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REGIONAL COPPER-NICKEL STUDY

AQUATIC TOXICOLOGY PROGRESS REPORT

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Aquatic Toxicology Progress Report

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SUMMARY

Fathead minnows and Daphnia pulicaria were exposed to mixtures of copper and nickel in water from Lake Superior and the South Kawishiwi River. The acutely toxic concentration of a copper-nickel mixture to the fathead minnow was approximately half of what it would have been if copper and nickel were additive in their joint effect. This observation held true in both test waters despite the difference in the relative potency of copper and nickel in the two test waters.

The joint lethal effect of copper and nickel on Daphnia pulicaria was slightly more than additive in Lake Superior water, and additive or slightly less than additive in South Kawishiwi River water, although these departures from additivity were probably not large enough to have practical significance. Variations in the ratio of copper concentration to nickel concentration among bioassays conducted with Daphnia in South Kawishiw River water were unrelated to variations in LC50.

Results of copper and nickel bioassays using fathead minnows and Daphnia pulicaria in different surface waters were used to develop models for predicting copper and nickel toxicity to these species in natural waters. Hardness, alkalinity, pH and total organic carbon (TOC) were considered as potential predictors of LC50. TOC was the best predictor of copper toxicity to both species. Hardness was the best predictor of acute nickel toxicity to the fathead minnow. For Daphnia pulicaria, which were exposed to nickel in an additional surface water, hardness together with TOC were the best predictors of nickel toxicity. Dummy x-variables were used to show that for both species there was significant variation in copper toxicity among test waters which was not explained by variations in TOC.

Toxicity prediction models were also fitted after deletion of the data from bioassays in Lake Superior water. Wtihout the Lake Superior data, TOC remained the best predictor of copper toxicity to both species and assumed more importance in prediction of nickel toxicity, replacing hardness as the best predictor of nickel toxicity to the fathead minnow.

Bioassays of three copper-nickel leachates were conducted with Daphnia pulicaria. In none of the nine bioassays was a leachate shown to be more toxic than predicted, although the first three bioassays of the U.S. Steel leachate, in which all test animals died, could not have detected greater-than-predicted toxicity. In six of the bioassays, toxicity was less than that predicted on the basis of total metal concentrations. Four of these discrepancies can be satisfactorily explained by the presence of substantial proportions of suspended copper and nickel, but in the last two tests of U.S. Steel leachate, no ready explanation can be found for the difference between observed and predicted toxicity.

In three separate 30-day experiments, fathead minnows were exposed to copper, nickel and cobalt in Lake Superior water from 1 day after fertilizatior until the early juvenile life stage. Copper reduced growth of young fish at a concentration of 13.1 μg/l. A nickel concentration of 433.5 μg/l reduced survival of embryos and young fish. Survival of young fish was reduced at a cobalt concentration of 48.7 μg/l.

ACUTE TOXICITY OF COPPER-NICKEL MIXTURES

The likelihood that copper, nickel and other heavy metals would occur together in water effluents from copper-nickel mining and ore processing necessitates an understanding of the joint toxicity of heavy metals. In a previous report, the literature dealing with toxic effects of metal mixtures was surveyed (Regional Copper-Nickel Study 1978). In the present study we examined the acute toxicity of copper-nickel mixtures to the fathead minnow, *Pimephales promelas*, and the cladoceran *Daphnia pulicaria* in water from Lake Superior at the ERL-Duluth intake and from the South Kawishiwi River near the State Highway 1 crossing.

96-hour LC50's of copper sulfate, nickel sulfate and copper-nickel mixtures for the fathead minnow were determined using continuous-flow dilution apparatus at a temperature of 25 C. for *Daphnia pulicaria*, 48-hour LC50's of copper sulfate, nickel sulfate and copper-nickel mixtures were determined in unrenewed solutions at 18 C. Dissolved oxygen concentrations were near saturation in all experiments. Total metal concentrations and pH in all treatment levels, and hardness, alkalinity and total organic carbon (TOC) in controls were determined at least once in each bioassay. Experimental and analytical methods were described in greater detail in the Aquatic Toxicology Operations Manual.

With each test species two or more bioassays of each metal were conducted. Weighted mean LC50's were calculated for each species and each metal. Because the precision of individual LC50 estimates varied, each LC50 was assigned a weighting coefficient equal to the LC50 divided by its 95% confidence interval. For each species and each test water a copper-nickel potency ratio (copper LC50/nickel LC50) was calculated from these weighted means.

The relative concentrations of copper and nickel in bioassays of copper-nickel mixtures were set in most cases according to their relative potencies. For each treatment level, the desired nickel concentration multiplied by the potency ratio equaled the desired copper concentration. For a variety of reasons, the ratios of measured copper concentration to measured nickel concentration in some mixture bioassays were quite different from the potency ratios, but concentration ratios within a bioassay were consistent. Total toxicant concentrations in mixture bioassays were expressed as copper. The nickel concentration in each treatment was multiplied by the potency ratio and the product was added to the copper concentration. If a mixture LC50, expressed as copper, equaled the weighted mean copper LC50 determined earlier, it was concluded that the joint effects of copper and nickel were additive.

Table 1 gives the weighted means of the copper LC50's and nickel LC50's for both test species in both test waters, as well as the potency ratio (copper LC50/nickel LC50) for each species in each water. Tables 2 and 3 give the individual LC50's from all bioassays of copper-nickel mixtures.

It is apparent from Table 2 that the joint toxicity of copper and nickel to the fathead minnow is more than additive in both test waters, since the ratio (mixture LC50 as Cu/copper LC50) would be unity if the combined toxicity were additive. The ratios of copper concentration to nickel concentration in each water were held fairly close to the copper-nickel potency ratios given in Table 1 for the fathead minnow.

The joint toxicity of copper and nickel to Daphnia pulicaria (Table 3) appears to be somewhat more than additive in Lake Superior water but additive or somewhat less than additive in South Kawishiwi River water. The ratios of copper concentration to nickel concentration in mixture bioassays conducted in South Kawishiwi River water varied above and below the copper-nickel potency ratio given in Table 1 for Daphnia pulicaria, but were not significantly correlated with LC50(r= -.369). TOC and hardness remained nearly constant during the entire experimental period in the South Kawishiwi River.

RELATIONSHIP OF RECEIVING WATER CHEMISTRY TO THE ACUTE TOXICITY OF COPPER AND NICKEL

Predictions of the vulnerability of aquatic communities to heavy metal pollution must take into account the relationship of water chemistry to the toxicity of heavy metals. A previous report surveyed the literature dealing with the effects of hardness, alkalinity, pH and organic substances on the toxicity of selected heavy metals to aquatic life. In this study we attempted to develop models for predicting the acute toxicity of copper and nickel to the fathead minnow Pimephales promelas and the cladoceran Daphnia pulicaria in surface waters of the copper-nickel study area, based on these water chemistry parameters.

96-hour LC50's of copper and nickel sulfates for the fathead minnow were determined using continuous-flow dilution apparatus at a temperature of 25 C. For Daphnia pulicaria, 48-hour LC50's of copper and nickel sulfates were determined in unrenewed solutions at 18 C. Dissolved oxygen concentratio were near saturation in all experiments. Total metal concentrations and pH in all treatment levels, and hardness, alkalinity and total organic carbon (TOC) in controls were determined at least once in each bioassay. Experimenta and analytical methods were described in greater detail in the Aquatic Toxicolo Operations Manual.

Continuous-flow bioassays with fathead minnows were conducted in water from Lake Superior, the South Kawishiwi River and the St. Louis River. Bioassays with Daphnia were conducted in these waters as well as in samples from Lake One, Colby Lake, Cloquet Lake and Greenwood Lake. Water chemistry parameters and LC50's are presented in Tables 4-7. For each metal and test organism, a forward selection procedure (Draper and Smith, 1966) was followed to choose the best model for the prediction of LC50 from the four water chemistry parameters. Transformations of variables were not considered. Because the precision of LC50 estimates was variable, each LC50 was given a weighting coefficient equal to the LC50 divided by its 95% confidence interval.

In copper bioassays with the fathead minnow (Table 4), TOC was the chemical variable most highly correlated with LC50 ($r=.9982$). LC50 was regressed on TOC along with each of the other variables made a significant contribution to the regression at the .10 level. Therefore, the best predictive model for acute copper toxicity to the fathead minnow was:

$$\text{LC50}(\mu\text{g Cu/l}) = -67.3 + 44.8\ (\text{TOC in mg/l})$$
$$R^2 = .9964$$

Dummy x-variables (Draper and Smith, 1966) were used to determine whether or not there were differences among the three test waters in their effects on LC50 which were not fully explained by the chosen model. The dummy variables made a significant contribution to the model ($\alpha = .05$), increasing R^2 to .9997. Inclusion of these dummy variables would therefore improve the fit of the model to the existing data but could not be used to enhance its predictive capability for other waters.

In copper bioassays with Daphnia (Table 5), TOC was the chemical variable best correlated with LC50 ($r=.7010$). Deletion of an outlier from the St. Louis River increased the correlation coefficient to .9637. Hardness, alkalinity and pH did not contribute significantly ($\alpha = .10$) to the regression of LC50 on TOC, so the best model for prediction of acute copper toxicity to Daphnia pulicaria was:

LC50 = -12.5 + 5.48 (TOC)
R^2 = .9287

Dummy x-variables were used to isolate the differences in LC50 among test waters which were not explained by TOC. Only Lake Superior, the South Kawishiwi River and the St. Louis River, where multiple bioassays were conducted, were i-cluded in this analysis. For the eight bioassays considered, the regression of LC50 on TOC had an R^2 value of .9519. Inclusion of the dummy variables increased R^2 to .9833. The contribution of the dummy variables was significant at the .025 level.

