., respiration). Such studies with copper would provide an indication of whether BLM-based copper criteria are adequately protective.

In one mesocosm study, periphtyon, aquatic plants, benthic invertebrates, zooplankton, and a predatory fish (three-spined stickleback) were exposed to mean dissolved Cu concentrations of <0.5, 4, 20, and 57 µg/L in lotic (flowing) mesocosms for 18 months (Joachim et al. 2017). Evaluation of invertebrate community-based metrics resulted in a no-observed-effect concentration (NOEC) of 4 µg/L and a lowest-observed-effect concentration (LOEC) of 20 µg/L. Based on the grand mean of the mesocosm water chemistry conditions across treatments, the study authors calculated a BLM-based chronic copper criterion of 6.8 µg/L. This criterion is near the NOEC of 4 µg/L and well below the LOEC of 20 µg/L. The pH was monitored weekly during the 18-month experiment, suggesting that the long-term average used for the BLM calculations did not account for the regular diurnal variability in pH (and, specifically, the daily pH minima). Nevertheless, even without accounting for diurnal minima in pH, the BLM-based criteria were found to be protective of a range of invertebrate community indices.

Copper Toxicity Based on Fluctuating and Constant Concentrations

As noted above, an additional way to evaluate whether short-term increases in bioavailable copper concentrations during daily periods of low pH is sufficient to cause increase toxicity is to consider the toxicity of fluctuating metal concentrations compared with constant metal concentrations, and whether toxicity is associated with the short-term maxima of the fluctuating concentrations (which may be considered representative of increased bioavailability of copper concentrations during short-term minima in pH) or constant concentrations (which may be considered representative of an average condition). One of the clearest examples for metals is Nimick et al. (2007), who conducted acute field bioassays in mining-influenced steams to westslope cutthroat trout under diel conditions when metal concentrations varied and under constant metal concentrations. Cadmium and zinc concentrations showed diel variability, increasing by up to 61 and 125%, respectively, during the night, while copper concentrations did not show variability (in this study toxicity was primarily attributed to zinc). Cutthroat trout survival was greater under diel metal exposure compared to constant exposures, despite the mean metal concentrations being very similar. The authors suggested that higher survival in the diel exposure was greater due to the periods of lower metal concentrations during the day and because the night-time periods of higher metal concentrations also corresponded with colder water temperatures that would reduce the rate of metal uptake by the fish.

Building on the data from Nimick et al. (2007), Balistrieri et al. (2012) developed a bioavailability-based "Tox" function for predicting the toxicity of cadmium, copper, and zinc mixtures to cutthroat trout. They found that the same function could predict toxicity based on the average of the diel conditions and the constant conditions, indicating that the peak high metal bioavailability conditions were less important.

Exposure Duration and Copper Concentration Magnitude Are Not Independent

In addition to the empirical data examples above, it is important to consider toxicity as a function of both exposure time and the magnitude of the concentration. The effect of copper, or almost any chemical, on an aquatic organism is much more rapid at a high concentration than at a low concentration. In the case of aquatic life criteria, concentrations are of course not high, but low, as they are based on the 5th percentile of toxicity thresholds for all species tested. Further, the toxicity thresholds for each species, including those at and below the 5th percentile, are essentially "no-effect" concentrations.

In recommending 1 hour as the averaging time for assessing compliance with acute criteria, USEPA (1985) notes that "One hour is probably an appropriate averaging period because ***high*** [emphasis added] concentrations of ***some materials*** [emphasis added] can cause death in one to three hours." One major flaw in this reasoning is that this statement specifically refers to high concentrations of materials (chemicals), while criteria are of course associated with low concentrations. The more relevant question is how quickly toxicity may occur to sensitive species at near-criteria concentrations. The other piece of this statement to note is that it acknowledges this is true for some chemicals (i.e., not all).

The question then becomes: Is copper a "fast-acting" chemical at near-criteria concentrations?

