Effect of pH and Dissolved Organic Carbon on the Toxicity of Copper to Larval Fathead Minnow (*Pimephales promelas*) in Natural Lake Waters of Low Alkalinity

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The impacts of pH and dissolved organic carbon (DOC) on the acute toxicity of Cu to larval fathead minnow (*Pimephales promelas*) were determined using natural soft water from two Precambrian Shield lakes in south-central Ontario. By artificially manipulating the pH and DOC levels of the water, we demonstrated that both acidification and the removal of DOC increased the toxicity of Cu. The 96-h Cu LC50s were determined over a pH range from 5.4 to 7.3 and a DOC concentration range from 0.2 to 16 $mg\cdot L^{-1}$. The LC50s ranged from a low of 2 $\mu g\cdot L^{-1}$ (pH 5.6, DOC 0.2 $mg\cdot L^{-1}$) to a high of 182 $\mu g\cdot L^{-1}$ (pH 6.9, DOC 15.6 $mg\cdot L^{-1}$). A multiple regression model ($\log_{10}$96-h Cu LC50 = $-0.308 + 0.192$ pH + 0.136 (pH$\cdot\log_{10}$DOC)) was used to describe the relationship between Cu toxicity, pH, and DOC. The model was significant ($p < 0.00001$) and explained 93% of the variability in the toxicity data. These results suggest that current water quality objectives for Cu, and possibly for other metals, may not be sufficiently protective of aquatic life in soft, moderately acidic water containing low levels of DOC.

Les impacts du pH et du carbone organique dissous (COD) sur la toxicité aiguë du Cu pour les larves du tête-de-boule (*Pimephales promelas*) ont été déterminés au moyen d'une eau douce naturelle qui provient de deux lacs situés dans le Bouclier précambrien, dans la partie centre-sud de l'Ontario. En faisant varier artificiellement le pH et la teneur en COD de l'eau, nous avons montré que l'acidification ainsi que la suppression du COD se traduisaient par un accroissement de la toxicité du Cu. Les CL50 96-h ont été déterminées à l'intérieur d'une plage de pH et à l'aide de 5,4 à 7,3 et à l'interieur d'une plage de concentration du COD allant de 0,2 à 16 $mg\cdot L^{-1}$. La CL50 a varié entre 2 $\mu g\cdot L^{-1}$ (pH 5,6, COD 0,2 $mg\cdot L^{-1}$) et 182 $\mu g\cdot L^{-1}$ (pH 6,9 COD 15,6 $mg\cdot L^{-1}$). On a appliqué un modèle de régression multiple ($\log_{10}$CL50 96-h pour le Cu = $-0,308 + 0,192$ pH + 0,136 (pH$\cdot\log_{10}$COD)) pour décrire la relation entre la toxicité du Cu, le pH et le COD. Le modèle donne des résultats significatifs ($p < 0,00001$) et permet d'expliquer 93 % de la variabilité des données sur la toxicité. Ces résultats indiquent que les objectifs actuels de qualité de l'eau, dans le cas du cuivre, et peut-être d'autres métaux, peut ne pas protéger adéquatement les organismes aquatiques dans une eau douce et modérément acide, qui contient peu de COD.

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Acidification of Ontario lakes has been associated with the disappearance of a variety of fish species, including sports fish at pH 5.0–5.5 (Kelso et al. 1987) and forage fish such as cyprinids at pH 5.5–6.0 (Matusek et al. 1990). The loss of fish populations has consistently been linked to recruitment failure, primarily through mortality at the egg and fry stages (Peterson et al. 1982; Gunn and Keller 1984; Mills et al. 1987). Exposure to elevated H^+ and Al concentrations during spring runoff (Gunn and Keller 1984), long-term depression of pH (Holtze and Hutchinson 1989), and the interaction of H^+ with Al, Zn, and Cu (Hutchinson and Sprague 1986; Hickie et al. 1993) have all been suggested as possible factors contributing to recruitment failure.

The fathead minnow (*Pimephales promelas*), an important forage species found in Precambrian Shield waters, is known to be sensitive to acidification. Surveys of Ontario lakes (Matusek et al. 1990), the experimental acidification of Lake 223 (Mills et al. 1987), and laboratory testing (McCormick et al. 1989) have all confirmed that pH 6.0 marks the lower pH threshold for reproduction and survival of the species. Since fathead minnow spawn during the summer (Scott and Crossman 1973), their reproductive processes are not subject to the same degree to the spring pulses of acid and Al that may impair those processes in lake trout (*Salvelinus namaycush*) (Gunn and Keller 1984). Recruitment failure in minnow populations is therefore more likely associated with long-term exposure to low pH in conjunction with metal toxicity.