Next, the five LC50's in St. Louis river water including the outlier initially rejected were regressed against TOC, yielding a predictably low R^2 value of .3297. Because the high turbidity associated with periods of high river flow seemed to increase copper LC50's for Daphnia, and frequent turbidity measurements were not available, flow rate as measured by a nearby gauging station during each bioassay was entered into the regression of LC50 on TOC. Inclusion of the second x-variable increased R^2 to .9967 although flow rate alone was not significantly correlated with LC50 (r = .5590).

Of the four chemical variables considered in nickel bioassays with the fathead minnow (Table 6), hardness was best correlated LC50 (r = .8716). Alkalinity, pH and TOC did not add significantly (α = .10) to the regression of LC50 on hardness; therefore the best predictive model for the acute toxicity of nickel to the fathead minnow was:

LC50 = -1969 + 170 (hardness in mg/l as $CaCO_3$)
R^2 = .7597; significant at .025 level

Adding dummy x-variables representing test waters did not significantly improve the regression of LC50 on hardness (α = .10), indicating that deviations between fitted and observed LC50's were as great within the three blocks of bioassays from the three test waters as they were among the blocks, i.e. hardness explained variations in LC50 equally well both within blocks and among blocks.

Hardness was the chemical variable best correlated with LC50 (r = .9056) in nickel bioassays with Daphnia (Table 7). At the .05 significance level, alkalinity, pH and TOC did not contribute significantly to the regression of LC50 on hardness, but TOC added significantly to the regression at the .10 level.

Alternative models for predicting the acute toxicity of nickel to Daphnia pulicaria are:

LC50 (μgNi/l) = 372 + 32.8 (hardness in mg/l as $CaCO_3$)
R^2 = .8201; significant at .005 level

LC50 = 188 + 27.4 (hardness) + 22.1 (TOC)
R^2 = .8723; contribution of TOC significant at .10 level

Dummy x-variables representing test waters did not significantly improve the regression of LC50 on hardness and TOC (α =.10). The single experiment in Greenwood Lake water was not included in the data set for this analysis. The importance of TOC as a predictor of nickel toxicity to Daphnia but not fathead minnows is related to the fact that toxicity tests in Greenwood Lake water, which has low hardness and high TOC, were conducted only with Daphnia. Bioassays with fathead minnows were conducted in waters where hardness and TOC were highly correlated (r-.8362) so that the individual importance of each is difficult to discern.

Since Lake Superior does not lie within the Study Area, and it has somewhat different characteristics from the other waters discussed here, regression analyses were performed with the Lake Superior LC50's deleted from the data set. Without the Lake Superior data, TOC was again shown to be the only significant water chemistry parameter with respect to copper toxicity. The best prediction models were:

Fathead minow: $LC50 = -112 + 46.4(TOC)$

$R^2 = .9949$

Daphnia pulicaria: $LC50 = -32.1 + 6.17\ (TOC)$

$R^2 = .8527$

Without the Lake Superior data, the best model for predicting acute nickel toxicity to the fathead minnow was:

$LC50 = -4371 + 544\ (TOC)$

$R^2 = .8382$; significant at .05 level

The regression of LC50 on hardness fit the data nearly as well:

$LC50 = -1670 + 167\ (\text{hardness})$

$R^2 = .7423$; significant at .10 level

This model was nearly identical to the prediction model derived from the full data set.

The best model for predicting the acute toxicity of nickel to Daphnia pulicaria with Lake Superior data deleted was:

$LC50 = -362 + 22.9\ (\text{hardness}) + 50.2\ (TOC)$

$R^2 = .9398$; contribution of TOC significant at .025 level.

The x-variables in this model were the same as those in the two-variable model derived from the full data set, although the contribution of TOC to this model was greater.

ACUTE TOXICITY OF COPPER-NICKEL LEACHATES

Bioassays of three copper-nickel leachates were conducted with *Daphnia pulicaria*. 48-hour LC50's were determined in unrenewed dilutions of leachates at 18 C. Dissolved oxygen concentrations were near saturation in all bioassays. Metal concentrations in treatments were either measured directly or calculated from measured concentrations in leachates. If the concentration of a trace metal in dilution water was less than 2 μg/ℓ, its contribution to trace metal levels in leachate dilutions was ignored in calculations. pH was measured in all treatments, and hardness and organic carbon (TOC) in treatments was calculated from levels in leachate and dilution water.

Table 8 summarizes the chemical characteristics of leachate dilutions representing 48-hour LC50's, where LC50's could be determined. For bioassays in which full strength leachate was not lethal, or in which the weakest dilution was lethal to all the test animals, the chemical characteristics of those concentrations are listed. Predicted copper LC50's and predicted nickel LC50's in the listed dilutions were calculated from hardness and organic carbon using the models given in a previous section. It must be noted that none of the test waters in which the prediction models were derived had hardness levels as high as the Seep 3 or INCO leachates. Model predictions were assumed to hold beyond the range of hardnesses actually tested. Organic carbon in leachates and in the EM-6 dilution water was measured only in the dissolved form, whereas, the prediction models are based on total organic carbon measurements. Dissolved organic carbon levels were usually more than 90% of total organic carbon levels in the waters from which the prediction models were derived.

The combined concentration of copper and nickel in the listed leachate dilutions was expressed as copper using potency ratios derived from the prediction models and listed in Table 8. Mortality observed at this combined concentration could then be compared with the predicted copper LC50. The joint toxicity of copper and nickel in leachates was assumed to be additive. This assumption was consistent with the findings of the copper-nickel mixture bioassays reported in a previous section.

Chemical analyses of leachates and prediction models for copper and nickel toxicity were not available at the time most of the leachate bioassays were conducted. This made it difficult to select leachate dilutions necessary for conclusive test results.

Two bioassays were run using leachate from Seep 3 at Erie Mining Company's Dunka Mine. In the first, no mortality occurred in the undiluted leachate despite a combined copper-nickel concentration 3 times as great as the predicted lethal level. An explanation may be found in the fact that the leachate was muddied during sample collection, probably causing adsorption of metals onto suspended sediment particles. It was found that only 20% of the copper and 30% of the nickel in the leachate sample were present in the dissolved* fraction.

While the toxicity prediction models were derived from LC50's expressed as total metal, dissolved copper concentrations in the prediction experiments ranged from 80% to 100% of total copper, and dissolved nickel ranged from 90% to 100% of total nickel. Because of the discrepancy in percentage of dissovled metal between this leachate and the waters used in toxicity prediction experiments, it seems more realistic to compare the predicted copper LC50 with the dissolved, rather than total, combined copper-nickel concentration in the leachate. Since the concentration of dissolved copper and nickel, expressed as copper is less than half of the predicted copper LC50, the lack of mortality in the leachate is consistent with model predictions.

*Dissolved metal and dissolved organic carbon are defined here as the fraction passing through a 0.45 micron membrane filter

In the second bioassay of Seep 3 leachate, the 48-hour LC50 was 2.2% leachate diluted in water from the Erie 011 discharge. The combined concentration of total copper and total nickel in this dulution was 50% greater than the predicted LC50. Since no metal filtrations were performed with this leachate sample, it cannot be determined how much of the difference between observed and predicted toxicity can be attributed to absorption of metals on suspended matter. However, in this case the substrate was not disturbed when the sample was collected. For all Seep 3 samples in which filtrations were performed (except the muddied sample) an average of 75% of total copper and 97% of total nickel was in the dissolved fraction. If this proportion of dissolved metal is assumed for the second Seep 3 sample, the combined dissolved copper-nickel concentration was equivalent to 32 μg copper/l, just 20% above the predicted copper LC50.

Five bioassays were conducted with leachate from the U.S. Steel bulk sample site. Filtered copper and nickel levels in this leachate averaged more than 95% of the total levels. The first two bioassays were run concurrently using different dilution waters, the South Kawishiwi River and the EM-6 site on Unnamed Creek. In both tests there were no survivors in the lowest treatment level, which contained 1.7% leachate. The combined copper-nickel concentration in this dilution was 4 times as great as the predicted LC50 in South Kawishiwi River water, and 8 times as great as the predicted LC50 in water from Unnamed Creek.

In the third bioassay of leachate from the U.S. Steel pit, the dilution water was from Lake Superior. Complete mortality occured in the lowest treatment level, containing 0.1% leachate. In this dilution, the combined copper-nickel concentration was 17% greater than the predicted LC50.

The first and second bioassays of U.S. Steel leachate in water from the South Kawishiwi River and EM-6 on Unnamed Creek were repeated using lower concentrations of leachate. The 48-hour LC50 in South Kawishiwi River water was 0.7% leachate. This dilution contained a combined copper-nickel concentration 1.8 times as great as the predicted LC50 of the mixture. In water from the EM-6 site the 48-hour LC50 was 1.0% leachate. In this dilution the combined concentrations of copper and nickel were nearly 8 times higher than the predicted LC50 of the copper-nickel mixture. No explanation can be offered for the unexpectedly low toxicity of the U.S. Steel leachate in the last two experiments.

The toxicity of leachate from the seep at the INCO Spruce Road bulk sample site was tested twice. In both bioassays, all test animals survived in the undiluted leachate. The combined concentration of total Cu and total Ni in the first leachate sample was 5% greater than the predicted LC50, and was 75% as great as the predicted LC50 in the second sample. Metal filtrations were performed on the second sample, and showed that only 43% of the copper and 78% of the nickel in the sample were dissovled. When expressed in terms of filtered metal, the combined copper-nickel concentration in the second sample was 43% of the predicted LC50. It is probably safe to assume that a similar proportion of suspended copper and nickel was also present in the first INCO sample .

In addition to copper and nickel, the three leachates contained elevated concentrations of zinc, cobalt and manganese (Table 8). Biesinger and Christenson (1972) found that the 48-hour LC50 of zinc for unfed *Daphnia magna* in Lake Superior water was 100 μg/l, or 10 times the copper LC50. Tabata (1969) showed that the toxicity of zinc to *Daphnia* sp. was diminished more than the toxicity of copper or nickel by water hardness. Except for the second sample from the INCO seep, zinc concentrations in the leachates were substantially lower than copper concentrations. In the INCO sample, the concentration of dissolved zinc was 50% greater than the dissovled copper concentration, but nevertheless only 15% of the LC50 for a congeneric species in Lake Superior water. The low toxicity of zinc relative to copper in soft water and the effect of hardness on zinc toxicity suggest that zinc could have contributed little to the toxicity of this or any of the other leachates.