We previously addressed this question in our February 1, 2017 comments to IDEQ, in which we provided an analysis of empirical averaging times for copper toxicity to a variety of species over a wide range of exposure conditions.[1] That analysis supported that the acute averaging time of 24 hours, as recommended in USEPA (2007), was appropriate for acute copper criteria. Although that analysis was focused on the averaging time for quantifying the dissolved copper concentration at a site for comparison to a copper criterion, the same concept directly applies in considering the appropriate conditions for calculating BLM-based criteria (i.e., it can be thought of as the averaging time over fluctuating conditions that influence copper bioavailability). As such, we recommend that a 24-hour averaging time is also relevant for defining the pH levels, and other water chemistry conditions, that are used as inputs for deriving BLM-based copper criteria at a site.

Summary and Conclusions

We recognize the averaging times recommended in USEPA (1985) are also based on consideration of fluctuating concentrations of chemicals sometimes being more toxic (although this was not observed by Nimick et al. [2007], as discussed above). As such, acute and chronic averaging times of 1 hour and 96 hours for acute and chronic criteria are less than the exposure times in the acute and chronic studies used to derive criteria. The acute tests are all 48 to 96 hours in duration and the chronic tests are life cycle tests for invertebrates, and at least early life stage tests for fish (approximately 30 days or greater, depending on the species). Thus, there is already conservatism in these averaging times that is accounted for in the measurement of dissolved copper for determining compliance with BLM-based copper criteria. That conservatism is unnecessarily compounded in likewise assuming a short averaging time for pH in determining the BLM-based criterion, which does not appear to be supported by the data available for addressing this issue.

In closing:

- There is no evidence that short-term increases in copper bioavailability due to diurnal pH minima results in increased toxicity, which is also supported by the averaging time evaluations.

- The chronic BLM-based copper criterion based on average pH and other water quality conditions in a long-term mesocosm study was found to be protective of several invertebrate community metrics.

- If continuous data are collected for pH, we recommend use of the average pH for deriving BLM-based copper criteria, as the average condition appears to provide appropriate protection.

We appreciate the opportunity to provide these comments. Please feel free to let us know if you have any questions or if you would like to discuss further.

[1] For reference, that analysis has been attached to these comments as well.

Sincerely,
GEI CONSULTANTS, INC.

Robert W. Gensemer, Ph.D., GEI Consultants
Senior Ecotoxicologist

David DeForest, Windward Environmental
Senior Ecotoxicologist

Carrie Claytor, CDA
Director of Health, Environment and Sustainable Development

cc:

John Gondek, GEI Consultants
Scott Tobiason, Windward Environmental
Robert Santore, Windward Environmental
Eric Van Genderen, International Zinc Association

References

Balistrieri LS, Nimick DA, Mebane CA. 2012. Assessing time-integrated dissolved concentrations and predicting toxicity of metals during diel cycling in streams. Sci Total Environ 425:155-168.

IDEQ. 2017. DRAFT Implementation guidance for the Idaho copper criteria for aquatic life. Using the biotic ligand model. June 2017.

Joachim S, Roussel H, Bonzom J-M, Thybaud E, Mebane CA, Van den Brink P, Gauthier L. 2017. A long-term copper exposure in a freshwater ecosystem using lotic mesocosms: Invertebrate community responses. Environ Toxicol Chem DOI: 10.1002/etc.3822.

Nimick DA, Harper DD, Farag AM, Cleasby TE, MacConnell E, Skaar D. 2007. Influence of in-stream diel concentration cycles of dissolved trace metals on acute toxicity to one-year-old cutthroat trout (*Oncorhynchus clarki lewisi*). Environ Toxicol Chem 26:2667-2678.

USEPA. 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. U.S. Environmental Protection Agency. Washington, D.C.

USEPA. 2007. Aquatic life ambient freshwater quality criteria - copper. Washington, DC, USA. EPA 822-R-07-001.