Hickie et al. (1993), working on the toxicity of metal mixtures to rainbow trout (*Oncorhynchus mykiss*) in low-alkalinity waters, reported that mixture toxicity was caused by Cu alone at pH 5.8 and by Al alone at pH 4.9. They suggested that Cu could contribute to impacts on fish populations in moderately acidified systems (i.e., at pHs above those associated with Al toxicity), especially for pH-sensitive species including the fathead minnow. Since other species, such as the crustacean *Daphnia galeata mendotae*, are also susceptible to direct pH effects at levels <6.0 in soft water (Keller et al. 1990), they too may be vulnerable to Cu toxicity.

Several factors can alter metal toxicity in surface waters, primarily by controlling the speciation and complexation of metals, and hence, their bioavailability to aquatic organisms. Since the inorganic complexation of metals by bicarbonate and hydroxide ions is minimal in the low-alkalinity waters characteristic of acid-sensitive areas (Campbell and Stokes 1985), the bioavailability of metals in these waters will be primarily dependent on pH and the complexation potential of dissolved and particulate organic carbon. The organic complexation of Cu is well documented (Sprague 1985). In addition, Hutchinson and Sprague (1987) demonstrated that organic complexation reduced the toxicity of a mixture of Al, Zn, and Cu to flagfish (*Jordanella floridae*) at pH 5.8.

Dissolved organic carbon (DOC) and pH vary widely in Ontario lakes. Neary et al. (1990) reported DOC concentrations ranging from 0.1 to 58.0 $mg \cdot L^{-1}$ in 3340 Ontario lakes and pH levels from 3.03 to 9.80 in 5982 lakes; the median values were 5.2 mg $DOC \cdot L^{-1}$ and pH 6.68. Of these lakes, 1970 were softwater acid-sensitive systems with conductivity levels <50 $\mu S \cdot cm^{-1}$. Within the acid-sensitive group, a subset of 777 lakes showed DOC concentrations ranging from 0.1 to 23.5 $mg \cdot L^{-1}$, with a median of 3.8 $mg \cdot L^{-1}$, and widely varying levels of pH (Spry and Wiener 1991). Apparently the pH and DOC levels in Ontario lakes are sufficiently variable to allow for significant expression of metal toxicity to acid-sensitive species of aquatic organisms.

This study extends the work of Hickie et al. (1993) to more fully understand whether Cu toxicity is a factor in the survival of fathead minnow larvae in acidified soft waters. We determined the acute toxicity of total Cu to larval fathead minnow in low-alkalinity water as a function of both pH and DOC concentration. The null hypothesis was that the toxicity of Cu would be unaffected by pH and DOC levels typical of Ontario's softwater lakes.

Materials and Methods

Four-day static-exposure acute-lethality toxicity tests were conducted with larval fathead minnow (<1 d old) at the Dorset Research Centre, Ontario Ministry of Environment and Energy, Dorset, Ont. The fish were exposed to Cu at varying levels of DOC and pH in water from either St. Mary's Lake (45°06′N, 79°32′W) or Brandy Lake (45°13′N, 79°32′W), both of which are low-alkalinity systems (Table 1). LC50s were calculated for each treatment using measured total Cu concentrations and were related to levels of pH and/or DOC using geometric mean regression analysis.

All test fish were cultured in St. Mary's Lake water. Water from both lakes was used for the toxicity tests, either directly or after stripping of the DOC by charcoal filtration (Table 1). To achieve the latter, 20-L batches of water were pumped at 2 $L \cdot min^{-1}$ through 6- to 14-μm-mesh activated charcoal (Fisher Scientific Ltd., Toronto, Ont.) held in a 3-L rechargeable polypropylene filter, followed by a 3-μm paper-filter cartridge (Aqua-pure, CUNO, Meridin, Conn.). The water was recirculated until colour was no longer visible under fluorescent light, 15–24 and 40–48 h for St. Mary's Lake and Brandy Lake water, respectively. The water was aerated vigorously to remove carbon dioxide and to stabilize pH.

The pH of the water for the various treatments was adjusted with nitric acid or sodium hydroxide (both analytical grade, Fisher Scientific, Toronto, Ont.) before each bioassay and daily thereafter; pH never varied from nominal by >0.2 unit over a 24-h period. DOC concentrations required by the design were obtained by using filtered (0.4 mg $DOC \cdot L^{-1}$) or unfiltered (3.4 mg $DOC \cdot L^{-1}$) St. Mary's Lake water for the first series of experiments or, for later experiments, by combining filtered (0.8 mg $DOC \cdot L^{-1}$) and unfiltered (16 mg $DOC \cdot L^{-1}$) Brandy Lake water to give the following dilution series: 100% (16 mg $DOC \cdot L^{-1}$), 67% (10 mg $DOC \cdot L^{-1}$), 33% (5 mg $DOC \cdot L^{-1}$), and 0% (0.8 mg $DOC \cdot L^{-1}$).

