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REGIONAL COPPER-NICKEL STUDY

AQUATIC TOXICOLOGY STUDY

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Minnesota Environmental Quality Board

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TABLE OF CONTENTS

INTRODUCTION

The Regional Copper-Nickel Study has as one of its major objectives the task of predicting the potential impacts of copper-nickel development on the aquatic environment. One type of potential impact which deserves attention is the toxicity of effluents from copper-nickel mining, processing and refining operations. The purpose of the Aquatic Toxiciology Study was to assist in predicting the toxicity of such effluents by conducting experiments to (1) measure the acute toxicity of potential heavy metal pollutants and their mixtures, (2) assess the effect of water chemistry on metal toxicity, (3) evaluate the acute toxicity of existing copper-nickel leachates, (4) determine the chronic effects of metals on fish, and (5) predict the relative sensitivity of different species of aquatic animals to metals.

ACUTE TOXICITY OF SELECTED HEAVY METAL 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, (Regional Copper-Nickel Study, 1978) literature dealing with the toxic effects of selected metal mixtures on aquatic life was surveyed. The present study examined the acute toxicity of copper-nickel mixtures, copper-cobalt mixtures, and nickel-cobalt mixtures to the fathead minnow, *Pimephales promelas*, and the cladoceran *Daphnia pulicaria*. Experiments with all of the mixtures were conducted in water from Lake Superior at the U.S. EPA Environmental Research Laboratory-Duluth. In addition, water from the South Kawishiwi River near the State Highway 1 crossing upstream from Birch Lake was used for studies of copper-nickel mixtures (Map 1).

METHODS

For the fathead minnow, 96-hr LC50's of copper, nickel and cobalt sulfates and their binary mixtures were determined using continuous-flow dilution apparatus at a temperature of 25°C. For *D. pulicaria*, 48-hr LC50's of the three individual toxicants and three mixtures were determined in unrenewed solutions at 18°C. Total metal concentrations in all treatment levels were measured at least once in each toxicity test. Experimental and analytical methods are described in greater detail in the Aquatic Toxicology Operations Manual.

Two or more toxicity tests were conducted for each test species with each metal in each water. Weighted mean LC50's were calculated for each species-metal-test water combination. 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.

Results

Copper-Nickel Mixtures--The relative concentrations of copper and nickel in tests of copper-nickel mixtures were set in most cases according to their relative potencies. For each species and test water, a copper-nickel potency ratio (copper LC50/nickel LC50) was calculated from the weighted mean LC50's of copper and nickel. For each treatment level, the desired nickel concentration multiplied by the potency ratio equaled the desired copper concentration. Both components were thus expected to contribute equally to the toxicity of the mixture. In some experiments the ratios of measured copper concentration to measured nickel concentration deviated significantly from the potency ratios, but measured concentration ratios were consistent within an experiment.

The total copper-nickel concentrations in experiments with mixtures were expressed as copper, the more toxic component. The nickel concentration in each test chamber was multiplied by the potency ratio, and the product was added to the copper concentration. If the LC50 of a copper-nickel mixture, expressed as copper, equaled the weighted mean LC50 of copper which was determined earlier, it was concluded that the joint effects of copper and nickel were additive.

Table 1 lists the 96-hr LC50's of copper and nickel and their weighted means for fathead minnows in both test waters. Table 2 gives the 96-hr LC50's of copper-nickel mixtures for D. pulicaria in both test waters.

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

Forty-eight-hour LC50's of copper and nickel for D. pulicaria in both test waters, and their weighted means, are given in Table 3. Table 4 lists the 48-hr LC50's of copper-nickel mixtures for D. pulicaria in both test waters.

The joint toxicity of copper and nickel to Daphnia pulicaria (Table 4) appeared 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 the South Kawishiwi River experiments varied above and below the copper-nickel potency ratio given in the table footnotes, but do not appear to have been related to the

LC50 of the mixture. The levels of hardness and total organic carbon remained nearly constant for the duration of the experiments in South Kawishiwi River water.

Copper-Cobalt Mixtures--Toxicity experiments with copper-cobalt mixtures were conducted in Lake Superior water, using the same experimental and analytical procedures which were employed in the copper-nickel experiments. The 96-hr LC50's of copper and cobalt for the fathead minnows in Lake Superior water and their weighted means, are presented in Table 1. Table 5 lists the 96-hr LC50's of copper-cobalt mixtures for fathead minnows. For *D. pulicaria* the 48-hr LC50's of copper and cobalt individually, and then in mixtures, are given in Tables 3 and 6, respectively. The copper-cobalt concentrations in the mixture experiments were expressed as copper.

From Table 5 it appears that the joint toxicity of copper and cobalt to the fathead minnow was somewhat less than additive. For *D. pulicaria* (Table 6) the results of the two mixture experiments were quite dissimilar, probably because of the inconsistent toxicity of cobalt to this species, as shown in Table 3. However, the toxic interaction of copper and cobalt did not appear to be much more or much less than additive.