ATTACHMENT

A Review of Metal Toxicity and Exposure Duration for Determining the Suitability of a 24-Hour Averaging Period for Comparison with Acute Water Quality Criteria

Windward environmental LLC

200 West Mercer St. • Suite 401 • Seattle, WA 98119
Phone: 206.378.1364 • Fax: 206.973.3048 • www.windwardenv.com

TECHNICAL MEMORANDUM

To: Michael Campbell

From: Robert Santore, Adam Ryan, Kelly Croteau, David DeForest

Subject: A review of metal toxicity and exposure duration for determining the suitability of a 24-hour averaging period for comparison with acute water quality criteria

Date: September 15, 2016

SUMMARY

Although EPA's recommended acute water quality criterion (WQC) for copper is based on a 24-hour average concentration (US EPA 2007), EPA recently proposed an acute copper WQC for Oregon based on a 1-hour average. In addition, EPA has recently changed its recommended averaging period for assessing compliance with the acute WQC for cadmium from 24-hours to 1-hour (US EPA, 2016). These changes are a reversal of an earlier move from 1-hour to 24-hours used in both the 2001 cadmium document (US EPA 2001), and the 2007 copper document (US EPA 2007). The justification for moving to the longer 24-hour averaging period in the 2001 and 2007 documents was based on an analysis by US EPA that demonstrated a strong dependence of metal toxicity, including copper toxicity, on exposure duration, such that metals were more toxic (lower EC50 or LC50 values) in acute exposures with longer durations (US EPA 1995). EPA has recently stated in the 2016 cadmium criteria document that the reasons for going back to a 1-hour duration are that it is consistent with the guidelines for deriving aquatic life criteria (US EPA, 1985), and that the analysis of exposure duration (US EPA, 1995) had a "focus on fish" and therefore the 24-hour averaging period may not be protective for invertebrates (US EPA, 2016). No new analysis accompanied the recommendation for returning to a 1-hour averaging period in the 2016 cadmium document, so it was not clear why EPA determined that the 1995 analysis might not be protective. Was there simply a lack of supporting

evidence to suggest a 24-hour averaging period was protective for invertebrates, or was there evidence to suggest 24-hours was not protective?

To address these questions, we reviewed the 1995 analysis by EPA and subsequent toxicity literature to assess:

- Whether invertebrates were included in the 1995 analysis.
- Whether toxicity data for invertebrates that were published since 1995 could be used to provide additional evidence to strengthen the analysis.
- Whether invertebrate data would confirm or refute that a 24-hour averaging period would be protective for invertebrates as well as for fish.

As a result of our review we determined that invertebrates were part of the 1995 analysis, and therefore it was not accurate to characterize the analysis as having a "focus on fish". The invertebrate data included in US EPA 1995 showed a strong relationship between exposure duration and metal toxicity, including copper toxicity, similar to that observed in the toxicity data for fish.

Furthermore, we found additional toxicity studies published after US EPA (1995) that approximately tripled the number of invertebrate studies used to characterize the relationship between exposure duration and copper toxicity. These additional studies further support the conclusion that there is a pronounced reduction in acute copper toxicity with decreasing exposure duration using an approach recommended in US EPA (1991) for deriving a scientifically justifiable averaging period. Both the US EPA (1995) analysis, and the extended analysis presented here, show that the recommended 24-hour averaging period in the 2001 cadmium and 2007 copper criteria documents would be protective for invertebrates.

BACKGROUND ON RECENT CHANGES TO THE AVERAGING PERIOD

The 1985 EPA guidance on deriving water quality criteria recommended a 1-hour averaging period (USEPA 1985). Quoting from the guidance document:

> "*For the CMC the averaging period should again be substantially less than the lengths of the tests it is based on, i.e., substantially less than 48 to 96 hours. One hour is probably an appropriate averaging period because high concentrations of some materials can cause death in one to three hours*."

The language in this document acknowledges that there is uncertainty in the 1-hour recommendation. Consequently, a few relevant questions, as follows, should be addressed. What is the definition of "*substantially less than 48 to 96 hours*"? One hour is "*probably appropriate*" but could a different averaging period be appropriately protective? While "*high concentrations of some materials can cause death in one to three hours*", is a metal such as copper one of the materials for which this concern is relevant?