The fathead minnow brood stock for this study was donated by Dr. J.B. Sprague, University of Guelph, Guelph, Ont., from a culture originally developed at the Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, Minn. Fish culture followed the protocol of Benoit (1981). Twenty-two spawning pairs were maintained, one pair in each half of a divided 20-L glass aquarium. Each aquarium was provided with 100 $mL \cdot min^{-1}$ of sand-filtered St. Mary's Lake water at 22 ± 0.5°C. The fish were fed frozen brine shrimp three times daily. When daily checks revealed eggs deposited on the underside of a spawning substrate, the entire substrate was removed to a separate hatching tank of identical water quality where aeration ensured adequate circulation past the eggs. Fry that hatched on any one day were pooled and used in the toxicity tests. The mean hatching success of the eggs was 87% (SD 11%, n = 21 clutches).

For each toxicity test, groups of 10 fry <1 day old were

TABLE 1. Water quality characteristics (mean (SD)) for St. Mary's Lake water ($n = 6$), Brandy Lake water ($n = 6$), unfiltered St. Mary's Lake water ($n = 2$), and charcoal-filtered St. Mary's lake water ($n = 2$). Between St. Mary's Lake and Brandy Lake water and between filtered and unfiltered St. Mary's Lake water, means with an asterisk are significantly different ($p \leq 0.05$). Values below the detection limit are indicated by nd.

Water quality characteristic	St. Mary's Lake[a]	Brandy Lake	St. Mary's Lake unfiltered[a]	St. Mary's Lake filtered
Al ($\mu g \cdot L^{-1}$)	nd	140[b]	3.8 (6.1)*	22.5 (17.4)*
Cd ($\mu g \cdot L^{-1}$)	nd	0.11[b]	nd	nd
Cu ($\mu g \cdot L^{-1}$)	2.3 (1.8)	1.9 (0.2)	1.8 (1.5)	0.8 (0.3)
Pb ($\mu g \cdot L^{-1}$)	0.3 (0.4)	0.5 (0.1)	0.1 (0.2)	0.6 (1.0)
Zn ($\mu g \cdot L^{-1}$)	nd	nd	nd	11.3 (16.4)
Ca ($mg \cdot L^{-1}$)	4.2 (0.1)	5.1 (0.3)	4.4 (0.4)	4.7 (0.5)
Cl^- ($mg \cdot L^{-1}$)	4.7 (0.0)	4.3 (1.3)	5.9 (3.1)	6.7 (1.5)
Mg ($mg \cdot L^{-1}$)	0.8 (1.1)	1.5 (0.1)	1.6 (0.1)	1.6 (0.1)
K ($mg \cdot L^{-1}$)	0.9 (0.2)	0.9 (0.1)	0.8 (0.1)	0.9 (0.3)[c]
Na ($mg \cdot L^{-1}$)	2.9 (0.1)	3.2 (0.6)	3.5 (1.8)	4.3 (1.1)[c]
SO_4 ($mg \cdot L^{-1}$)	8.4 (1.4)	8.8 (2.6)	8.3 (1.2)	6.8 (3.4)
Alkalinity ($mg \cdot L^{-1}$ as $CaCO_3$)	6.9 (0.1)	7.1 (1.3)	3.7 (3.2)	4.6 (4.5)
Conductivity ($\mu S \cdot cm^{-1}$)	55.4 (0.4)	56.3 (0.1)	61.8 (10.6)	69.6 (8.2)
Total hardness ($mg \cdot L^{-1}$ as $CaCO_3$)	16.8 (0.4)	19.0 (0.7)	17.3 (0.9)	18.5 (1.7)[c]
Colour (true colour units)	13.0 (1.4)*	150.5 (9.2)*	13.0 (3.7)*	1.8 (1.8)*[,c]
DOC ($mg \cdot L^{-1}$)	3.4 (0.1)*	15.8 (0.3)*	3.4 (0.4)*[,d]	0.4 (0.1)*[,e]

[a]Although these two columns are for water from the same source, the means for unfiltered St. Mary's Lake water represent samples taken at the same time as those for filtered St. Mary's Lake water to allow for a direct comparison regarding filtration effects.
[b]$n = 1$; no statistical comparison possible.
[c]$n = 5$.
[d]$n = 9$.
[e]$n = 7$.

gently transferred by a large-bore dropper (two or three at a time) to 100-mL polyethylene weigh boats. To begin a test, each group of 10 fish was randomly distributed to one of six treatments (five concentrations of Cu plus a control). The mean (SD) wet weight of fish used during the study was 0.68 (0.08) mg. The exposures used 2-L polypropylene beakers in which the ratio by weight of water to fish of approximately 300 000 : 1 (74 $L \cdot g^{-1}$ $fish \cdot d^{-1}$) far exceeded the minimum recommended by Sprague (1969).