Nickel-Cobalt Mixtures--The 96-hr LC50's of nickel and cobalt for the fathead minnow in Lake Superior water, and their weighted means, are given in Table 1. In Table 7, the 96-hr LC50's of nickel-cobalt mixtures for fathead minnows are listed. For *D. pulicaria*, the 48-hr LC50's of nickel and cobalt, and of nickel-cobalt mixtures, are shown in Tables 3 and 8, respectively. The nickel-cobalt concentrations in mixture experiments with fathead minnows were expressed as cobalt, because of the higher toxicity of cobalt to the fathead minnow. However, nickel was slightly more toxic than cobalt to D. pulicaria, and nickel-cobalt concentrations in experiments with this species were therefore expressed as nickel.

The joint toxicity of nickel and cobalt to the fathead minnow (Table 7) was slightly less than additive, while to D. pulicaria (Table 8) it was additive to more than additive. The toxicity of nickel-cobalt mixtures to D. pulicaria was variable, as had been the toxicity of copper-cobalt mixtures to the same species.

DISCUSSION

All but one of the metal mixtures that were tested had approximately additive joint effects on both species. In the only exceptional case, copper-nickel mixtures were about twice as toxic to the fathead minnow as would have been expected on the basis of an additive interaction. For D. pulicaria, the toxic interaction of copper and nickel ranged from additive or slightly less, to slightly more than additive. The joint toxicity of copper-cobalt mixtures and nickel-cobalt mixtures to fathead minnow was slightly less than additive. Copper and cobalt displayed an approximately additive interaction in tests with D. pulicaria, while the joint toxicity of nickel-cobalt mixtures to this species was additive or slightly more than additive.

Only copper-cobalt mixtures acted in about the same way on both species; only D. pulicaria responded in a fairly consistent manner to all three mixtures. The two types of water in which copper-nickel mixtures were tested did not affect their toxic interaction on the fathead minnow, but appeared to change their joint toxicity to D. pulicaria from slightly more than additive in Lake Superior water, to additive or slightly less than additive in South Kawishiwi River water.

Of the three mixtures addressed here, only copper-nickel mixtures have been reported on in the aquatic toxicology literature. Anderson and Weber (1977) showed that copper and nickel were additive in their joint lethal effect on the guppy (Poecilia reticulata), in contrast to the results of our experiments with the fathead minnow. Muska (1978) studied the effects of copper-nickel mixtures on food consumption and growth of the guppy in 7-day experiments. Copper-nickel mixtures had a more than additive joint effect on food consumption rate, and an additive joint effect on food conversion efficiency. The joint effect of copper-nickel mixtures on the growth rate of the guppy was more than additive when rations were unlimited and growth rate depended on appetite, and additive when rations were restricted and growth rate depended on food conversion efficiency.

The effects of mixtures containing varying proportions of toxicants have not received a great deal of attention from aquatic toxicologists. If the reason for additive effects in a mixture is that all of the components act on the organism in the same way, thus behaving essentially as a single toxicant, a change in their relative proportions should not influence the way in which their individual effects add together. Less than additive or more than additive joint effects would seem likely to be the most pronounced in a mixture which contains equally toxic proportions of its components. The relative proportions of copper and nickel in mixture experiments with D. pulicaria in South Kawishiwi River water were quite variable, as stated earlier (Table 4). The joint toxicity of copper and nickel was approximately additive in all experiments, and does not appear to have been related to the copper-nickel concentration ratio.

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

Predictions of the vulnerability of aquatic communities to heavy metal pollution must take into account the relationship of receiving water chemistry to the toxicity of heavy metals. In a previous report, literature was surveyed which dealt with the effects of hardness, alkalinity, pH and organic substances on the toxicity of selected heavy metals to aquatic life. In this study models were developed using these parameters 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 Regional Copper-Nickel Study Area (Study Area).

METHODS

For the fathead minnow, 96-hr LC50's of copper and nickel sulfates were determined using continuous-flow dilution apparatus at a temperature of 25^{o}C. For *Daphnia pulicaria*, 48-hr LC50's of copper and nickel sulfates were determined in unrenewed solutions at 18^{o}C. Total metal concentrations and pH in all treatment levels, and hardness, alkalinity and total organic carbon in controls were measured at least once in each toxicity test. Experimental and analytical methods are described in greater detail in the Aquatic Toxicology Operations Manual.

Toxicity tests with fathead minnows were conducted in water from Lake Superior at the U.S. EPA Environmental Research Laboratory-Duluth, the South Kawishiwi River near the State Highway 1 crossing upstream from Birch Lake, the St. Louis River at the County Highway 100 crossing south of Aurora, Colby Lake near the Erie Mining Co. pump house, and the Embarrass River at the State Highway 135 crossing near Embarrass (Map 1). Tests with *D. pulicaria* were conducted in these waters as well as in water samples from near the public access points on Lake One, Cloquet Lake and Greenwood Lake, all in Lake County.

The LC50's and the hardness, alkalinity, pH and total organic carbon (TOC) measurements from all of the toxicity tests are listed in Tables 9-12. For each metal and test species, the best single-variable and (if significant) two-variable regression models were chosen for prediction of LC50 from the four water chemistry parameters. Because of the limited number of experiments, regression models with three independent variables were not considered. LC50, hardness, alkalinity and TOC were transformed to base 10 logarithms. 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.