Recent work by the present authors has shown that the weighted mean 48-hour LC50 of cobalt for unfed Daphnia pulicaria in Lake Superior water was 2253 μg/l, or 240 times the copper LC50. Tabata (1969) found that cobalt toxicity to Daphnia sp. was affected less by hardness than was the toxicity of copper or nickel. Cobalt was present in a higher concentration than copper only in the first Seep 3 sample and the second INCO sample, and the cobalt concentration in both samples was less than 1/4 of the LC50 determined in Lake Superior water, which had lower hardness and TOC than either leachate.

The 48-hour LC50 of manganese for unfed Daphnia magna in Lake Superior water was 9800 μg/l, or 1000 times the copper LC50, according to Biesinger and Christenson (1972). Tabata (1969) found that the toxicity of manganese to Daphnia sp. was affected less by hardness than was the toxicity of copper, nickel, zinc or cobalt. Manganese was present in a higher concentration than copper only in the two Seep 3 samples. (Manganese was not measured in the INCO seep). In the first sample, the concentration of filtered manganese was less than 1/10 of the LC50 measured for Daphnia magna in Lake Superior water, and in the second sample the total manganese concentration was about 2% of the LC50 in Lake Superior water.

CHRONIC TOXICITY OF COPPER, NICKEL AND COBALT

In an attempt to evaluate the chronic toxicity of heavy metals, fathead minnows were exposed for 30-day periods to copper, nickel and cobalt in Lake Superior water. In each of the three experiments, five test concentrations and a control were supplied by a continuous-flow dilution apparatus. Exposures began with embryos 1 day after fertilization and continued for 30 days. Temperature in all bioassays was 25 C and dissolved oxygen concentration was near saturation. Total metal concentrations and pH in all treatment levels, and hardness, alkalinity and total organic carbon in controls were determined at regular intervals in each bioassay. Experimental and analytical methods were described in greater detail in the Aquatic Toxicology Operations Manual.

Results of the three bioassays are summarized in Tables 9, 10 and 11. Copper did not significantly affect embryo survival at the highest concentration tested ($\alpha = .05$ for all significance tests), but survival of the young fish after hatch was reduced at 26.2μg Cu/l. Mean weight of surviving fish at the end of the exposure was significantly reduced at 13.1 μg Cu/l, which corresponds to 0.1211 of the 96-hour LC50 of copper for 8-week-old fathead minnows in Lake Superior water. Significant body accumulations of copper occurred at 9.0 μg Cu/l.

A nickel concentration of 433.5 μg Ni/l, 0.0836 of the 96-hour LC50, significantly reduced both embryo survival and young fish survival. No effect on mean fish weight after 30 days was detected at nickel concentrations permitting survival. Significant body accumulations of nickel were found at 44.4 μg Ni/l.

Duncan's new multiple range test was used in the statistical analysis of the cobalt bioassay. This test was used because embryos and larvae in control tanks suffered heavy mortality caused by fungus and controls were therefore not included in the statistical analysis. Fungus problems were not evident in the chambers with cobalt added which may indicate that cobalt acts as a fungus inhibitor. Cobalt did not affect embryo survival at the highest treatment level. A significant reduction in fish survival was found between the lowest treatment and 48.7 μg Co/l, which corresponds to 0.0918 of the 96-hr LC50. An F-test detected no significant treatment differences in fish growth. However, Duncans's test detected a difference between the lowest treatment and 223.2 μg Co/l. Body accumulation data are not yet available.

Mount and Stephan (1969) exposed fathead minnows to copper from hatch in a well water diluted with deionized water. They found that 18 μg Cu/l caused 100% mortality after 60 days. No effects on survival or growth were observed at a concentration of 10.6 μg Cu/l. The present tests had similar results in that survival was reduced at 26.2 μgCu/l but not affected at 13.1 μg Cu/l. However, an affect on growth was detected in the present study at 13.1 μg Cu/l.

Pickering (1974), using fathead minnow embryos which were spawned and incubated in nickel solutions, found a significant reduction in hatchability at 730 μg Ni/l. Mean length of young fish after 30 days also appeared to be reduced at this concentration although length differences were not tested statistically. No significant reduction in hatchability or fish length was found a 380 μg Ni/l. Fish survival was not affected at the highest concentration of 730μg Ni/l. The present study, which was conducted in a softer water, showed an effect on embryo and fish survival at 433.5 μg Ni/l but no significant effects at 108.9 μg Ni/l.

Shabalina (1964),using juvenile carp exposed to 5 μg Co/l found no significant reduction in weight after 70 days although growth rate was initially depressed. Characteristics of the test water were not described. The present tests showed growth effects at 223.2 μg Co/l but no significant effects at 112.5 μg Co/l.

TABLE 1. Toxicity of Copper and Nickel to the Fathead Minnow and *Daphnia pulicaria* in water from Lake Superior and the South Kawishiwi River.

SPECIES	TEST WATER	TOXICANT	WEIGHTED MEAN LC50(μg/l)	POTENCY RATIO
fathead minnow	L. Superior	copper	108.2(3)*	.02087
		nickel	5186 (2)	
	So. Kawishiwi R.	copper	477.8(2)	.1636
		nickel	2920 (20	
D. pulicaria	L. Superior	copper	9.291(4)	.004887
		nickel	1901 (3)	
	So. Kawishiwi R.	copper	54.47(3)	.05521
		nickel	986.6(30	

*numbers of observations contributing to means are parenthesized

TABLE 2. Toxicity of Copper-Nickel Mixtures to the Fathead Minnow in Water from Lake Superior and the South Kawishiwi River

TEST WATER	TEST DATE	$\frac{\text{COPPER CONC.}}{\text{NICKEL CONC.}}$	96-hour LC50 as Cu(µg/ℓ)	$\frac{\text{MIXTURE LC50}}{\text{COPPER LC50}}$
Lake Superior	4/4/77	.0255	66.0	.610
Lake Superior	4/21/77	.0313	46.3	.428
Lake Superior	5/9/77	.0280	45.5	.421
So. Kawishiwi R.	8/29/77	.156	229	.478
So. Kawishiw R.	9/5/77	.124	241	.503

TABLE 3. Toxicity of Copper-Nickel Mixtures to *Daphnia pulicaria* in Water from Lake Superior and the South Kawishiwi River

TEST WATER	TEST DATE	COPPER CONC. / NICKEL CONC.	48-hour LC50 as Cu(μg/ℓ)	MIXTURE LC50 / COPPER LC50
Lake Superior	3/30/77	.00697	7.77	.836
Lake Superior	4/5/77	.00634	8.12	.874
Lake Superior	4/13/77	.00652	7.18	.773
S. Kawishiwi R.	8/2/77	.0883	65.0	1.193
S. Kawishiwi R.	8/9/77	.0628	58.6	1.077
S. Kawishiwi R.	8/9/77	.0617	60.0	1.101
S. Kawishiwi R.	8/16/77	.0245	67.0	1.230
S. Kawishiwi R.	8/23/77	.0208	57.1	1.049
S. Kawishiwi R.	8/23/77	.0256	68.2	1.252
S. Kawishiwi R.	8/30/77	.0284	74.7	1.372

TABLE 4. Acute Toxicity of Copper to the Fathead Minnow in Different Surface Waters.

TEST WATER	TEST DATE	96-hr. L(50(μg/ℓ)	95% Confidence interval on LC50 (μg/ℓ)	HARDNESS (mg/ℓ as $CaCO3$)	ALKALINITY (mg/ℓ as $CaCO_3$)	pH	TOC (mg/ℓ)
Lake Superior	2/14/77	114	33.3	48	44	8.03	3.7
Lake Superior	3/7/77	121	33.5	45	44	8.04	3.5
Lake Superior	3/21/77	88.5	27.5	46	41	7.98	3.5
S. Kawishiwi R.	7/12/77	436	103	30	21	6.82	12
So. Kawishiwi R.	8/1/77	516	111	27	21	7.28	13
St. Louis R.	9/26/77	1586	302	87	20	7.11	36
St. Louis R.	10/17/77	1129	269	73	18	6.94	28

TABLE 5. Acute Toxicity of Copper to <u>Daphnia pulicaria</u> in different surface waters.

TEST WATER	TEST DATE	48-hr L(50(μg/ℓ)	95% Confidence interval on LC50(μg/ℓ)	Hardness (mg/ℓ as $CaCO_3$)	Alkalinity (mg/ℓ as $CaCO_3$)	pH	TOC (mg/ℓ)	Flow Rate (m^3/sec)
Lake Superior	1/26/77	11.4	3.59	48	42	8.025*	2.6	
Lake Superior	1/26/77	9.06	1.56	48	42	8.025*	3.2	
Lake Superior	2/16/77	7.24	1.25	48	44	8.01	3.1	
Lake Superior	3/16/77	10.8	2.39	44	42	8.04	3.5	
So. Kawishiwi R.	7/6/77	55.4	32.8	31	27	6.66	14	
So. Kawishiwi R.	7/13/77	55.3	15.3	29	27	6.97	13	
So. Kawishiwi R.	7/26/77	55.3	13.9	28	22	7.20	13	
St. Louis R.	9/6/77	97.2	30.7	88*	20*	7.01	28	30.8
St. Louis R.	9/22/77	199	46.3	100	20	7.55	34	15.5
St. Louis R.	9/27/77	627	169	86	22	7.25	34	32.2
St. Louis R.	10/5/77	213	114	82	18.	6.99	32*	20.8
St. Louis R.	10/12/77	165	12.9	84	17	7.01	32	20.4
Lake One	4/2/77	35.5	20.6	16	11	7.39	12	
Colby Lake	4/2/77	78.8	53.5	151	44	7.76	13	
Gloquet Lake	4/2/77	113	83.9	96	91	8.10	28	
Greenwood Lake	8/30/77	76.4	40.0	26	4	7.24	25	

*Means of known values substituted for missing data.