The 1991 Technical Support Document for Water Quality-based Toxics Control (EPA 1991) provides a further explanation of the acute averaging period as follows:

> "*For acute criteria, EPA recommends an averaging period of 1 hour. That is, to protect against acute effects, the 1-hour average exposure should not exceed the CMC. The 1-hour acute averaging period was derived primarily from data on response time for toxicity to ammonia, a fast-acting toxicant. The 1-hour averaging period is expected to be fully protective for the fastest-acting toxicants, and even more protective for slower-acting toxicants. Scientifically justifiable alternative (site-specific) averaging periods*
>
> *can be derived from (1) data relating toxic response to exposure time, if coupled with considerations of delayed mortality (mortality occurring after exposure has ended), or (2) models of toxicant uptake and action, such as presented by Erickson [5] and Mancini et al. [4].*"

To address a lack of data supporting the 1-hour averaging period, EPA conducted an analysis of the speed of action of metal toxicity to aquatic organisms (US EPA, 1995) using the approach in recommended in US EPA (1991). This analysis evaluated how the toxicity of various metals changed with increasing exposure duration. The analysis involved tabulating the median lethal or median effect concentrations (LC50 or EC50) of metals at various exposure durations. An exponential function was then fit to the data, such that:

$$LC50_t = LC50_\infty * \frac{1}{1-e^{-kt}} \qquad \text{(Equation 1)}$$

Where t is the exposure duration (hours), $LC50_t$ is the measured LC50 at exposure t (µg/L), k is an exponential constant (1/hours), and $LC50_\infty$ is the asymptotic value of the LC50. For each experiment, values of $LC50_t$ and t were tabulated and the value of k and $LC50_\infty$ were determined by non-linear regression. An example of the data and exponential function is shown in Figure 1, where the strong relationship between copper exposure duration and toxicity is evident. The calculated k for this example is 0.0008, and the averaging period (1/k) is 1250 hours, which in the EPA analysis was reported as >120 hours, thereby constrained by the total exposure duration. The strong relationship between toxicity and exposure duration means that copper toxicity at 24 hours occurs at a concentration that is approximately twice that at 48 hours (Figure 1).

To understand the relevance of this information for understanding the selection of averaging period, it is helpful to review again the quote from the EPA guidance on deriving water quality criteria. The reason that "*averaging period should again be substantially less than the lengths of the tests it is based on*" is because shorter averaging periods are inherently more conservative. The new information in the 1995 speed of action analysis provided a quantitative assessment as to the length of exposure duration that would be appropriate and yet still be "*substantially less*" than the 48 hours used to derive toxicity data for invertebrates in the WQC documents.

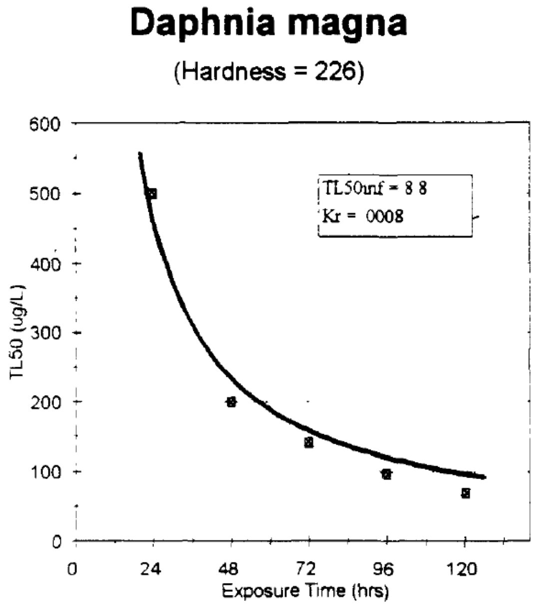

Figure 1. Example data showing copper toxicity to *D. magna*, and the exponential function (equation 1) fit to determine k and $LC50_{\infty}$.

Although the 1995 speed of action analysis was not cited in the 2001 cadmium or 2007 copper WQC documents, both of these documents changed the averaging period for acute WQC to 24-hours. In the 2016 cadmium WQC, this change was reversed back to 1-hour, but this time the 1995 speed of action analysis was referred to, although it was still not explicitly cited. Quoting from the 2016 cadmium document (US EPA, 2016):