RESULTS AND DISCUSSION

In tests of the acute toxicity of copper to the fathead minnow (Table 9), log TOC was the chemical variable most highly correlated with the log 96-hr LC50. The relationship of the two variables is described by:

$$\log 96\text{-hr LC50} = 1.37 + 1.20 \log \text{TOC} \quad R^2 = .957$$

Of the remaining chemical variables, log alkalinity made the only significant contribution to the regression of log 96-hr LC50 on log TOC ($\alpha = .05$), yielding this model:

$$\log 96\text{-hr LC50} = .610 + 1.32 \log \text{TOC} + .443 \log \text{alkalinity} \quad R^2 = .985$$

In copper experiments with Daphnia pulicaria (Table 10), log TOC was better correlated with the log 48-hr LC50 than were the other chemical variables. The best model for this relationship is:

$$\log 48\text{-hr LC50} = .301 + 1.33 \log \text{TOC} \quad R^2 = .925$$

None of the remaining three chemical variables contributed significantly to the regression of log 48-hr LC50 on log TOC ($\alpha = .05$).

The exceptionally high LC50 from the experiment at the St. Louis River which began on 9/27/77 was judged to be an outlier by using a t-test of the corresponding studentized residual. The significance level for the t-test was adjusted for selection bias to provide a true error rate of .05.

With the outlier deleted TOC remained the best single predictor of the 48-hr LC50, and the fit of the model was markedly improved:

$$\log \text{48-hr LC50} = .342 + 1.27 \log \text{TOC} \quad R^2 = .955$$

None of the other chemical variables added significantly to the regression of log 48-hr LC50 on log TOC ($\alpha = .05$).

In nickel toxicity tests with the fathead minnow (Table 11), log hardness was the chemical variable best correltated with the log 96-hr LC50. The model for this relationship is:

$$\log \text{96-hr LC50} = 1.95 + 1.05 \log \text{hardness} \quad R^2 = .707$$

None of the other variables made a significant contribution to the regression of log 96-hr LC50 on log hardness ($\alpha = .05$).

Hardness was also the variable most highly correlated with LC50 in nickel experiments with <u>Daphnia pulicaria</u> (Table 12). The relationship of log 48-hr LC50 with log hardness is described by this equation:

$$\log \text{48-hr LC50} = 2.07 + .710 \log \text{hardness} \quad R^2 = .705$$

Again, none of the possible two-variable regressions fit the experimental data significantly better than the simple model relating log 48-hr LC50 to log hardness ($\alpha = .05$).

Th LC50 from the experiment conducted in Greenwood Lake water was judged to be an outlier by the criterion outlined earlier. Greenwood Lake is different from the other test waters in that it has relatively low hardness and relatively high TOC; hardness and TOC in the 15 tests run in other waters were positively correlated (r=.580; significant at .05 level). The fact that the 48 hour LC50 of nickel in Greenwood Lake water is much higher than would be predicted on the basis of hardness suggests that high concentrations of TOC may reduce nickel toxicity, but that the effect of TOC in this series of experiments is difficult to distinguish because of its positive correlation with hardness. Because of the possibility that the outlying LC50 value contains valid and worthwhile information which also pertains to the other experiments, and because there was no evidence of unusual errors or circumstances in the experiment, it was decided not to delete the outlier from the data set.

Dummy X- variables (Draper and Smith 1966) were used to determine whether, for any of the four sets of toxicity data, different test waters had effects on copper and nickel LC50's which were not fully explained by the chosen regression models. Only those waters in which two or more toxicity experiments had been conducted were included in these analyses. In none of the four data sets did the addition of dummy variables significantly improve the fit of the regression models to the toxicity data. It was, therefore, concluded that the chosen regression models explained variations in LC50's equally well both among experiments in a particular test water and among the test waters themselves.

An attempt was made to experimentally verify the effect of hardness on nickel toxicity and its lack of effect on copper toxicity, which had been demonstrated in natural waters. A series of test were run in Lake Superior water to determine the affect of added calcium and magnesium sulfates on the toxicity of copper and nickel to D. pulicaria in Lake Superior water. The results of these experiments are given in Tables 13 and 14, and show that calcium and magnesium sulfates reduced the toxicity of both copper and nickel, although the effect of magnesium sulfate on nickel toxicity was relatively small. Increased additions of calcium and magnesium sulfates did not always produce further decreases in copper and nickel toxicity. The mitigating effect of calcium and magnesium sulfates on copper and nickel toxicity was most likely due to the cations, since, in another test, the addition of 100 mg/l of sodium sulfate (expressed as the anion) reduced the 48 hour LC50 of copper to Daphnia pulicaria only from 9.3 ug/l to 10.8 ug/l.

In light of the field experiments in natural waters, the affect of added hardness on copper toxicity was unexpected, although the effect was substantial only in terms of percentage change in the LC50. It is not known whether this effect is multiplicative or only additive, in which case it would be less noticeable in a waters with a higher value of TOC, where the copper LC50 would already be high.