TABLE 6. Acute Toxicity of Nickel to the Fathead Minnow in Different Surface Waters.

TEST WATER	TEST DATE	96-hr LC50(μg/ℓ)	95% Confidence interval on LC50 (μg/ℓ)	Hardness (mg/ℓ as $CaCO_3$)	Alkalinity (mg/ℓ as $CaCO_3$)	pH	TOC (mg/ℓ)
Lake Superior	2/28/77	5209	1521	45	43	8.05	4.2
Lake Superior	3/14/77	5163	1491	44	42	8.01	3.7
So. Kawishiwi R.	7/18/77	2916	685	29	20	6.50	12
So. Kawishiwi R.	7/25/77	2923	631	28	21	7.00	14
St. Louis R.	10/3/77	12356	2893	77	19	6.99	32*
St. Louis R.	10/10/77	17678	5459	89	20	7.09	33
St. Louis R.	10/25/77	8617	2398	91	19	7.04	30

*Mean of known values substituted for missing datum.

TABLE 7. Acute Toxicity of Nickel to *Daphnia pulicaria* in different surface waters.

TEST WATER	TEST DATE	48-hr LC50(μg/ℓ)	95% Confidence interval on LC50 (μg/ℓ)	Hardness (mg/ℓ as $CaCO_3$)	Alkalinity (mg/ℓ as $CaCO_3$)	pH	TOC (mg/ℓ)
Lake Superior	1/26/77	2182	1932	48	42	8.07*	2.6
Lake Superior	2/16/77	1813	785	48	44	8.10	2.8
Lake Superior	3/16/77	1836	1055	44	42	8.04	2.7
So. Kawishiwi R.	7/13/77	697	301	29	26	6.77	13
So. Kawishiwi R.	7/26/77	1140	336	28	22	7.23	15
So. Kawishiwi R.	8/2/77	1034	330	28	20	7.36	13
St. Louis R.	9/6/77	3414	1779	73	18	6.94	28
St. Louis R.	9/22/77	3757	1029	100	20	7.55	34
St. Louis R.	9/27/77	3316	1141	86	22	7.25	34
St. Louis R.	10/12/77	3014	875	84	17	7.01	32
St. Louis R.	10/19/77	2325	732	74	17	7.09	28
Greenwood Lake	8/12/77	2171	770	25	2.5	5.88	39

*Mean of known values substituted for missing datum.

Table 8. Acute Toxicity of Copper-Nickel Leachates to *Daphnia pulicaria*.

Leachate	Dilution Water	Test Date	Percentage leachate	Effect	Cu (μg/ℓ)	Ni (μg/ℓ)	Zn (μg/ℓ)	Co (μg/ℓ)	Mn (μg/ℓ)	Hardness (mg/ℓ as CaCo$_3$)	Organic Carbon (mg/ℓ)	pH	Predicted Cu LC50	Predicted Potency ratio	Cu-Ni Concentration as Cu
Seep 3	EM-6	4/6/77	100%	No mortality	T[a] 270 F[b] 52	T 3200 F 930	T 73	T 440	T 2740 F 840	446	F 26	8.30	132	.0102	T 303 F 61
Seep 3	O11	9/14/77	2.2%	48-hr LC50	T(40)[c]	T(660)	T(16)	T(35)	T(220)	(263)	F (7.3)	8.09	27	.00358	T 42
U.S. Steel	S. Kawishiwi R.	4/6/77	1.7%	Complete mortality	T(210)	T(294)	T(2.3)	T(12)	T(89)	(31)	F (13)	7.45	54[d]	.0548	T 226
U.S. Steel	EM-6	4/6/77	1.7%	Complete mortality	T(210)	T(294)	T(5.3)	T(12)	T(170)	(164)	F (6.8)	8.32	25	.00518	T 212
U.S. Steel	Lake Superior	4/26/77	0.1%	Complete mortality	T(11)	T(22)	T(<2)	T(<2)	T(6.0)	(45)	T(3.0)	7.99	9.3[d]	.00489	T 11
U.S. Steel	EM-6	10/26/77	1.0%	48-hr LC50	T 150	T 130	T(6.7)	T(11)	T(67)	(118)	F(6.0)	8.20	20	.00577	T 151
U.S. Steel	S. Kawishiwi R.	10/27/77	0.7%	48-hr LC50	T 114	T 101	T(2.6)	T(7.7)	T(78)	(23)	T(15)	7.04	69	.0731	T 121
INCO Seep	S. Kawishiwi R.	7/28/77	100%	No mortality	T 96	T 65	T 2.7	T 17	-	765	F 19	8.31	92	.00427	T 96
INCO Seep	S. Kawishiwi R.	11/29/77	100%	No mortality	T 23 F 9.9	T4500 F3500	T 13	T 250 F 210	-	490	F 12	8.35	53	.00384	T 40 F 23

[a] Total

[b] Filtered

[c] Calculated from measurements in leachate and dilution water.

[d] Chemical characteristics were similar when prediction bioassays were run in this water. Weighed mean of those LC50's is used here rather than LC50 predicted by model.

Table 9 Chronic Toxicity of Copper to Fathead Minnow Embryos and Larvae in Lake Superior Water

Mean Cu^{++} Concentration (µg/ℓ)	Fraction of 96-hr LC50[a]	Mean Cu Body Accumulation (µg/g dry wt)	Mean Percentage Embryo Survival to hatch[b]	Mean Percentage Fish Survival after hatch[b]	Mean Fish Weight after 30 days (mg)[c]
< 5	-	5.1375	93	99	107
5.0	0.04621	11.590	89	99	112
9.0	0.08318	19.7675[d]	97	97	96
13.1	0.1211	25.2250[d]	91	88	14[d]
26.2	0.2421		92	62[d]	3[d]
52.1	0.4315		90	15[d]	2[d]

[a] Acute tests run in Lake Superior water using 8-week-old fathead minnows. Weighted mean 96-hr LC50 = 108.2 µg Cu^{++}/ℓ.

[b] Arcsin-$\sqrt{X}$ transformation used on all percentages.

[c] Log X transformation used on weights.

[d] Significantly different from control at $\alpha = 0.05$. (Dunnett)

Table 10 Chronic Toxicity of Nickel to Fathead Minnow Embryos and Larvae in Lake Superior Water

Mean Cu^{++} Concentration (µg/ℓ)	Fraction of 96-hr LC50[a]	Mean Cu Body Accumulation (µg/g dry wt)	Mean Percentage Embryo Survival to hatch[b]	Mean Percentage Fish Survival after hatch[b]	Mean Fish Weight after 30 days (mg)[c]
< 6	-	2.7225	98	97	105
21.0	0.0040	11.1300	93	95	97
44.4	0.0086	17.6000[d]	92	100	97
108.9	0.0210	25.4750[d]	92	99	98
433.5	0.0836	-	71[d]	9[d]	123
1532.1	0.2954	-	0[d]	-	-

[a] Acute tests run in Lake Superior water using 8-week-old fathead minnows. Weighted mean 96-hr LC50 = 5186 µg Ni^{++}/ℓ.

[b] $\arcsin\sqrt{X}$ transformation used on all percentages.

[c] Log X transformation used on weights.

[d] Significantly different from control at $\alpha = 0.05$. (Dunnett)

Table 11 Chronic Toxicity of Cobalt to Fathead Minnow Embryos and Larvae in Lake Superior Water

Mean Cu^{++} Concentration (μg/ℓ)	Fraction of 96-hr LC50[a]	Mean Percentage Embryo Survival to hatch[b]	Mean Percentage Fish Survival after hatch[b]	Mean Fish Weight after 30 days (mg)[c]
< 1[d]	-	78[d]	21[d]	71[d]
14.7	0.0277	94	96	82
29.5	0.0556	88	85	78
48.7	0.0918	91	86[e]	77
112.5	0.2120	91	78[e]	66
223.2	0.42066	81	61[e]	65[e]

[a] Acute test run in Lake Superior water using 8-week-old fathead minnows. Weighted mean 96-hr LC50 = 530.6 μg Co/ℓ.

[b] $\text{Arcsin}\sqrt{X}$ transformation used on all percentages.

[c] Log X transformation used on weights.

[d] Control data which was not included in statistical analysis.

[e] Significantly different from lowest treatment at $\alpha = 0.05$ (Duncans new multiple range test).

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REGIONAL COPPER-NICKEL STUDY

Aquatic Toxicology Progress Report

Draft Report

Minnesota Environmental Quality Board

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Kevin Alto
Steven Chatterton

April 10, 1978

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SUMMARY

Fathead minnows and Daphnia pulicaria were exposed to mixtures of copper and nickel in water from Lake Superior and the South Kawishiwi River. The acutely toxic concentration of a copper-nickel mixture to the fathead minnow was approximately half of what it would have been if copper and nickel were additive in their joint effect. This observation held true in both test waters despite the difference in the relative potency of copper and nickel in the two test waters.

The joint lethal effect of copper and nickel on Daphnia pulicaria was slightly more than additive in Lake Superior water, and additive or slightly less than additive in South Kawishiwi River water, although these departures from additivity were probably not large enough to have practical significance. Variations in the ratio of copper concentration to nickel concentration among bioassays conducted with Daphnia in South Kawishiw River water were unrelated to variations in LC50.