> *"For the 2016 acute cadmium criteria, EPA has changed the duration to 1-hour from the 24 hours EPA applied in the 2001 final cadmium criteria document. EPA made this change to the 2016 criteria to reflect the acute criteria duration recommended in the 1985 Guidelines. The draft 2001 cadmium criteria document used a 1-hour duration, which EPA subsequently revised to 24 hours in the final criteria document. The final cadmium criteria document did not detail the rationale for this change, and EPA has further examined this issue as part of the 2016 criteria update.*
>
> *The 24-hour duration used in the 2001 final cadmium criteria document was based on a limited number of fish toxicity studies that were conducted in the mid-1990s and which suggested that cadmium time-to-effect may be longer than reflected by the 1-hour averaging period. These studies were focused on fish and did not address trends in duration for other aquatic species, such as invertebrates. Because of the limited nature of these investigations and absence of additional supporting information, EPA decided to revise the acute duration in this document to be consistent with the more protective 1-*

> *hour duration, which is generally supported by and consistent with the 1985 Guidelines."*

The quote characterizes the 1995 analysis as "*focused on fish*". The implication is that there is uncertainty in whether the longer averaging period would be protective for other organisms, and especially invertebrates. However, this characterization is inaccurate, because fish and invertebrates were represented in US EPA (1995). For copper, the invertebrate data in US EPA (1995) included 14 observations for nine species (Table 1). The mean effective averaging period for these invertebrate tests with copper was 44 hours, which is well over the 24-hour averaging period recommended in EPA 2001 and 2007. With one of EPAs concerns being the "*absence of additional supporting information*" this review considered whether additional information published since US EPA (1995) could further extend the analysis to include a greater number and types of aquatic invertebrate species.

REVIEW OF ADDITIONAL TOXICITY DATA

A review was conducted to identify additional toxicity data to extend the analysis in US EPA (1995). This review focused on copper toxicity to freshwater invertebrate species to provide a significant number of additional studies to supplement those included in the original analysis.

Datasets were analyzed in Microsoft Excel, using the Solver add-in to fit Equation 1 to the data. The best fit was determined by minimizing the sum of squared residuals (SSR) between the reported and estimated LC50s in Equation 1. The Excel Solver tool was set to use the GRG Nonlinear method for minimizing the SSR, with $LC50_{\infty}$ and 1/k set as the variables, with the constraint of $1/k \geq 1$. This constraint was used to prevent errors from occurring in the minimization, since a value of $1/k \leq 0$ would result in a divide-by-zero error in some of the equations. Furthermore, $1/k$ was calibrated rather than k because the GRG Nonlinear method, being a gradient optimization method, performs better when the variables being optimized are in a similar numerical space to each other.

Before analyzing the new data, the procedure used in the current analysis was applied to some of the datasets from EPA 1995 (Dave 1984; Pickering & Henderson 1966) to ensure that the procedure would yield equivalent results. The calculated $LC50_{\infty}$ and k_r values were less than a 10% difference from the values reported in EPA 1995, and so we concluded that this numerical approach was equivalent to the approach used in EPA 1995.

The optimization was performed five times for each toxicity test, with five different pairs of starting values for $LC50_{\infty}$ and k so that the minimum SSR found by Solver was more likely to be the global minimum rather than a local minimum. The starting values for the two variables are shown in Table 2, and were selected to cover a wide range of the variable space. The $LC50_\infty$ and *k* values resulting from the calibrations that yielded the lowest SSR are reported in Table 1.

The new datasets provided averaging period information for an additional 32 toxicity tests covering an additional 19 species. These results combined with the copper invertebrate tests included in US EPA (1995) provide information from 46 tests, with 27 invertebrate species. These additional data triple the number of averaging period estimates, and triple the number of invertebrate species used to evaluate the suitability of a 24-hour averaging period. The copper invertebrate data from US EPA (1995) and from this review are summarized in Table 1. Table 1 also includes the calculated averaging periods for the studies in the updated dataset. Averaging periods for these additional data ranged from 18 to 240 hours with a mean of 76 hours.

CONCLUSION

The new data provided in this analysis provide similar results to those presented in EPA 1995. Averaging periods for a wide range of invertebrate species calculating using the approach recommended in US EPA (1991), ranged from 17 to 240 hours with an overall mean averaging period of 66 hours. This range in averaging periods demonstrate that a 1-hour averaging period is overly conservative, and that the 24-hour averaging period recommended in the 2007 copper criteria document would be suitably protective for sensitive invertebrates.

Table 1. Summary of calculated averaging periods from copper toxicity tests for freshwater invertebrate species in US EPA 1995, and in additional literature included in this review.