Test solutions were prepared with copper sulphate ($CuSO_4 \cdot 5H_2O$, reagent grade; Fisher Scientific, Toronto, Ont.) 3–12 h before each test to allow pH and Cu to equilibrate. Five contiguous concentrations of Cu were selected from the geometric series 560, 320, 180, 100, 56, 33, 18, 10, 5.6, 3.2, and 1.8 $\mu g \cdot L^{-1}$ as indicated by preliminary range-finding tests. The test beakers were covered with glass plates to limit evaporation and airborne contamination and held in a water bath to maintain temperature (measured daily) at 22.0 ± 0.2°C. Although the beakers were not aerated, oxygen levels never fell below 80% saturation. The pH in each beaker was measured daily with a pH meter (model PHM82, Radiometer, Copenhagen, Denmark) attached to a chart recorder. Values were recorded when a stable reading had been established in still water after stirring. Fry were inspected for mortality after 1, 3, 6, 24, 48, 72, 96, and (in some cases) 120 h and were considered dead when fin or heart movement was no longer detectable under a dissecting microscope. The fish were not fed during the toxicity tests. Since fathead minnow have limited yolk reserves, feeding during chronic toxicity tests is usually initiated within 12 h of hatch (Environment Canada 1992). We chose not to feed the fish during these acute tests, since the metal-chelating effects of food in the test containers could have confounded the toxicity-modifying effects of DOC. Food deprivation probably contributed to the control mortality outlined below.

Batch samples of dilution water were taken prior to the start of each toxicity test (after pH adjustment) for full chemical characterization, including determination of DOC concentration and alkalinity (Table 1). Water samples for analysis of total Cu concentration were collected from each test container at the beginning and end of each toxicity test. All samples were stored in linear polyethylene bottles pending analysis by the Laboratory Services Branch, Ontario Ministry of Environment and Energy, Rexdale, Ont. All sample containers, as well as the test beakers, were soaked for approximately 1 min in 5% nitric acid and rinsed 7–10 times in glass-distilled water before use. Cu concentrations in the rinse water were typically <1 $\mu g \cdot L^{-1}$ and were often undetectable. Total Cu concentrations were determined by graphite-furnace atomic absorption spectrophotometry (detection limit 0.3 $\mu g \cdot L^{-1}$, sensitivity ± 1.0 $\mu g \cdot L^{-1}$), alkalinity by inflection-point acid titration, and DOC by colorimetric reaction after inorganic carbon was removed and the sample digested by ultraviolet light. All analytical procedures conformed to standard protocols of the Laboratory Services Branch, Ontario Ministry of Environment and Energy (Locke and Scott 1986). Analytical blanks appropriate to the design were completed in conjunction with all chemical analyses.

During the toxicity tests, background Cu levels in the controls were consistently <3 $\mu g \cdot L^{-1}$. Assayed Cu concentrations were, on balance, 96% of nominal. The percent difference between actual and nominal values ranged between −70 and 30% at concentrations ≤10 μg·L⁻¹ and between −10 and 20% at concentrations >10 μg Cu·L⁻¹.

The waters from St. Mary's and Brandy lakes were similar, except that Brandy Lake had significantly higher levels of colour ($p < 0.05$) and DOC ($p < 0.01$) (Table 1). Brandy Lake also had elevated levels of Al, although, owing to low sample sizes, it was not possible to test for the statistical significance of the difference. Charcoal filtration of St. Mary's Lake water resulted in significant decreases in colour (sevenfold) and DOC concentration (eightfold) ($p < 0.001$) and a significant increase (fivefold) in Al concentration ($p < 0.05$). All other measured water characteristics did not change significantly (Table 1). The large standard deviations associated with alkalinity can be attributed to the pH adjustment of the water before each test. Low variability in the hardness and conductivity values, however, indicates that the "softness" of the water did not change appreciably. While Al was the only metal to increase significantly after charcoal filtration, an increasing trend was also observed with Pb and Zn. This increase of the three metals in the filtered water indicates that they were being leached out of the charcoal filter. The concentrations of Cu and Cd in the water were not significantly different after filtering (Table 1). Although similar changes in water quality were evident in Brandy Lake water after filtration, a sample size of 1 precluded statistical comparisons.