The observed affect of hardness on nickel toxicity, while not unexpected, was somewhat inconsistent with predictions from the nickel toxicity model for D. pulicaria. On the basis of this model, if 50, 100, and 200 mg/l of hardness were added to Lake Superior water (mean hardness=47 mg/l), the corresponding 48 hour LC50's of nickel would be 2781, 4037, and 5835 ug/l. At 50 mg/l of added hardness, the prediction falls between the two LC50's corresponding to that level in Table 14, but at 100 mg/l of added hardness, beyond the range of hardnesses encountered in field experiments, the model gives a moderate overestimate. At 200 mg/l of added hardness, the overestimate is substantial. The calcium:magnesium ratios (as $CaCO_3$) in the waters from which the toxicity model was derived ranged from about 1 Ca:1 Mg to about 2 Ca:1 Mg. These overestimates could have been related to the effect of TOC on nickel toxicity, the possibility of which was discussed earlier. If high levels of TOC did indeed reduce nickel toxicity in the prediction experiments, where the affect of TOC would have been confounded with that of hardness, nickel would be less toxic than predicted in hardened Lake Superior water, which has an average TOC level of only 3 mg/l.

Several investigators have studied the relationship of hardness to the toxicity of metals. Tabata (1969) found that the 24 hour LC50 of nickel to carp (Cyprinus carpio) and Daphnia spp. was increased more on a percentage basis than the 24 hour LC50 of copper by raising hardness from 25 mg/l to 100 mg/l as $CaCO_3$. In a study by Geckler et al. (1977), the 48 hour LC50 of copper for bluntnose minnows (Pimephales notatus) increased when hardness was increased up to a level of 276 mg/l as $CaCO_3$, without altering the Ca:Mg ratio. Above this level, the LC50 stayed the same or decreased. Zitko and Carson (1977) found that copper toxicity to Atlantic salmon was not affected by raising calcium hardness from 9 mg/l to 45 mg/l as $CaCO_3$, or by raising magnesium hardness from 4 to 34 mg/l as $CaCO_3$.

The affect of alkalinity on copper toxicity has been demonstrated by several investigators. Andrew (1976) reported that the addition of 100 mg/l of alkalinity as $CaCO_3$ to a copper solution in Lake Superior water increased the median survival time of fathead minnows tenfold in the solution. Andrew et al. (1977) found that the median survival time of Daphnia magna in a copper solution increased from less than 50 min. to 60 min. when 100 mg/l (as $CaCO_3$) of alkalinity was added, and to 73 min. when 200 mg/l of alkalinity was added. These observations corroborate the findings in this study that alkalinity affected the toxicity of copper to the fathead minnow but not D. pulicaria.

The findings from the present study that copper toxicity was highly dependent on the level of total organic carbon do not mean that any source of organic carbon would have the identical effect. Humic materials comprise most of the organic carbon in the surface waters of the Study Area, and the affinity of copper for these substances is well documented. It has also been shown previously that copper is less toxic to aquatic animals when complexed with humic substances. According to Carson and Carson (1972), adding 10 mg/l of humic acid to copper solutions quadrupled the 96-hr LC50 of copper for Atlantic salmon. Brown et al. (1974) showed that the median survival time of rainbow trout in a copper solution doubled when 4.5 mg/l of humic substances (equal parts of peat extract and humic acid) were added.

ACUTE TOXICITY OF COPPER-NICKEL LEACHATES TO DAPHNIA PULICARIA

The acute toxicities of five copper-nickel leachates were assessed in a series of experiments with *Daphnia pulicaria*. The leachates were from Seep 3 and the EM-8 sampling station at the Erie Mining Company Dunka Mine east of Babbitt, from the U.S. Steel bulk sample site south of Babbitt, from the INCO bulk sample site near the South Kawishiwi River upstream from Birch Lake, and from the FL-4 sampling station at the Minnamax exploration site southeast of Babbitt (Map 1). Dilution water was taken from near the 011 mine discharge pipe and the EM-6 sampling station on Unnamed Creek at the Dunka Mine, from the South Kawishiwi River upstream from Birch Lake, and from the North Branch of the Partridge River at the Dunka Mine road crossing.

METHODS

Forty-eight-hour LC50's were determined in unrenewed dilutions of leachates at 18°C. Heavy metal concentrations, hardness alkalinity, and organic carbon concentrations were either measured in the treatment dilutions, or calculated from measured levels in leachate and dilution water. The pH was measured in all treatments and controls.

Table 15 summarizes the chemical characteristics of leachate dilutions representing 48-hr LC50's, where LC50's could be determined. For tests in which full strength leachate was lethal to less than half the test animals, or in which the weakest dilution was lethal to more than half the animals, the chemical characteristics of those concentrations are listed.

Copper LC50's and nickel LC50's were predicted from hardnesses and organic carbon concentrations in the listed dilutions, using the models given in the previous section. It should be noted that many of the listed leachate dilutions had higher hardness levels than did the test waters from which the toxicity prediction models were derived. The model for nickel toxicity was assumed to be valid beyond the range of hardnesses which had actually been tested. Organic carbon in some leachates and dilution waters was measured only in the dissolved form, whereas the copper toxicity prediction model is based on total organic carbon measurements. (Dissolved organic carbon and dissolved metal are defined here as the portion passing through a 0.45 micron membrane filter.) Dissolved organic carbon levels were usually more than 90% of total organic carbon levels in the waters from which the prediction model was derived.