Citation	**Species**	**Comments**	**Averaging period (hours)**
Rehwoldt 1973	*Amnicola sp.*	Included in US EPA 1995	28
Gutierrez 2012	*Argyrodiaptomus falcifer*		>48
Strode & Balode 2013	*Bathyporeia pilosa (Lindstrom, 1855)*		>96
Bellavere & Gorbi 1981	*Biomphlaria glabrata*		19
Rehwoldt 1973	*Caddisfly*	Included in US EPA 1995	37
Gutierrez 2012	*Ceriodaphnia duba*		>48
Taylor et al. 1991	*Chironomus riparius*		>240
Rehwoldt 1973	*Chironomus sp.*	Included in US EPA 1995	>48
Martin & Holdich 1986	*Crangonyx pseudogracilis*	Included in US EPA 1995	>96
Rehwoldt 1973	*Damselfly*	Included in US EPA 1995	50
Gutierrez 2012	*Daphnia magna*		<24
Adema & Degroot 1972	*Daphnia magna*	Included in US EPA 1995	22
Adema & Degroot 1972	*Daphnia magna*	Included in US EPA 1995	31
Dave 1984	*Daphnia magna*	Included in US EPA 1995	>48
Cabejszek & Stasiak 1960	*Daphnia magna*	Included in US EPA 1995	>48
Dave 1984	*Daphnia magna*	Included in US EPA 1995	>48
Cairns et al. 1978	*Daphnia magna*	Included in US EPA 1995	<24
Cairns et al. 1978	*Daphnia pulex*	Included in US EPA 1995	>48
Charles et al. 2013	*Gammarus pulex*		<24
Güven et al. 1999	*Gammarus pulex*		33
Taylor et al. 1991	*Gammarus pulex*		<48

Citation	Species	Comments	Averaging period (hours)
Vincent et al. 1986 (via Charles et al. 2013)	*Gammarus pulex*		73
Strode & Balode 2013	*Gammarus pulex*		>96
Strode & Balode 2013	*Gammarus pulex (Linnaeus, 1758)*		75
Rehwoldt 1973	*Gammarus sp.*		17
Moon & Wozniewski	*Gammarus sp. (female)*		>96
Moon & Wozniewski	*Gammarus sp. (male)*		>96
Strode & Balode 2013	*Gammarus tigrinus (Sexton, 1939)*		>96
Stephenson 1983	*Gammerus pulex*	Included in US EPA 1995	>48
Stephenson 1983	*Gammerus pulex*	Included in US EPA 1995	>48
Strode & Balode 2013	*Hyalella azteca*		<48
Karntanut & Pasco 2002	*Hydra oligactis*		41
Karntanut & Pasco 2002	*Hydra viridissima*		>96
Karntanut & Pasco 2002	*Hydra vulgaris*	there are two stains of Hydra vulgaris	85
Beach & Pascoe 1998	*Hydra vulgaris*		>96
Karntanut & Pasco 2002	*Hydra vulgaris (Zurich)*	there are two stains of Hydra vulgaris	>96
Khangarot & Ray 1988	*Lymnaea luteola*		>96
Strode & Balode 2013	*Monoporeia affinis*		>96
Strode & Balode 2013	*Monoporeia affinis (Lindstrom, 1855)*		>96
Rehwoldt 1973	*Nais sp.*	Included in US EPA 1995	>48
Gutierrez 2012	*Notodiaptomus conifer*		>48
Strode & Balode 2013	*Pontogammarus robustoides*		>96

Citation	**Species**	**Comments**	**Averaging period (hours)**
Strode & Balode 2013	*Pontogammarus robustoides (Sars, 1894)*		<48
Gutierrez 2012	*Pseudosida variabilis*		>48
Rathore & Khangarot 2003	*Tubifex tubifex (Hard)*		>96
Rathore & Khangarot 2003	*Tubifex tubifex (Soft)*		>96
Rathore & Khangarot 2003	*Tubifex tubifex (Very hard)*		>96
Rathore & Khangarot 2003	*Tubifex tubifex (Very soft)*		57

Table 2. Values for five different initial conditions used for the optimization of $LC50_\infty$ and 1/k.