Median lethal concentrations (96-h LC50s) were calculated with mortality data and measured total Cu concentrations by the trimmed Spearman–Karber method (Hamilton et al. 1977). For two of the tests, 96-h LC50s could not be calculated, since the lowest exposure concentration showed >50% mortality at 96 h. The lowest exposure concentrations were substituted; the actual 96-h LC50s would be lower. No corrections were made for control mortality, which occurred in 11 of the 21 tests with Cu. Of the 11 tests in which control fish died, eight showed a mortality level of 10% (1 fish); the remaining three had mortality of 10–30%. In all cases, control mortality occurred at the end of the tests (between 72 and 120 h) while mortality in the Cu-exposed groups occurred before 96 h and usually before 72 h. Since control mortality occurred at the end of the tests, and since the fish were not fed, we consider the mortality to be the result of starvation as opposed to an undefined toxicosis. To make correction for control mortality unnecessary, all test results (with two exceptions outlined above) are reported as 96-h LC50s. Since mortality ceased by 96 h in all of the Cu-treated groups, the LC50s are considered to be incipient lethal levels. The treatment LC50s were related to DOC and/or pH using geometric mean regression analysis (Halfon 1985), which assumes that both the X and Y variables are measured with error. The interactive effects of acidity and DOC were assessed by comparing the slopes of the relationship between LC50 and pH at the two DOC levels by analysis of covariance. The relationship between DOC and pH with Cu LC50 from both St. Mary's and Brandy lakes was determined by simple and stepwise multiple regression. Water characteristics (Table 1) were compared using Student t-tests. All statistical analysis were completed using SYSTAT (SYSTAT 5.0; Systat Inc., Evanston, Ill.).

Results

The acute toxicity of Cu to larval fathead minnow ranged from a low of 2 μg·L⁻¹ (96-h LC50 at pH 5.6 and 0.2 mg DOC·L⁻¹) to a high of 182 μg·L⁻¹ (96-h LC50 at pH 6.9 and 15.6 mg DOC·L⁻¹) (Table 2). Toxicity was a function of both the pH and the DOC of the test water. There was a significant positive relationship between the logarithm of the 96-h Cu LC50 and the pH of the test water at 3.4 mg DOC·L⁻¹ ($p = 0.00003$, $r^2 = 0.93$, $n = 9$). At 0.4 mg DOC·L⁻¹, however, the relationship between log LC50 and pH was marginally insignificant ($p = 0.066$, $r^2 = 0.52$, $n = 7$). At both DOC concentrations, the LC50s decreased (toxicity increased) as the pH of the water decreased (Fig. 1). At 0.4 mg DOC·L⁻¹ the LC50s ranged from 2.0 μg Cu·L⁻¹ at pH 5.6 to 8.2 μg Cu·L⁻¹ at pH 7.3, while at 3.4 mg DOC·L⁻¹, they ranged from 9.9 μg Cu·L⁻¹ at pH 5.5 to 60 μg Cu·L⁻¹ at pH 7.2. The slopes of the regression lines between LC50 and pH at the high and low DOC levels were not significantly different ($p = 0.06$). The potential differences in slope between the high- and low-DOC relationships were difficult to evaluate because of the elevated variability in the low-DOC relationship relative to the high-DOC relationship. The variability in the former is due largely to the LC50 value at pH 5.8. With this point removed, the relationship

TABLE 2. The 96-h Cu LC50s for larval fathead minnow over ranges of both pH and DOC in water from two different softwater lakes.

LC50 (μg·L⁻¹)	95% fiducial limits (μg·L⁻¹)	DOC (mg·L⁻¹)	Mean (SD) pH[b]
Low DOC,[a] St. Mary's Lake			
2.0	1.5–2.7	0.2	5.64 (0.03)
4.9	3.6–6.5	0.3	5.83 (0.06)
2.8	2.5–3.1	0.5	6.20 (0.08)
<3.5[c]	—	0.4	6.66 (0.01)
<4.5[c]	—	0.5	7.02 (0.06)
4.8	3.4–6.8	0.6	7.06 (0.02)
8.2	6.3–10.8	0.4	7.25 (0.02)
High DOC,[d] St. Mary's Lake			
11.1	8.9–13.8	3.3	5.40 (0.05)
9.9	8.0–12.1	3.1	5.49 (0.08)
15.7	13.3–18.5	3.1	5.85 (0.03)
15.1	12.1–18.8	3.3	5.85 (0.11)
31.6	26.6–37.5	3.3	6.36 (0.05)
21.1	18.1–24.6	3.1	6.42 (0.04)
36.0	31.3–41.3	4.3	6.38 (0.03)
47.7[e]	—	3.3	7.10 (0.06)
59.8	51.7–69.3	3.4	7.15 (0.02)
Brandy Lake			
4.8	3.9–6.0	0.8	7.16 (0.03)
70.3	57.2–86.4	5.1	7.13 (0.02)
85.6	69.1–101.1	10.5	7.06 (0.02)
105.4	89.2–124.7	16.0	7.05 (0.05)
182.0	146.0–225.5	15.6	6.90 (0.04)

[a]Mean (SD, n) DOC level: 0.4 (0.1, 7) mg·L⁻¹.

[b]The pH was measured daily in each test breaker and a mean calculated. These are the grand means for the six beakers used in each toxicity test.

[c]Calculation of a 96-h LC50 was not possible, since mortality at 96 h exceeded 50% in the lowest Cu exposure concentration. The value given here is the lowest exposure concentration; the 96-h LC50 would be lower.

[d]Mean (SD, n) DOC level: 3.4 (0.4, 9) mg·L⁻¹.