Results of copper and nickel bioassays using fathead minnows and Daphnia pulicaria in different surface waters were used to develop models for predicting copper and nickel toxicity to these species in natural waters. Hardness, alkalinity, pH and total organic carbon (TOC) were considered as potential predictors of LC50. TOC was the best predictor of copper toxicity to both species. Hardness was the best predictor of acute nickel toxicity to the fathead minnow. For Daphnia pulicaria, which were exposed to nickel in an additional surface water, hardness together with TOC were the best predictors of nickel toxicity. Dummy x-variables were used to show that for both species there was significant variation in copper toxicity among test waters which was not explained by variations in TOC.

Toxicity prediction models were also fitted after deletion of the data from bioassays in Lake Superior water. Wtihout the Lake Superior data, TOC remained the best predictor of copper toxicity to both species and assumed more importance in prediction of nickel toxicity, replacing hardness as the best predictor of nickel toxicity to the fathead minnow.

Bioassays of three copper-nickel leachates were conducted with *Daphnia pulicaria*. In none of the nine bioassays was a leachate shown to be more toxic than predicted, although the first three bioassays of the U.S. Steel leachate, in which all test animals died, could not have detected greater-than-predicted toxicity. In six of the bioassays, toxicity was less than that predicted on the basis of total metal concentrations. Four of these discrepancies can be satisfactorily explained by the presence of substantial proportions of suspended copper and nickel, but in the last two tests of U.S. Steel leachate, no ready explanation can be found for the difference between observed and predicted toxicity.

In three separate 30-day experiments, fathead minnows were exposed to copper, nickel and cobalt in Lake Superior water from 1 day after fertilization until the early juvenile life stage. Copper reduced growth of young fish at a concentration of 13.1 μg/l. A nickel concentration of 433.5 μg/l reduced survival of embryos and young fish. Survival of young fish was reduced at a cobalt concentration of 48.7 μg/l.

ACUTE TOXICITY OF COPPER-NICKEL MIXTURES

The likelihood that copper, nickel and other heavy metals would occur together in water effluents from copper-nickel mining and ore processing necessitates an understanding of the joint toxicity of heavy metals. In a previous report, the literature dealing with toxic effects of metal mixtures was surveyed (Regional Copper-Nickel Study 1978). In the present study we examined the acute toxicity of copper-nickel mixtures to the fathead minnow, Pimephales promelas, and the cladoceran Daphnia pulicaria in water from Lake Superior at the ERL-Duluth intake and from the South Kawishiwi River near the State Highway 1 crossing.

96-hour LC50's of copper sulfate, nickel sulfate and copper-nickel mixtures for the fathead minnow were determined using continuous-flow dilution apparatus at a temperature of 25 C. for Daphnia pulicaria, 48-hour LC50's of copper sulfate, nickel sulfate and copper-nickel mixtures were determined in unrenewed solutions at 18 C. Dissolved oxygen concentrations were near saturation in all experiments. Total metal concentrations and pH in all treatment levels, and hardness, alkalinity and total organic carbon (TOC) in controls were determined at least once in each bioassay. Experimental and analytical methods were described in greater detail in the Aquatic Toxicology Operations Manual.

With each test species two or more bioassays of each metal were conducted. Weighted mean LC50's were calculated for each species and each metal. Because the precision of individual LC50 estimates varied, each LC50 was assigned a weighting coefficient equal to the LC50 divided by its 95% confidence interval. For each species and each test water a copper-nickel potency ratio (copper LC50/nickel LC50) was calculated from these weighted means.

The relative concentrations of copper and nickel in bioassays of copper-nickel mixtures were set in most cases according to their relative potencies. For each treatment level, the desired nickel concentration multiplied by the potency ratio equaled the desired copper concentration. For a variety of reasons, the ratios of measured copper concentration to measured nickel concentration in some mixture bioassays were quite different from the potency ratios, but concentration ratios within a bioassay were consistent. Total toxicant concentrations in mixture bioassays were expressed as copper. The nickel concentration in each treatment was multiplied by the potency ratio and the product was added to the copper concentration. If a mixture LC50, expressed as copper, equaled the weighted mean copper LC50 determined earlier, it was concluded that the joint effects of copper and nickel were additive.

Table 1 gives the weighted means of the copper LC50's and nickel LC50's for both test species in both test waters, as well as the potency ratio (copper LC50/nickel LC50) for each species in each water. Tables 2 and 3 give the individual LC50's from all bioassays of copper-nickel mixtures.

It is apparent from Table 2 that the joint toxicity of copper and nickel to the fathead minnow is more than additive in both test waters, since the ratio (mixture LC50 as Cu/copper LC50) would be unity if the combined toxicity were additive. The ratios of copper concentration to nickel concentration in each water were held fairly close to the copper-nickel potency ratios given in Table 1 for the fathead minnow.

The joint toxicity of copper and nickel to Daphnia pulicaria (Table 3) appears to be somewhat more than additive in Lake Superior water but additive or somewhat less than additive in South Kawishiwi River water. The ratios of copper concentration to nickel concentration in mixture bioassays conducted in South Kawishiwi River water varied above and below the copper-nickel potency ratio given in Table 1 for Daphnia pulicaria, but were not significantly correlated with LC50(r= -.369). TOC and hardness remained nearly constant during the entire experimental period in the South Kawishiwi River.

RELATIONSHIP OF RECEIVING WATER CHEMISTRY TO THE ACUTE TOXICITY OF COPPER AND NICKEL

Predictions of the vulnerability of aquatic communities to heavy metal pollution must take into account the relationship of water chemistry to the toxicity of heavy metals. A previous report surveyed the literature dealing with the effects of hardness, alkalinity, pH and organic substances on the toxicity of selected heavy metals to aquatic life. In this study we attempted to develop models for predicting the acute toxicity of copper and nickel to the fathead minnow Pimephales promelas and the cladoceran Daphnia pulicaria in surface waters of the copper-nickel study area, based on these water chemistry parameters.

96-hour LC50's of copper and nickel sulfates for the fathead minnow were determined using continuous-flow dilution apparatus at a temperature of 25 C. For Daphnia pulicaria, 48-hour LC50's of copper and nickel sulfates were determined in unrenewed solutions at 18 C. Dissolved oxygen concentratio were near saturation in all experiments. Total metal concentrations and pH in all treatment levels, and hardness, alkalinity and total organic carbon (TOC) in controls were determined at least once in each bioassay. Experimenta and analytical methods were described in greater detail in the Aquatic Toxicol Operations Manual.

Continuous-flow bioassays with fathead minnows were conducted in water from Lake Superior, the South Kawishiwi River and the St. Louis River. Bioassays with Daphnia were conducted in these waters as well as in samples from Lake One, Colby Lake, Cloquet Lake and Greenwood Lake. Water chemistry parameters and LC50's are presented in Tables 4-7. For each metal and test organism, a forward selection procedure (Draper and Smith, 1966) was followed to choose the best model for the prediction of LC50 from the four water chemistry parameters. Transformations of variables were not considered. Because the precision of LC50 estimates was variable, each LC50 was given a weighting coefficient equal to the LC50 divided by its 95% confidence interval.

In copper bioassays with the fathead minnow (TAble 4), TOC was the chemical variable most highly correlated with LC50 (r=.9982). LC50 was regressed on TOC along with each of the other variables made a significant contribution to the regression at the .10 level. Therefore, the best predictive model for acute copper toxicity to the fathead minnow was:

$$LC50(\mu g\ Cu/l) = -67.3 + 44.8\ (TOC\ in\ mg/l), R^2 = .9964.$$

Dummy x-variables (Draper and Smith, 1966) were used to determine whether or not there were differences among the three test waters in their effects on LC50 which were not fully explained by the chosen model. The dummy variables made a significant contribution to the model ($\alpha = .05$), increasing R^2 to .9997. Inclusion of these dummy variables would therefore improve the fit of the model to the existing data but could not be used to enhance its predictive capability for other waters.

In copper bioassays with Daphnia (Table 5), TOC was the chemical variable best correlated with LC50 (r=.7010). Deletion of an outlier from the St. Louis River increased the correlation coefficient to .9637. Hardness, alkalinity and pH did not contribute significantly ($\alpha = .10$) to the regression of LC50 on TOC, so the best model for prediction of acute copper toxicity to Daphnia pulicaria was:

$LC50 = -12.5 + 5.48\ (TOC)$

$R^2 = .9287$

Dummy x-variables were used to isolate the differences in LC50 among test waters which were not explained by TOC. Only Lake Superior, the South Kawishiwi River and the St. Louis River, where multiple bioassays were conducted, were included in this analysis. For the eight bioassays considered, the regression of LC50 on TOC had an R^2 value of .9519. Inclusion of the dummy variables increased R^2 to .9833. The contribution of the dummy variables was significant at the .025 level.

Next, the five LC50's in St. Louis river water including the outlier initially rejected were regressed against TOC, yielding a predictably low R^2 value of .3297. Because the high turbidity associated with periods of high river flow seemed to increase copper LC50's for Daphnia, and frequent turbidity measurements were not available, flow rate as measured by a nearby gauging station during each bioassay was entered into the regression of LC50 on TOC. Inclusion of the second x-variable increased R^2 to .9967 although flow rate alone was not significantly correlated with LC50 ($r = .5590$).

Of the four chemical variables considered in nickel bioassays with the fathead minnow (Table 6), hardness was best correlated LC50 ($r = .8716$). Alkalinity, pH and TOC did not add significantly ($\alpha = .10$) to the regression of LC50 on hardness; therefore the best predictive model for the acute toxicity of nickel to the fathead minnow was:

$LC50 = -1969 + 170\ (\text{hardness in mg/l as } CaCO_3)$

$R^2 = .7597$; significant at .025 level

Adding dummy x-variables representing test waters did not significantly improve the regression of LC50 on hardness (α = .10), indicating that deviations between fitted and observed LC50's were as great within the three blocks of bioassays from the three test waters as they were among the blocks, i.e. hardness explained variations in LC50 equally well both within blocks and among blocks.

Hardness was the chemical variable best correlated with LC50 (r = .9056) in nickel bioassays with Daphnia (Table 7). At the .05 significance level, alkalinity, pH and TOC did not contribute significantly to the regression of LC50 on hardness, but TOC added significantly to the regression at the .10 level.