Calibration	**Starting $LC50_\infty$ (µg/L)**	**Starting 1/k (hours)**
1	= min(reported LC50s) / 2	= 1 / 24
2	= min(reported LC50s) * 5	= 2
3	= min(reported LC50s) * 5	= 1000
4	= min(reported LC50s) / 200	= 2
5	= min(reported LC50s) / 200	= 1000

References

Beach, M. J. and D. Pascoe, 1998. The role of *Hydra vulgaris (pallas)* in assessing the toxicity of freshwater pollutants, Water Research, Vol. 32, iss. 1, pp. 101-106.

Bellavere, C. and J. Gorbi, 1998. A comparative analysis of acute toxicity of chromium, copper and cadmium to *Daphnia magna, Biomphlaria glabrata*, and *Brachydanio rerio*, Environmental Technology Letters, Vol. 2, pp. 119-128.

Charles, J., G. Crini, F. Degiorgi, B. Sancey, N. Morin-Crini, and P. Badot, 2013. Unexpected toxic interactions in the freshwater amphipod *Gammarus pulex (L.)* exposed to binary copper and nickel mixtures, Environ Sci Pollut Res, doi: 10.1007/s11356-013-1978-1

Gutierrez, M. F., 2012. Impacto de xenobióticos y comunicadores químicos sobre algunos procesos biológicos en organismos del zooplancton [Impact of xenobiotics and chemical communicators on some biological processes in zooplankton organisms] (Thesis), Universidad Nacional Del Litoral, Santa Fe, Argentina.

Güven, K., C. Özbay, E. Ünlü, A. Satar, 1999. Acute lethal toxicity and accumulation of copper in *Gammarus pulex (L.) (Amphipoda)*, Tr. J. of Biology, Vol. 23, pp. 513-521.

Karntanut, W. and D. Pascoe, 2002. The toxicity of copper, cadmium and zinc to four different Hydra *(Cnidaria: Hydrozoa)*, Chemosphere, Vol. 47, 1059-1064.

Khangarot, B. S., and P. K. Ray, 1988. Sensitivity of freshwater pulmonate snails, *Lymnaea luteola L.,* to heavy metals, Bulletin of Environmental Contamination and Toxicology, vol. 41, pp. 208-213.

Moon, Y. J. and L. Wozniewski. Evaluation of the acute toxicity of copper sulfate to *Gammarus*, a freshwater amphipod over 4 days.

Rathore, R. S., and B. S. Khangarot, 2003. Effects of water hardness and metal concentration on a freshwater *Tubifex tubifex* Muller, Water Air and Soil Pollution, Vol. 142, pp. 341-356.

Strode, E. and M. Balode, 2013. Toxico-resistance of Baltic amphipod species to heavy metals, Crustaceana, Vol. 86, Iss. 7-8, pp. 1007-1024.

Taylor, E. J., S. J. Maund, and D. Pascoe, 1991. Toxicity of four common pollutants to the freshwater macroinvertebrates *Chironomus riparius Meigen (Insecta:Diptera)* and *Gammarus pulex (L.) (Crustacea:Amphipoda)*, Archives of Environmental Contamination and Toxicology, Vol. 21, pp. 371-376.

US EPA, (1985). Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. PB85-227049. Washington, DC, Office of Research and Development, US Environmental Protection Agency**:** 98.

US EPA (1991). Technical support document for water quality-based toxics control. EPA/505/2-90-001. PB91-127415. Washington, DC, Office of Water, US Environmental Protection Agency.

EPA (2001). 2001 Update of ambient water quality criteria for cadmium. EPA-822-R-01-001. Washington, DC, US Environmental Protection Agency.

EPA, U. (2007). Aquatic life ambient freshwater quality criteria - copper, 2007 revision. EPA-822-R-07-001. Washington, DC, Office of Water, US Environmental Protection Agency

USEPA (1985). Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. PB85-227049. Washington, DC, Office of Research and Development, US Environmental Protection Agency**:** 98.

Vincent M, Debord J, Penicaut B (1986) Comparative study of the toxicity of metal chlorides and a synthetic organic molluscacide, N-tritylmorpholine, in 2 fresh-water amphipods, *Gammarus pulex* and *Echinogammarus berilloni.* Ann Rech Vet 17:441–446