[e]There was an insufficient number of exposure concentrations with partial mortality to allow calculation of confidence limits by Spearman–Karber analysis.

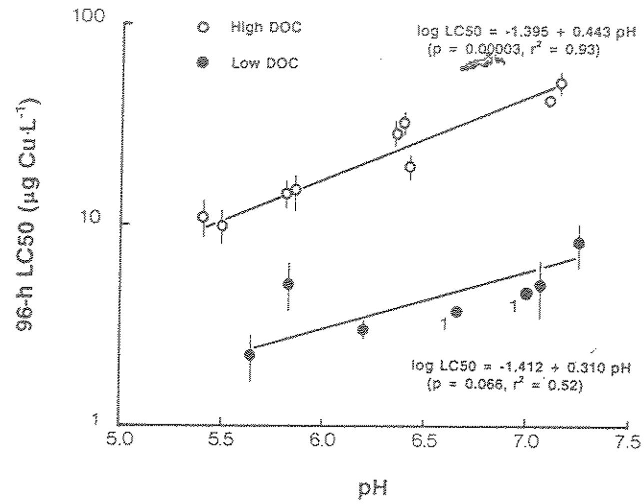

FIG. 1. Relationship between pH and the acute toxicity of Cu to larval fathead minnow at either a low (0.4 $mg \cdot L^{-1}$) or a high (3.4 $mg \cdot L^{-1}$) concentration of DOC in St. Mary's Lake water. With two exceptions, all values are given as 96-h LC50s with 95% fiducial limits. Two values (denoted by the number 1) are the lowest exposure concentrations in the toxicity tests, concentrations that showed >50% mortality at 96 h.

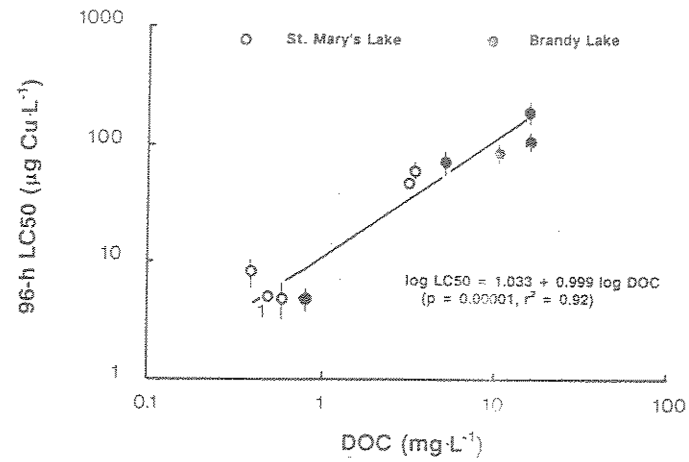

FIG. 2. Relationship between DOC concentration and the acute toxicity of Cu to larval fathead minnow at pH 7.0. The toxicity tests used water from either St. Mary's Lake or Brandy Lake. With one exception, all values are given as 96-h LC50s with 95% fiducial limits. One value (denoted by the number 1) is the lowest exposure concentration in the toxicity test, a concentration that showed > 50% mortality at 96 h.

between log LC50 and pH at the low DOC level was both significant and less variable ($p = 0.005$, $r^2 = 0.89$, $n = 6$). This second relationship was also not significantly different in slope from the high-DOC relationship ($p = 0.204$).

For two of the toxicity tests in filtered St. Mary's Lake water (Fig. 1 and 2), the lowest exposure concentrations were substituted for the 96-h LC50s; the actual 96-h LC50s would, of course, be lower. We included these values because of the low number of toxicity tests in filtered St. Mary's Lake water.

The logarithm of the 96-h Cu LC50 also had a significant linear relationship with the logarithm of the test water DOC concentration ($p = 0.00001$, $r^2 = 0.92$, $n = 10$) (Fig. 2). This relationship was determined at pH 7.0 using both St. Mary's Lake and Brandy Lake water; toxicity consistently increased (LC50 decreased) with decreasing levels of DOC. The LC50s ranged from 4.8 $\mu g\ Cu \cdot L^{-1}$ at 0.6 $mg\ DOC \cdot L^{-1}$ to 182 $\mu g\ Cu \cdot L^{-1}$ at 15.6 $mg\ DOC \cdot L^{-1}$.

A stepwise multiple regression model was used to describe the modification of the 96-h LC50 of Cu for fathead minnow by pH and DOC concentration. The equation for the model, which was significant ($p = 0.00001$) and explained 93% of the variability in the data, was

$$\log(96\text{-h LC50}) = -0.308 + 0.192\text{pH} + 0.136(\text{pH} \cdot \log_{10}\text{DOC}).$$

The model applies over the pH range from 5.4 to 7.3 and the DOC range from 0.2 to 16.0 $mg \cdot L^{-1}$. Although the main effects model (i.e., no interaction term) was also highly significant, it had slightly less predictive capacity ($p = 0.00001$, $r^2 = 0.91$).