Alternative models for predicting the acute toxicity of nickel to Daphnia pulicaria are:

LC50 (μgNi/l) = 372 + 32.8 (hardness in mg/l as $CaCO_3$)
R^2 = .8201; significant at .005 level

LC50 = 188 + 27.4 (hardness) + 22.1 (TOC)
R^2 = .8723; contribution of TOC significant at .10 level

Dummy x-variables representing test waters did not significantly improve the regression of LC50 on hardness and TOC (α=.10). The single experiment in Greenwood Lake water was not included in the data set for this analysis. The importance of TOC as a predictor of nickel toxicity to Daphnia but not fathead minnows is related to the fact that toxicity tests in Greenwood Lake water, which has low hardness and high TOC, were conducted only with Daphnia. Bioassays with fathead minnows were conducted in waters where hardness and TOC were highly correlated (r=.8362) so that the individual importance of each is difficult to discern.

Since Lake Superior does not lie within the Study Area, and it has somewhat different characteristics from the other waters discussed here, regression analyses were performed with the Lake Superior LC50's deleted from the data set. Without the Lake Superior data, TOC was again shown to be the only significant water chemistry parameter with respect to copper toxicity. The best prediction models were:

Fathead minow: $\text{LC50} = -112 + 46.4(\text{TOC})$

$R^2 = .9949$

<u>Daphnia pulicaria</u>: $\text{LC50} = -32.1 + 6.17(\text{TOC})$

$R^2 = .8527$

Without the Lake Superior data, the best model for predicting acute nickel toxicity to the fathead minnow was:

$\text{LC50} = -4371 + 544\ (\text{TOC})$

$R^2 = .8382$; significant at .05 level

The regression of LC50 on hardness fit the data nearly as well:

$\text{LC50} = -1670 + 167\ (\text{hardness})$

$R^2 = .7423$; significant at .10 level

This model was nearly identical to the prediction model derived from the full data set.

The best model for predicting the acute toxicity of nickel to <u>Daphnia pulicaria</u> with Lake Superior data deleted was:

$\text{LC50} = -362 + 22.9\ (\text{hardness}) + 50.2\ (\text{TOC})$

$R^2 = .9398$; contribution of TOC significant at .025 level.

The x-variables in this model were the same as those in the two-variable model derived from the full data set, although the contribution of TOC to this model was greater.

ACUTE TOXICITY OF COPPER-NICKEL LEACHATES

Bioassays of three copper-nickel leachates were conducted with Daphnia pulicaria. 48-hour LC50's were determined in unrenewed dilutions of leachates at 18 C. Dissolved oxygen concentrations were near saturation in all bioassays. Metal concentrations in treatments were either measured directly or calculated from measured concentrations in leachates. If the concentration of a trace metal in dilution water was less than 2 μg/ℓ, its contribution to trace metal levels in leachate dilutions was ignored in calculations. pH was measured in all treatments, and hardness and organic carbon (TOC) in treatments was calculated from levels in leachate and dilution water.

Table 8 summarizes the chemical characteristics of leachate dilutions representing 48-hour LC50's, where LC50's could be determined. For bioassays in which full strength leachate was not lethal, or in which the weakest dilution was lethal to all the test animals, the chemical characteristics of those concentrations are listed. Predicted copper LC50's and predicted nickel LC50's in the listed dilutions were calculated from hardness and organic carbon using the models given in a previous section. It must be noted that none of the test waters in which the prediction models were derived had hardness levels as high as the Seep 3 or INCO leachates. Model predictions were assumed to hold beyond the range of hardnesses actually tested. Organic carbon in leachates and in the EM-6 dilution water was measured only in the dissolved form, whereas the prediction models are based on total organic carbon measurements. Dissolved organic carbon levels were usually more than 90% of total organic carbon levels in the waters from which the prediction models were derived.

The combined concentration of copper and nickel in the listed leachate dilutions was expressed as copper using potency ratios derived from the prediction models and listed in Table 8. Mortality observed at this combined concentration could then be compared with the predicted copper LC50. The joint toxicity of copper and nickel in leachates was assumed to be additive. This assumption was consistent with the findings of the copper-nickel mixture bioassays reported in a previous section.

Chemical analyses of leachates and prediction models for copper and nickel toxicity were not available at the time most of the leachate bioassays were conducted. This made it difficult to select leachate dilutions necessary for conclusive test results.

Two bioassays were run using leachate from Seep 3 at Erie Mining Company's Dunka Mine. In the first, no mortality occurred in the undiluted leachate despite a combined copper-nickel concentration 3 times as great as the predicted lethal level. An explanation may be found in the fact that the leachate was muddied during sample collection, probably causing adsorption of metals onto suspended sediment particles. It was found that only 20% of the copper and 30% of the nickel in the leachate sample were present in the dissolved* fraction.

While the toxicity prediction models were derived from LC50's expressed as total metal, dissolved copper concentrations in the prediction experiments ranged from 80% to 100% of total copper, and dissolved nickel ranged from 90% to 100% of total nickel. Because of the discrepancy in percentage of dissolved metal between this leachate and the waters used in toxicity prediction experiments, it seems more realistic to compare the predicted copper LC50 with the dissolved, rather than total, combined copper-nickel concentration in the leachate. Since the concentration of dissolved copper and nickel, expressed as copper is less than half of the predicted copper LC50, the lack of mortality in the leachate is consistent with model predictions.

*Dissolved metal and dissolved organic carbon are defined here as the [illegible] 0.45 micron membrane filter.

In the second bioassay of Seep 3 leachate, the 48-hour LC50 was 2.2% leachate diluted in water from the Erie 011 discharge. The combined concentration of total copper and total nickel in this dulution was 50% greater than the predicted LC50. Since no metal filtrations were performed with this leachate sample, it cannot be determined how much of the difference between observed and predicted toxicity can be attributed to absorption of metals on suspended matter. However, in this case the substrate was not disturbed when the sample was collected. For all Seep 3 samples in which filtrations were performed (except the muddied sample) an average of 75% of total copper and 97% of total nickel was in the dissolved fraction. If this proportion of dissolved metal is assumed for the second Seep 3 sample, the combined dissolved copper-nickel concentration was equivalent to 32 μg copper/l, just 20% above the predicted copper LC50.

Five bioassays were conducted with leachate from the U.S. Steel bulk sample site. Filtered copper and nickel levels in this leachate averaged more than 95% of the total levels. The first two bioassays were run concurrently using different dilution waters, the South Kawishiwi River and the EM-6 site on Unnamed Creek. In both tests there were no survivors in the lowest treatment level, which contained 1.7% leachate. The combined copper-nickel concentration in this dilution was 4 times as great as the predicted LC50 in South Kawishiwi River water, and 8 times as great as the predicted LC50 in water from Unnamed Creek.

In the third bioassay of leachate from the U.S. Steel pit, the dilution water was from Lake Superior. Complete mortality occured in the lowest treatment level, containing 0.1% leachate. In this dilution, the combined copper-nickel concentration was 17% greater than the predicted LC50.

The first and second bioassays of U.S. Steel leachate in water from the South Kawishiwi River and EM-6 on Unnamed Creek were repeated using lower concentrations of leachate. The 48-hour LC50 in South Kawishiwi River water was 0.7% leachate. This dilution contained a combined copper-nickel concentration 1.8 times as great as the predicted LC50 of the mixture. In water from the EM-6 site the 48-hour LC50 was 1.0% leachate. In this dilution the combined concentrations of copper and nickel were nearly 8 times higher than the predicted LC50 of the copper-nickel mixture. No explanation can be offered for the unexpectedly low toxicity of the U.S. Steel leachate in the last two experiments.

The toxicity of leachate from the seep at the INCO Spruce Road bulk sample site was tested twice. In both bioassays, all test animals survived in the undiluted leachate. The combined concentration of total Cu and total Ni in the first leachate sample was 5% greater than the predicted LC50, and was 75% as great as the predicted LC50 in the second sample. Metal filtrations were performed on the second sample, and showed that only 43% of the copper and 78% of the nickel in the sample were dissovled. When expressed in terms of filtered metal, the combined copper-nickel concentration in the second sample was 43% of the predicted LC50. It is probably safe to assume that a similar proportion of suspended copper and nickel was also present in the first INCO sample.

In addition to copper and nickel, the three leachates contained elevated concentrations of zinc, cobalt and manganese (Table 8). Biesinger and Christenson (1972) found that the 48-hour LC50 of zinc for unfed Daphnia magna in Lake Superior water was 100 μg/l, or 10 times the copper LC50. Tabata (1969) showed that the toxicity of zinc to Daphnia sp. was diminished more than the toxicity of copper or nickel by water hardness. Except for the second sample from the INCO seep, zinc concentrations in the leachates were substantially lower than copper concentrations. In the INCO sample, the concentration of dissolved zinc was 50% greater than the dissovled copper concentration, but nevertheless only 15% of the LC50 for a congeneric species in Lake Superior water. The low toxicity of zinc relative to copper in soft water and the effect of hardness on zinc toxicity suggest that zinc could have contributed little to the toxicity of this or any of the other leachates.

Recent work by the present authors has shown that the weighted mean 48-hour LC50 of cobalt for unfed Daphnia pulicaria in Lake Superior water was 2253 µg/l, or 240 times the copper LC50. Tabata (1969) found that cobalt toxicity to Daphnia sp. was affected less by hardness than was the toxicity of copper or nickel. Cobalt was present in a higher concentration than copper only in the first Seep 3 sample and the second INCO sample, and the cobalt concentration in both samples was less than 1/4 of the LC50 determined in Lake Superior water, which had lower hardness and TOC than either leachate.