For each listed leachate dilution, the measured copper concentration divided by the predicted copper LC50 was added to the measured nickel concentration divided by the predicted nickel LC50. If the sum equaled 1, the percentage of leachate in the dilution was equal to the predicted 48-hr LC-50 of leachate, using the copper and nickel toxicity models, therefore 50%

mortality in that dilution was predicted. If the sum was greater than 1, more than 50% mortality was predicted, and if it was less than 1, less than 50% mortality was predicted. In order to use this approach, it must be assumed that the joint toxicity of copper and nickel to D. pulicaria is additive at different concentration ratios and in different types of water, and that other toxic metals made negligible contributions to the toxicity of the five leachates. The first assumption is fairly well supported by the data in Table 4, and the second assumption will be addressed later in this section.

Because of the nature of the distribution of percentage mortality with respect to toxicant concentration, the calculations required to predict the percentage mortality, if different from 50%, in a leachate dilution would be overly complex and could be misleading, considering the limited size of each experiment. Prediction of the leachate concentration which would cause 50% mortality would likewise require overly complex calculations, since hardness and total organic carbon, and thus the predicted LC50s, vary with dilution of a leachate.

Chemical analyses of leachates and prediction models for copper and nickel toxicity were not available when most of the leachate toxicity tests were conducted. This made it difficult to select leachate dilutions which would give conclusive test results.

RESULTS AND DISCUSSION

Table 15 shows that in most of the experiments, from which conclusive results were obtained, leachates were less toxic than predicted. Some of the differences between observed and predicted toxicity could be at least partially explained by the presence of suspended metals in leachates, since only dissolved metals are believed to be biologically active (Davies, 1976). In the first sample of leachate from Seep 3, which was inadvertently muddied as it was taken, only about 20% of the copper and 30% of the nickel was dissolved. While the toxicity prediction models were derived from LC50's which were expressed as total metal, dissolved copper concentrations averaged about 90%, and dissolved nickel concentrations about 95%, of total levels in the prediction experiments.

Dissolved metal concentrations in the other two samples from Seep 3 were not determined, but the substrate was not disturbed when these samples were taken. A large number of other leachate samples were taken from Seep 3 during 1977 by the Copper-Nickel Study. In these, an average of 75% of total copper and 97% of total nickel was dissolved. It appears, then, that the relationship of dissolved metal to total metal cannot completely explain the unexpectedly low toxicity of the second and third leachate samples.

The two samples of leachate from the INCO site were not lethal to any of the test animals, even though the total concentrations of copper and nickel in both samples were high enough to cause about 50% mortality according to the toxicity prediction models. In the second sample, concentrations of dissolved copper and nickel were determined. Only 43% of the copper and 78% of the nickel in the sample were dissolved. It is probably safe to assume that similar proportions of dissolved copper and nickel were also present in the first sample, which was taken in the same manner 4 months before.

Dissolved copper and nickel levels in the toxicity test samples of the other three leachates were not measured, but samples of three leachates were taken routinely by the Copper-Nickel Study and analyzed for total and dissolved metals. Dissolved copper and nickel both averaged more than 95% of total levels in samples of the U.S. Steel leachate from 1977; thus the low toxicity of the last three samples of this leachate was probably not caused by suspended copper and nickel.

In samples of the EM-8 leachate taken during the same period, dissolved copper levels averaged 70% of total copper levels, and dissolved nickel averaged 97% of total nickel. The total copper-nickel concentration in the LC50 dilution of this leachate was nevertheless twice as high as the predicted lethal level.

In samples of the Minnamax leachate that were taken during 1977, dissolved copper levels averaged 88% of total copper levels, and dissolved nickel averaged 90% of total nickel. Since such high proportions of copper and nickel in this leachate were dissolved, the occurrence of 20% mortality in a sample containing .68 of the predicted lethal copper-nickel level, based on total metal, appears reasonable.

Since the presence of suspended metals does not fully account for the low toxicity of the second and third samples of seep 3 leachate, the last three samples of U.S. Steel leachate, and the EM-8 leachate, another factor or factors must be responsible for the differences between predicted and observed toxicity. The levels of hardness in the Seep 3 and EM-8 leachates, and in the 011 and EM-6 dilution waters were higher than those in the waters from which the copper and nickel toxicity prediction models were derived, and might have unexpected effects on toxicity. The data of Tables 13 and 14, however, indicate that successively higher levels of hardness do not necessarily cause corresponding decreases in copper and nickel toxicity.

Another factor may be the presence of metal-complexing substances in leachates which were not encountered or not recognized in toxicity prediction experiments. Furthermore, a longer time may be available for the formation of metal complexes in a natural leachate than in a prepared toxicity test solution. Overestimates of toxicity were nonetheless just as severe for the U.S. Steel leachate, which contained low levels of organic and inorganic ligands, as for the Seep 3 and EM-8 leachates where the ligands were more concentrated.

In addition to copper and nickel, leachate samples often contained elevated concentrations of zinc, cobalt and manganese (Table 15). Biesinger and Christensen (1972) found that the 48-hr LC50 of zinc for unfed Daphnia magna in Lake Superior water was 100 μg/l, or 10 times the copper LC50 for that species. Tabata (1969) showed that the toxicity of zinc to Daphnia spp. was diminished more than the toxicity of copper or nickel by increased water hardness. In all of the leachate samples except those from EM-8 and Minnamax, zinc concentrations were lower than copper concentrations (Table 15), and not likely to have noticeable toxic effects. The zinc concentrations in the EM-8 and Minnamax samples, especially in the latter, may have contributed to their toxicity if the LC50's of zinc for D. magna and D. pulicaria are similar, although the hardness of both leachates was extremely high.