Discussion

By selectively removing DOC from low-alkalinity lake water, we generated conditions that allowed us to determine the impact of DOC on the toxicity of Cu to fathead minnow over a pH range from circumneutral to the lower range of H^+ tolerance for the species, pH 5.5. The results indicate that we can reject our original null hypothesis and affirm that both pH and DOC affect the toxicity of Cu and do so at levels typical of softwater lakes.

The linear relationships between log toxicity and both pH and log DOC indicate that the H^+ concentration and the binding properties of the Cu molecule to DOC are the primary factors associated with the acute toxicity of Cu to fathead minnow in soft water. This observation is reinforced by the high correlation coefficient and significance that was obtained with the multiple regression model examining the combined effects of both pH and DOC concentration. An interaction between pH and DOC was detected in describing the toxicity of Cu in both Brandy Lake and St Mary's Lake water. This interaction indicates that the binding specificity of the DOC molecules to Cu changes with increases in H^+ concentration. Since the addition of the interaction term to the multiple regression model only marginally increased the predictive power over the main effects model, the effect of H^+ appears to be relatively limited. We did not detect interaction between pH and DOC when Cu toxicity was examined at the two levels of DOC in St. Mary's Lake water (i.e, the slopes of the lines in Fig. 1 were not significantly different). The inability to detect an interaction might have been due to the sensitivity of the Cu analysis ($\pm 1\ \mu g \cdot L^{-1}$) combined with the small differences in measured LC50s at low DOC. Although Meador (1991) and O'Shea and Mancy (1978) also provided some evidence for a pH effect on Cu complexation by DOC, the evidence is inconclusive.

All of the toxicity tests performed in filtered St. Mary's Lake water (DOC 0.4 $mg \cdot L^{-1}$) gave 96-h LC50s below 10 $\mu g\ Cu \cdot L^{-1}$. For the most extreme conditions (DOC 0.2 $mg \cdot L^{-1}$, pH 5.6), acute toxicity was observed at 2 $\mu g\ Cu \cdot L^{-1}$. Similar levels of Cu toxicity were reported by Cusimano et al. (1986) in artificial soft water (alkalinity 11 $mg \cdot L^{-1}$). They observed acute toxicity with 3-mo-old rainbow trout at 4.2 and 2.8 $\mu g\ Cu \cdot L^{-1}$ at pH 5.7 and 7.0, respectively. The bulk of the studies on the significance of organic molecule impacts on metal ion toxicity were completed with synthetic ligands in artificial or reconstituted water at pH >7.0 (Borgmann 1983; Winner 1985; Starodub et al. 1987; Meador 1991). Despite these differences from the work reported here, some interesting parallels are apparent. Meador (1991) found that both pH and naturally derived DOC were important factors controlling the amount of free Cu ion (Cu^{2+}) present in a synthetic water at pH >7.0 and that both were important in predicting Cu toxicity to *Daphnia magna*. When toxicity was expressed as free Cu^{2+} (as opposed to total Cu), however, no relationship between toxicity and DOC was apparent. Hutchinson and Sprague (1987), working with a mixture of Al, Zn, and Cu, also found no relationship between DOC and the toxicity of Al and Cu when toxicity was expressed in terms of free (dialysed) metal. However, both of the studies outlined above reported decreasing toxicity with increasing DOC concentration when toxicity was expressed in terms of total metal, as does our work. Although it is apparent that organic complexation of metals will reduce their acute toxicity to aquatic organisms, the potential contribution of some organometallic complexes to toxicity, particularly during chronic exposure, remains unclear (Borgmann 1983; Meador 1991).

Changes in the amount of free Cu^{2+} in solution will affect the amount of Cu that is bioavailable and hence its toxicity. The reduction in toxicity with increased DOC is probably due to a decrease in the amount of the toxic free metal ion through complexation with DOC. The amount of Cu bound to the DOC molecule would be expected to decrease as the H^+ concentration increased, owing to competition for binding sites. If H^+ displaces Cu^+ from the sites, an increase in free Cu^+, and hence toxicity, would be expected to occur with decreased pH. H^+ itself may, of course, be exerting a direct toxic impact on fish. We determined the incipient lethal level (ILL) of pH for the fathead minnow to be approximately 5.3 (unpublished data). Hickie et al. (1993) reported an ILL of 5.54 for the same species. Therefore, at the low-pH Cu tests in our study, we were close to the lower limit of lethal pH tolerance. Although we did not observe any direct toxicity from H^+ alone, sublethal stress due to H^+ probably occurred in the tests. Since both H^+ and Cu^{2+} are ionoregulatory toxicants, the LC5Os in the low pH range are probably a function of joint H^+–Cu^{2+} toxicity. Interaction of sublethal levels of H^+ with other ionoregulatory stressors, including Al (Holtze and Hutchinson 1989) and mixtures of Al, Zn, and Cu (Hutchinson and Sprague 1989), has been reported for several fish species. Hickie et al. (1993) demonstrated that lethality in the latter study could be attributed to Cu–H^+ interactions. Similarly, the very low ILLs that we obtained in the low-pH, low-DOC treatments might have been due to H^+ stress.