The 48-hour LC50 of manganese for unfed Daphnia magna in Lake Superior water was 9800 µg/l, or 1000 times the copper LC50, according to Biesinger and Christenson (1972). Tabata (1969) found that the toxicity of manganese to Daphnia sp. was affected less by hardness than was the toxicity of copper, nickel, zinc or cobalt. Manganese was present in a higher concentration than copper only in the two Seep 3 samples. (Manganese was not measured in the INCO seep). In the first sample, the concentration of filtered manganese was less than 1/10 of the LC50 measured for Daphnia magna in Lake Superior water, and in the second sample the total manganese concentration was about 2% of the LC50 in Lake Superior water.

CHRONIC TOXICITY OF COPPER, NICKEL AND COBALT

In an attempt to evaluate the chronic toxicity of heavy metals, fathead minnows were exposed for 30-day periods to copper, nickel and cobalt in Lake Superior water. In each of the three experiments, five test concentrations and a control were supplied by a continuous-flow dilution apparatus. Exposures began with embryos 1 day after fertilization and continued for 30 days. Temperature in all bioassays was 25 C and dissolved oxygen concentration was near saturation. Total metal concentrations and pH in all treatment levels, and hardness, alkalinity and total organic carbon in controls were determined at regular intervals in each bioassay. Experimental and analytical methods were described in greater detail in the Aquatic Toxicology Operations Manual.

Results of the three bioassays are summarized in Tables 9, 10 and 11. Copper did not significantly affect embryo survival at the highest concentration tested (α= .05 for all significance tests), but survival of the young fish after hatch was reduced at 26.2μg Cu/l. Mean weight of surviving fish at the end of the exposure was significantly reduced at 13.1 μg Cu/l, which corresponds to 0.1211 of the 96-hour LC50 of copper for 8-week-old fathead minnows in Lake Superior water. Significant body accumulations of copper occurred at 9.0 μg Cu/l.

A nickel concentration of 433.5 μg Ni/l, 0.0836 of the 96-hour LC50, significantly reduced both embryo survival and young fish survival. No effect on mean fish weight after 30 days was detected at nickel concentrations permitting survival. Significant body accumulations of nickel were found at 44.4 μg Ni/l.

Duncan's new multiple range test was used in the statistical analysis of the cobalt bioassay. This test was used because embryos and larvae in control tanks suffered heavy mortality caused by fungus and controls were therefore not included in the statistical analysis. Fungus problems were not evident in the chambers with cobalt added which may indicate that cobalt acts as a fungus inhibitor. Cobalt did not affect embryo survival at the highest treatment level. A significant reduction in fish survival was found between the lowest treatment and 48.7 μg Co/l, which corresponds to 0.0918 of the 96-hr LC50. An F-test detected no significant treatment differences ~~for~~ in fish growth. However, Duncan's test detected a difference between the lowest treatment and 223.2 μg Co/l. Body accumulation data ~~is~~ are not yet available.

Mount and Stephan (1969) exposed fathead minnows to copper from hatch in a well water diluted with deionized water. They found that 18 μg Cu/l caused 100% mortality after 60 days. No effects on survival or growth were observed at a concentration of 10.6 μg Cu/l. The present tests had similar results in that survival was reduced at 26.2 μgCu/l but not affected at 13.1 μgCu/l. However, an effect on growth was detected in the present study at 13.1 μgCu/l.

Pickering (1974), using fathead minnow embryos which were spawned and incubated in nickel solutions, found a significant reduction in hatchability at 730 μgNi/l. Mean length of young fish after 30 days also appeared to be reduced at this concentration although length differences were not tested statistically. No significant reduction in hatchability or fish length was found at 380 μgNi/l. Fish survival was not affected at the highest concentration of 730μg Ni/l. The present study, which was conducted in a softer water, showed an effect on embryo and fish survival at 433.5 μg Ni/l but no significant effects at 108.9 μg Ni/l.

Shabalina (1964), using juvenile carp exposed to 5 µg Co/l found no significant reduction in weight after 70 days although growth rate was initially depressed. Characteristics of the test water were not described. The present tests showed growth effects at 223.2 µg Co/l but no significant effects at 112.5 µg Co/l.

TABLE 1. Toxicity of Copper and Nickel to the Fathead Minnow and *Daphnia pulicaria* in water from Lake Superior and the South Kawishiwi River.

SPECIES	TEST WATER	TOXICANT	WEIGHTED MEAN LC50(μg/l)	POTENCY RATIO
fathead minnow	L. Superior	copper	108.2(3)*	.02087
		nickel	5186 (2)	
	So. Kawishiwi R.	copper	477.8(2)	.1636
		nickel	2920 (20	
D. pulicaria	L. Superior	copper	9.291(4)	.004887
		nickel	1901 (3)	
	So. Kawishiwi R.	copper	54.47(3)	.05521
		nickel	986.6(30	

*numbers of observations contributing to means are parenthesized

TABLE 2. Toxicity of Copper-Nickel Mixtures to the Fathead Minnow in Water from Lake Superior and the South Kawishiwi River.

TEST WATER	TEST DATE	COPPER CONC. NICKEL CONC.	96-hour LC50 as Cu(μg/ℓ)	MIXTURE LC50 COPPER LC50
Lake Superior	4/4/77	.0255	66.0	.610
Lake Superior	4/21/77	.0313	46.3	.428
Lake Superior	5/9/77	.0280	45.5	.421
So. Kawishiwi R.	8/29/77	.156	229	.478
So. Kawishiw R.	9/5/77	.124	241	.503

TABLE 3. Toxicity of Copper-Nickel Mixtures to *Daphnia pulicaria* in Water from Lake Superior and the South Kawishiwi River.

TEST WATER	TEST DATE	COPPER CONC. / NICKEL CONC.	48-hour LC50 as Cu(μg/ℓ)	MIXTURE LC50 / COPPER LC50
Lake Superior	3/30/77	.00697	7.77	.836
Lake Superior	4/5/77	.00634	8.12	.874
Lake Superior	4/13/77	.00652	7.18	.773
S. Kawishiwi R.	8/2/77	.0883	65.0	1.193
S. Kawishiwi R.	8/9/77	.0628	58.6	1.077
S. Kawishiwi R.	8/9/77	.0617	60.0	1.101
S. Kawishiwi R.	8/16/77	.0245	67.0	1.230
S. Kawishiwi R.	8/23/77	3 .0[illegible]08	57.1	1.049
S. Kawishiwi R.	8/23/77	.0256	68.2	1.252
Kawishiwi R.	8/30/77	.0284	74.7	1.372

TABLE 4. Acute Toxicity of Copper to the Fathead Minnow in Different Surface Waters.

TEST WATER	TEST DATE	96-hr. L(50(μg/ℓ)	95% Confidence interval on LC50 (μg/ℓ)	HARDNESS (mg/ℓ as CaCO3)	ALKALINITY (mg/ℓ as $CaCO_3$)	pH	TOC (mg/ℓ)
Lake Superior	2/14/77	114	33.3	48	44	8.03	3.7
Lake Superior	3/7/77	121	33.5	45	44	8.04	3.5
Lake Superior	3/21/77	88.5	27.5	46	41	7.98	3.5
S. Kawishiwi R.	7/12/77	436	103	30	21	6.82	12
So. Kawishiwi R.	8/1/77	516	111	27	21	7.28	13
St. Louis R.	9/26/77	1586	302	87	20	7.11	36
St. Louis R.	10/17/77	1129	269	73	18	6.94	28

TABLE 5. Acute Toxicity of Copper to <u>Daphnia pulicaria</u> in different surface waters.

TEST WATER	TEST DATE	48-hr L(50(μg/ℓ)	95% Confidence interval on LC50(μg/ℓ)	Hardness (mg/ℓ as $CaCO_3$)	Alkalinity (mg/ℓ as $CaCO_3$)	pH	TOC (mg/ℓ)	Flow Rate (m^3/sec)
Lake Superior	1/26/77	11.4	3.59	48	42	8.025*	2.6	
Lake Superior	1/26/77	9.06	1.56	48	42	8.025*	3.2	
Lake Superior	2/16/77	7.24	1.25	48	44	8.01	3.1	
Lake Superior	3/16/77	10.8	2.39	44	42	8.04	3.5	
So. Kawishiwi R.	7/6/77	55.4	32.8	31	27	6.66	14	
So. Kawishiwi R.	7/13/77	55.3	15.3	29	27	6.97	13	
So. Kawishiwi R.	7/26/77	55.3	13.9	28	22	7.20	13	
St. Louis R.	9/6/77	97.2	30.7	88*	20*	7.01	28	30.8
St. Louis R.	9/22/77	199	46.3	100	20	7.55	34	15.5
St. Louis R.	9/27/77	627	169	86	22	7.25	34	32.2
St. Louis R.	10/5/77	213	114	82	18	6.99	32*	20.8
St. Louis R.	10/12/77	165	12.9	84	17	7.01	32	20.4
Lake One	4/2/77	35.5	20.6	16	11	7.39	12	
Colby Lake	4/2/77	78.8	53.5	151	44	7.76	13	
Gloquet Lake	4/2/77	113	83.9	96	91	8.10	28	
Greenwood Lake	8/30/77	76.4	40.0	26	4	7.24	25	

*Means of known values substituted for missing data.

TABLE 6. Acute Toxicity of Nickel to the Fathead Minnow in Different Surface Waters.

TEST WATER	TEST DATE	96-hr LC50(µg/ℓ)	95% Confidence interval on LC50 (µg/ℓ)	Hardness (mg/ℓ as $CaCO_3$)	Alkalinity (mg/ℓ as $CaCO_3$)	pH	TOC (mg/ℓ)
Lake Superior	2/28/77	5209	1521	45	43	8.05	4.2
Lake Superior	3/14/77	5163	1491	44	42	8.01	3.7
So. Kawishiwi R.	7/18/77	2916	685	29	20	6.50	12
So. Kawishiwi R.	7/25/77	2923	631	28	21	7.00	14
St. Louis R.	10/3/77	12356	2893	77	19	6.99	32*
St. Louis R.	10/10/77	17678	5459	89	20	7.09	33
St. Louis R.	10/25/77	8617	2398	91	19	7.04	30

*Mean of known values substituted for missing datum.