It was shown in the present study that the weighted mean 48-hr LC50 of cobalt for D. pulicaria in Lake Superior water was 2025 μg/l, or over 200 times the LC50 of copper (Table 3). According to Tabata (1969), the toxicity of cobalt to Daphnia spp. was affected less by raising the level of hardness than was the toxicity of copper or nickel. Cobalt was present in a higher concentration than copper in four leachate samples: the first sample from Seep 3, the EM-8 sample, the second INCO sample and the Minnamax sample

(Table 15). In none of these samples was the cobalt concentration more than 14% of the level of nickel, which had about the same toxicity as cobalt to D. pulicaria in Lake Superior water (Table 3). The highest listed cobalt concentration was 440 µg/l in the Seep 3 sample, and corresponded to 22% of the 48-hr LC50 in Lake Superior water. A substantial proportion of the cobalt may not have been dissolved, as was the case with the copper, nickel and manganese in the sample. Because of its low concentrations in relation to its LC50 and in relation to nickel concentrations, it is unlikely that cobalt could have added significantly to the toxicity of any of the leachate samples.

The 48-hr LC50 of manganese for unfed D. magna in Lake Superior water was 9800 µg/l, or 1000 times the copper LC50 and 19 times the nickel LC50 for that species (Biesinger and Christensen, 1972). Despite its relatively low toxicity in Lake Superior water, manganese was not detoxified as readily by elevated hardness levels as were copper, nickel and cobalt (Tabata, 1969). Manganese was present in a higher concentration than copper in all samples from Seep 3, EM-8 and Minnamax (manganese was not measured in the INCO seep). If suspended manganese in the first Seep 3 sample is ignored, the highest manganese concentration in any of the leachate dilutions listed in Table 15 was 1439 µg/l, in the dilution of EM-8 leachate. This level corresponds to just 15% of the LC50 of manganese for D. magna in Lake Superior water. If the toxicity of manganese to D. pulicaria is similar, manganese probably would not have contributed significantly to the toxicity of any of the leachates in which it was measured.

In summary, the toxic effects of three of the five leachates could be attributed almost entirely to copper and nickel, while zinc may have had significant toxic effects in two leachates, the EM-8 leachate and the Minnamax leachate.

The Seep 3, EM-8, and U.S. Steel leachates were less toxic than was expected on the basis of their copper and nickel concentrations and their levels of hardness and TOC. The reasons for these discrepancies could likely be found with further experimentation. The preparation and biological testing of "synthetic" leachtes which duplicate as closely as possible the leachates in question might be one way to resolve or at least pinpoint the problem.

CHRONIC TOXICITY OF COPPER, NICKEL AND COBALT TO THE FATHEAD MINNOW

Concentrations of toxic metals which are not sufficient to kill aquatic animals rapidly, e.g. in 96 hours, may nonetheless decimate aquatic populations by reducing long-term rates of survival, growth and reproduction. In a study of toxic pollutants, the potential for long-term toxic effects must therefore be considered.

METHODS

McKim (1977) proposed the wider use of toxicity tests with embryo-larval and early juvenile life stages of fish to predict life-cycle chronic toxicity when longer tests were impractical, since the early life stages were often the most sensitive to toxicants. 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 and to copper in Embarrass River water. In each of the four experiments, five test concentrations and a control were supplied by a continuous-flow dilution apparatus. Exposure began with embryos 1 day after fertilization and continued for 30 days. Temperature in all experiments was 25^{o}C, and dissolved oxygen concentration was near saturation. Total metal concentrations in all treatment levels were determined at regular intervals during each test. At the end of each of the three tests in Lake Superior water, a sample of five fish was taken from each replicate chamber for analysis of metal accumulation, from only those treatments in which all replicates contained at least 15 fish. The samples were frozen, then digested whole and analyzed for the test metal. The remaining fish were preserved in formalin and weighed. Experimental and analytical methods are described in greater detail in the Aquatic Toxicology Operations Manual.

RESULTS AND DISCUSSION

Results of the four experiments are summarized in Tables 16-19. In Lake Superior water 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 26.2 μg Cu/l (Table 16). Mean weight of the 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-hr LC50 of copper for 8-week-old fathead minnows in Lake Superior water. Fish exposed to 9.0 μg Cu/l accumulated significantly more copper than control fish.

A nickel concentration of 433.5 μg Ni/l, 0.0836 of the 96-hour LC50, significantly reduced the survival rates of both embryos and young fish (Table 17). No significant effect on mean fish weight after 30 days was detected at nickel concentrations permitting survival. Significantly more nickel accumulated in fish exposed to 44.4 μg Ni/l, than in control fish.

Duncan's new multiple range test was used in the statistical analysis of data from the cobalt experiment (Table 18). This test was used because embryos and larvae in control tanks suffered heavy mortality caused by fungus which originated in the fish culture unit from which the embryos had been taken; controls were therefore not included in the statistical analysis.