There is also the possibility, although slight, that the elevated background levels of Al in Brandy Lake and St. Mary's Lake water (Table 1) contributed to the high toxicity noted at the lower end of the DOC and pH ranges tested. We previously reported (Hickie et al. 1993) on the toxicity of metal mixtures to rainbow trout in soft, acidic water. Tests with individual metals showed that mixture toxicity was caused by Cu alone at pH 5.8 and by Al alone at pH 4.9. Measures of metal bioavailability at pH 4.9 and 5.8 using dialysis supported these findings. It can be inferred that as pH rises from 4.9 to 5.8, a shift from Al to Cu toxicity occurs. Since some of the toxicity tests reported here were completed between pH 5.4 and 5.8, some contribution from Al is possible. Above pH 5.8, however, Al would not be a factor in observed toxicity. A more extensive discussion of the toxic interactions of Al, Cu, and H^+ is given in Hickie et al. (1993).

Our findings illustrate that acute Cu toxicity increases as H^+ concentration increases (pH decreases) with the DOC held at either 0.4 or 3.4 $mg \cdot L^{-1}$ (Fig. 1). At no point did H^+ protect the fish against Cu toxicity. Campbell and Stokes (1985) noted that at higher concentrations of H^+, the H^+ should increasingly protect the fish from metal toxicosis by protonating the gill surface and thereby decreasing metal uptake. Cusimano et al. (1986) were able to demonstrate this reduction in metal toxicity at pHs lower than those used in this study. The effect was observed between pH 5.7 and 4.7, where the 96-h LC50s were 4.2 and 66 $\mu g\ Cu \cdot L^{-1}$, respectively. Since Cusimano et al. (1986) used steelhead trout, a more acid-tolerant species than the fathead minnow, the lethality of H^+ did not overshadow the gill protonation effect. For an acid-sensitive species at sublethal levels of H^+, however, the joint action of H^+ and Cu to produce toxicity will be more important than H^+–Cu competition for gill-binding sites.

It has been clearly established that free Cu^{2+} and copper hydroxides (at pH >7.0) are the primary Cu species resulting in toxicity to fish. At the pHs used in this study, toxicity will be a function of free Cu^{2+}, or more specifically, of competition between Cu^{2+} and H^+ for DOC binding sites. As the binding sites on the DOC molecule become saturated with Cu, the free Cu^{2+} concentration increases to the threshold of toxicity. The work reported here suggests a threshold between 2 and 4.8 $\mu g\ Cu \cdot L^{-1}$ and most likely closer to 4.8 $\mu g \cdot L^{-1}$ in the absence of H^+ stress (pH 7.0). This is based on the LC50 of 4.8 $\mu g\ Cu \cdot L^{-1}$ at pH 7.0 and minimal complexation (0.6 mg $DOC \cdot L^{-1}$) as well as the LC50 of 2.0 $\mu g\ Cu \cdot L^{-1}$ at pH 5.6 and 0.4 mg $DOC \cdot L^{-1}$. A threshold of 2–4.8 $\mu g \cdot L^{-1}$ is also consistent with the work of Hutchinson and Sprague (1987) who reported an LC50 of 2.3 $\mu g\ Cu \cdot L^{-1}$ for flagfish at pH 5.8 and <0.3 mg $DOC \cdot L^{-1}$.

Few studies examining the toxicity of Cu to aquatic organisms report results obtained in waters with an alkalinity <30 $mg \cdot L^{-1}$ as $CaCO_3$, a level that exceeds those in thousands of Canadian lakes. One of the most troublesome aspects of the work reported here, from the perspective of defining and limiting the environmental impacts of metals, is the extreme toxicity of Cu in water of low alkalinity (7 $mg \cdot L^{-1}$ as $CaCO_3$), pH, and DOC; the 96-h LC50 at pH 5.6 and 0.2 mg $DOC \cdot L^{-1}$ was 2 $\mu g\ Cu \cdot L^{-1}$. This level of acute toxicity is the same or very close to criteria for the protection of aquatic life established by several federal and provincial regulatory agencies (CCREM 1987). Since criteria are normally based on chronic toxicity data, which tend to provide the most sensitive endpoint, our acute lethality data bring into question the level of protection that current regulatory guidelines afford to aquatic life in softwater systems.

In summary, we have a model, based on pH and DOC, that will predict the toxicity of Cu to the fathead minnow in low-alkalinity systems. We are now in a position to test the model predictions by undertaking Cu toxicity tests with fathead minnow in water from a number of lakes with naturally different (as opposed to experimentally manipulated) levels of DOC.

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