TABLE 7. Acute Toxicity of Nickel to *Daphnia pulicaria* in different surface waters.

TEST WATER	TEST DATE	48-hr LC50(µg/ℓ)	95% Confidence interval on LC50 (µg/ℓ)	Hardness (mg/ℓ as $CaCO_3$)	Alkalinity (mg/ℓ as $CaCO_3$)	pH	TOC (mg/ℓ)
Lake Superior	1/26/77	2182	1932	48	42	8.07*	2.6
Lake Superior	2/16/77	1813	785	48	44	8.10	2.8
Lake Superior	3/16/77	1836	1055	44	42	8.04	2.7
So. Kawishiwi R.	7/13/77	697	301	29	26	6.77	13
So. Kawishiwi R.	7/26/77	1140	336	28	22	7.23	15
So. Kawishiwi R.	8/2/77	1034	330	28	20	7.36	13
St. Louis R.	9/27/77	3316	1141	86	22	7.25	34
St. Louis R.	10/12/77	3014	875	84	17	7.01	32
St. Louis R.	10/19/77	2325	732	74	17	7.09	28
St. Louis R.	9/6/77 ~~10/19/77~~	3414	1779	73	18	6.94	28
St. Louis R.	9/22/77	3757	1029	100	20	7.55	34
Greenwood Lake	8/12/77	2171	770	25	2.5	5.88	39

*Mean of known values substituted for missing datum.

Table 8. Acute toxicity of copper-nickel leachates to *Daphnia pulicaria*.

Leachate	Dilution water	Test date	Percentage leachate	Effect	Cu (µg/l)	Ni (µg/l)	Zn (µg/l)	Co (µg/l)	Mn (µg/l)	Hardness (mg/l as $CaCO_3$)	Organic carbon (mg/l)	pH	Predicted Cu LC50	Predicted potency ratio	Cu-Ni concentration as Cu
Seep 3	EM-6	4/6/77	100%	no mortality	T^a 270 F^b 52	T 3200 F 930	T 73	T 440	T 2740 F 840	446	F 26	8.30	132	.0102	T 303 F 61
Seep 3	O11	9/14/77	2.2%	48-hr LC50	T (40)^c	T (660)	T (16)	T (35)	T (220)	(263)	F (7.3)	8.09	27	.00358	T 42
U.S. Steel	So. Kawishiwi R.	4/6/77	1.7%	complete mortality	T (210)	T (294)	T (2.3)	T (12)	T (89)	(31)	F (13)	7.45	54^d	.0548	T 226
U.S. Steel	EM-6	4/6/77	1.7%	complete mortality	T (210)	T (294)	T (5.3)	T (12)	T (170)	(164)	F (6.8)	8.32	25	.00518	T 212
U.S. Steel	Lake Superior	4/26/77	0.1%	complete mortality	T (11)	T (22)	T (<2)	T (<2)	T (6.0)	(45)	T (3.0)	7.99	9.3^d	.00484	T (11)
U.S. Steel	EM-6	10/26/77	1.0%	48-hr LC50	T 150	T 130	T (6.7)	T (11)	T (67)	(118)	F (6.0)	8.20	20	.00577	T 151
U.S. Steel	So. Kawishiwi R.	10/27/77	0.7%	48-hr LC50	T 114	T 101	T (2.6)	T (7.7)	T (78)	(23)	T (15)	7.04	69	.0781	T 121
INCO Seep	So. Kawishiwi R.	7/28/77	100%	no mortality	T 96	T 65	T 2.7	T 17	—	(765)	F 19	8.31	92	.00427	T 96
INCO Seep	So. Kawishiwi R.	11/29/77	100%	no mortality	T 23 F 9.9	T 4500 F 3500	T 13	T 250 F 210	—	490	F 12	8.35	53	.00384	T 40 F 23

[a] Total

[b] Filtered

[c] Calculated from measurements in leachate and dilution water.

[d] Chemical characteristics were similar when prediction bioassays were run in this water. Weighted mean of those LC50's is used here rather than LC50 predicted by model.

Table 9. Chronic toxicity of copper to fathead minnow embryos and larvae in Lake Superior water.

Mean Cu^{++} concentration ($\mu g/l$)	Fraction of 96-hr LC50[a]	Mean Cu Body Accumulation ($\mu g/g$ dry wt)	Mean percentage embryo survival to hatch[b]	Mean percentage fish survival after hatch[b]	Mean fish weight after 30 days (mg)[c]
<5	-	5.1375	93	99	107
5.0	0.04621	11.590	89	99	112
9.0	0.08318	19.7675[d]	97	97	96
13.1	0.1211	25.2250[d]	91	88	14[d]
26.2	0.2421		92	62[d]	3[d]
52.1	0.4815		90	15[d]	2[d]

[a] Acute tests run in Lake Superior water using 8-week-old fathead minnows. Weighted mean 96-hr LC50 = 1082μg Cu^{++}/l

[b] arcsin $\sqrt{x}$ transformation used on all percentages

[c] log x transformation used on weights

[d] significantly different from control at $\alpha = 0.05$ (Dunnett)

Table TO. Chronic toxicity of nickel to fathead minnow embryos and larvae in Lake Superior water

Mean Ni⁺⁺ concentration (µg/l)	Fraction of 96-hr LC50[a]	Mean Ni Body Accumulation (µg/g dry wt)	Mean percentage embryo survival to hatch[b]	Mean percentage fish survival after hatch[b]	Mean fish weight after 30 days (mg)[c]
<6	-	2.7225	98	97	105
21.0	0.0040	11.1300	93	95	97
44.4	0.0086	17.6000[d]	92	100	97
108.9	0.0210	25.4750[d]	92	99	98
433.5	0.0836	-	71[d]	9[d]	123
1532.1	0.2954	-	0[d]	-	-

[a] Acute tests run in Lake Superior water using 8-week-old fathead minnows. Weighted mean 96-hr LC50 = 5186 µg Ni⁺⁺/l.

[b] arcsin $\sqrt{x}$ transformation used on all percentages.

[c] log x transformation used on weights

[d] significantly different from control at $\alpha = 0.05$ (Dunnett)

Table 11. Chronic toxicity of cobalt to fathead minnow embryos and larvae in Lake Superior water.

Mean Co^{++} concentration (µg/l)	Fraction of 96-hr LC50[a]	Mean percentage embryo survival to hatch[b]	Mean percentage fish survival after hatch[b]	Mean fish weight after 30 days (mg)[c]
<1[d]	-	78[d]	21[d]	71[d]
14.7	0.0277	94	96	82
29.5	0.0556	88	85	78
48.7	0.0918	91	86[e]	77
112.5	0.2120	91	78[e]	66
223.2	0.42066	81	61[e]	65[e]

[a] Acute test run in Lake Superior water using 8-week-old fathead minnows. Weighted mean 96-hr LC50 = 530.6 µg Co/l.

[b] arcsin $\sqrt{x}$ transformation used on all percentages.

[c] log x transformation used on weights

[d] Control data which was not included in statistical analysis.

[e] significantly different from lowest treatment at $\alpha = 0.05$ (Duncans new multiple range test)

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[b] Filtered

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9.0	0.08318	19.7675[d]	97	97	96
13.1	0.1211	25.2250[d]	91	88	14[d]
26.2	0.2421		92	62[d]	3[d]
52.1	0.4815		90	15[d]	2[d]

[a] Acute tests run in Lake Superior water using 8-week-old fathead minnows. Weighted mean 96-hr LC50 = 1082 µg Cu^{++}/l

[b] arcsin $\sqrt{x}$ transformation used on all percentages

[c] log x transformation used on weights

[d] significantly different from control at $\alpha = 0.05$ (Dunnett)

Table 1C. Chronic toxicity of nickel to fathead minnow embryos and larvae in Lake Superior water

Mean Ni^{++} concentration (μg/l)	Fraction of 96-hr LC50[a]	Mean Ni Body Accumulation (μg/g dry wt)	Mean percentage embryo survival to hatch[b]	Mean percentage fish survival after hatch[b]	Mean fish weight after 30 days (mg)[c]
<6	-	2.7225	98	97	105
21.0	0.0040	11.1300	93	95	97
44.4	0.0086	17.6000[d]	92	100	97
108.9	0.0210	25.4750[d]	92	99	98
433.5	0.0836	-	71[d]	9[d]	123
1532.1	0.2954	-	0[d]	-	-

[a] Acute tests run in Lake Superior water using 8-week-old fathead minnows. Weighted mean 96-hr LC50 = 5186 μg Ni^{++}/l.

[b] $\arcsin\sqrt{x}$ transformation used on all percentages.

[c] log x transformation used on weights

[d] significantly different from control at $\alpha = 0.05$ (Dunnett)

Table 11. Chronic toxicity of cobalt to fathead minnow embryos and larvae in Lake Superior water.

Mean Co^{++} concentration ($\mu g/l$)	Fraction of 96-hr LC50[a]	Mean percentage embryo survival to hatch[b]	Mean percentage fish survival after hatch[b]	Mean fish weight after 30 days (mg)[c]
<1[d]	-	78[d]	21[d]	71[d]
14.7	0.0277	94	96	82
29.5	0.0556	88	85	78
48.7	0.0918	91	86[e]	77
112.5	0.2120	91	78[e]	66
223.2	0.42066	81	61[e]	65[e]

[a] Acute test run in Lake Superior water using 8-week-old fathead minnows. Weighted mean 96-hr LC50 = 530.6 μg Co/l.

[b] arcsin $\sqrt{X}$ transformation used on all percentages.

[c] log X transformation used on weights

[d] Control data which was not included in statistical analysis.

[e] significantly different from lowest treatment at $\alpha = 0.05$ (Duncans new multiple range test)

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