Fungus was not evident in the chambers to which cobalt was added. Cobalt did not significantly affect embryo survival rate at the highest treatment level. A significant reduction in fish survival rate and mean fish weight relative to the lowest treatment level occurred at 112.5 μg Co/l and above, which corresponds to 0.212 of the 96-hr LC50. Fish exposed to 48.7 μg Co/l accumulated significantly more cobalt than those in the lowest treatment level.

Mount and Stephan (1969) exposed fathead minnows to copper for 60 days beginning at hatch, in well water diluted with deionized water to a hardness of 31 mg/l and an alkalinity of 30 mg/l, both as $CaCO_3$. They found that 18.4 μg Co/l caused 100% mortality over the test period. No effects on survival or growth rates were observed at a concentration of 10.6 μg Co/l. The present tests had similar results in that survival rate was reduced at 26.2 μg Co/l, but not affected at 13.1 μg Co/l. However, an effect on growth was detected in the present study at 13.1 μg Co/l. In the study by Mount and Stephan, 6-week-old fathead minnows were exposed to copper in an experiment that continued through the reproductive phase. Half of the fish exposed to 18.4 μg Co/l survived the test, but grew relatively poorly and failed to spawn. No detrimental effects were observed at 10 μg Co/l.

Pickering (1974) using fathead minnow embryos which had been spawned and incubated in nickel solutions, found a significant reduction in survival of the embryos at 730 μg Ni/l, at which concentration the production of embryos had also been reduced. The mean length of surviving young fish after 30 days also appeared to be reduced at this concentration, although length differences were not tested statistically. No significant reduction in fecundity, embryo survival or fish length was found at 380 μg Ni/l. The survival rate of the young fish was not affected by the highest concentration of 730 μg Ni/l.

The hardness of the test water was 210 mg/l, and alkalinity was 161 mg/l, both as $CaCO_3$. In the present study, which was conducted in a softer water, embryo and young fish survival were affected by 433.5 μg Ni/l, but not by 108.9 μg Ni/l.

Shabalina (1964) exposed carp fry to levels of 50, 500 and 5000 μg Co/l in an experiment lasting about 70 days. The rates of growth of the fish in all three treatments were depressed relative to controls during the first 20 days. The fish exposed to cobalt grew more rapidly than control fish during the next 10 days, however, and weighed about as much as the controls at the end of the test. Characteristics of the test water were not described, and survival rates were not discussed in the English abstract and summary of Shabalina's paper. In the present experiment, both growth and survival rates were decreased significantly by concentrations of 112.5 μg Co/l and above.

In the copper experiment, which was run in Embarrass River water (Table 19), there were no significant differences in embryo survival rate between treatments and controls. Although Table 19 shows large differences in mean percentage survival of fish after hatch between treatments and control, no statistically significant differences were found because of high variability between duplicates of treatments and control. (Survival rates in control duplicates were 20% and 64%.)

The mean weights of surviving fish were lowest in the control, possibly because of the high survival rate in the control, combined with the proliferation of algae in the control chambers and lower treatment levels, which could have made it more difficult for the fish to recognize and capture their food (brine shrimp nauplii). In the test chambers containing higher levels of copper, algae were not as abundant. Because of these factors, the direct detrimental effects of copper on growth which had been seen in the previous copper experiment were not recognizable in this test, if indeed they were present at all.

The weighted mean 96-hr LC50 of copper for the fathead minnow in Embarrass River water, at 2202 μg/l, is about 20 times higher than the weighted mean of 108 μg/l which was determined for the same species in Lake Superior water. Comparison of chronic effects in the two waters is more difficult, but it can be seen despite the complications discussed above, that the growth rate at 221.8 μg Cu/l in Embarrass River water was better than the growth rate at 13.1 μg Cu/l in Lake Superior water, and that the survival rate at 221.8 μg/l in Embarrass River water was similar to the survival rate at 52.1 μg Cu/l in Lake Superior water. These results suggest that the acute and chronic toxicities of copper are affected similarly by variations in water chemistry, and that acute copper toxicity tests can be used to predict the chronic toxicity of copper in different waters.

Comparison of the findings of Mount (1968) with those of Mount and Stephan (1969) seems to show that the chronic toxicity of copper is less dependent than acute toxicity on water chemistry. These authors found that the copper concentration which decreased egg production in the fathead minnow was between 1.3 and 3.1 times as high in a water with a hardness of 198 mg/l as $CaCO_3$ and an alkalinity of 161 mg/l as $CaCO_3$, as in a water with a hardness and alkalinity of 31 mg/l and 20 mg/l as $CaCO_3$, respectively. The 96-hr LC50 of copper, however, was more than 6 times as high in the hard water as in the soft water. This discrepancy may have been caused by precipitation of copper in the hard-water acute toxicity test, but no measurements of dissolved copper were reported. In the present study, dissolved copper levels were between 83% and 97% of total copper levels in both waters.

The observed effects of copper, nickel and cobalt on early survival and growth rates can be important in natural fish populations. The degree of importance depends not only on the degree to which the individuals are affected, but on certain characteristics of the aquatic ecosystem. For example, if the number of young fish in a population consistently exceeds the number of adults which can be supported by the ecosystem, partial mortality of embryos, larvae or juveniles from pollution may not be harmful to the population. Even if survivors are fewer than what the ecosystem can support, the available habitat may be occupied by immigrants from other populations.

Reduced growth rates can affect the success or usefulness of natural populations by diminishing the biomass available to predators, including man, or by postponing the age at which reproduction begins. Deleterious effects of metals on the growth of young fish may not persist into adulthood, as observed in copper and cobalt toxicity tests by Mount (1968) and Shabalina (1964), respectively. Slower-growing young fish, however, remain vulnerable to predation or competition for a longer time, and may not be able to take advantage of seasonal change in sizes and types of food.

ACUTE TOXICITY OF COPPER AND NICKEL TO THE ROCKBASS

METHODS

Tests of the acute toxicity of copper and nickel to young-of-the-year rockbass (Ambloplites rupestris) were undertaken in August, 1977. The fish were seined from the South Kawishiwi River and kept in holding tanks for 1 to 4 days before the toxicity tests began. South Kawishiwi River water was used in both the copper experiment and the nickel experiment.

Ninety-six-hour LC50's of copper and nickel sulfates were determined using continuous-flow dilution apparatus at a temperature of 25°C. Total metal concentrations and pH in all treatment levels, and hardness, alkalinity, and total organic carbon were measured at least once in each toxicity test. Experimental and analytical methods are described in greater detail in the Aquatic Toxicology Operations Manual.

Results and Discussion

Results of the copper and nickel experiments are summarized in Table 18. The 96-hr LC50 of copper for rockbass was about 3 times as high as the weighted mean 96-hr LC50 of copper for the fathead minnow in the same water, but the LC50 of nickel for rockbass was only 85% of the nickel LC50 for the fathead minnow in the same water (Table 1).

Relatively high resistance to copper in short experiments seems to be common among members of the centrarchid family. Benoit (1975) found that the mean 96-hr LC50 of copper for juvenile bluegill (*Lepomis macrochirus*) was 1100 μg Cu/l in Lake Superior water. However, in the same study, a copper concentration of just 12 μg/l was shown to significantly decrease the survival rate of bluegill larvae. This concentration is nearly the same as the lowest copper concentration which affected the growth rate of fathead minnows, as shown in Table 14.

Previous investigators have also found that the acute toxicity of nickel to bluegill is similar to that for fathead minnows. According to Pickering and Henderson (1966) the 96-hr LC50 of nickel for the bluegill was 5270 μg/l, while for the fathead minnow it was 4880 μg/l. Water with hardness of 20 mg/l as $CaCO_3$ and alkalinity of 18 mg/l as $CaCO_3$ was used in both tests.

SUMMARY

The fathead minnow (*Pimephales promelas*) and the cladoceran *Daphnia pulicaria* were exposed to copper-nickel, copper-cobalt, and nickel cobalt mixtures in Lake Superior water. In addition, both species were exposed to copper-nickel mixtures in water from the South Kawishiwi River. In both test waters, the acutely toxic copper concentration for the fathead minnow was about half of what it would have been if the combined effect of copper and nickel were additive. The joint effect of copper and nickel on *D. pulicaria* was slightly more than additive in Lake Superior water, and additive or slightly less than additive in South Kawishiwi River water. Variations in the ratio of copper concentration to nickel concentration in experiments with *D. pulicaria* in South Kawishiwi River water did not appear to be related to variations in the combined effect of the two metals.

The joint effects of copper and cobalt on the fathead minnow were slightly less than additive, while for *D. pulicaria* they were approximately additive. The toxic interaction of nickel and cobalt on the fathead minnow was slightly less than additive, but for *D. pulicaria* it was additive or slightly more than additive.

The results of acute copper and nickel toxicity tests with fathead minnows and *D. pulicaria* in different surface waters were used to develop regression 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 the LC50. For both species, TOC was the best single predictor of copper toxicity. For the fathead

minnow, TOC together with alkalinity had significantly better relationship to LC50 than did TOC alone. Hardness alone was the best predictor of nickel toxicity to both species. Dummy x-variables were used to show that variations in copper and nickel toxicity among test waters were explained as well by these predictors as were variations among experiments in a single test water.

The acute toxicities of five copper-nickel leachates to D. pulicaria were assessed, and the results of these tests were compared with toxicity predictions derived from the regression models, and from the experiments with copper-nickel mixtures. In most experiments, which produced conclusive results, leachates were less toxic than predicted. Some of the discrepancies could be traced to the presence of suspended (rather than dissolved) copper and nickel in some of the leachate samples. High concentrations of zinc in two leachates may have contributed to their toxicities.

In four separate 30-day experiments, fathead minnows were exposed to nickel and cobalt in Lake Superior water, and to copper in water from Lake Superior and the Embarrass River. Each experiment was started with embryos 1 day after fertilization. A nickel concentration of 433.5 μg/l reduced the survival rate of embryos and young fish. The survival and growth rates of young fish were reduced in a cobalt concentration of 112.5 μg/l. In Lake Superior water, copper reduced the growth rate of young fish at a concentration of 13.1 μg/l. In Embarrass River water, the effects of copper were not statistically significant.

The acute toxicities of copper and nickel to the rockbass (Ambloplites rupestris) in South Kawishiwi River water were compared to those for the fathead minnow in the same water. The rockbass was less sensitive to copper than the fathead minnow, and slightly more sensitive than the fathead minnow to nickel.

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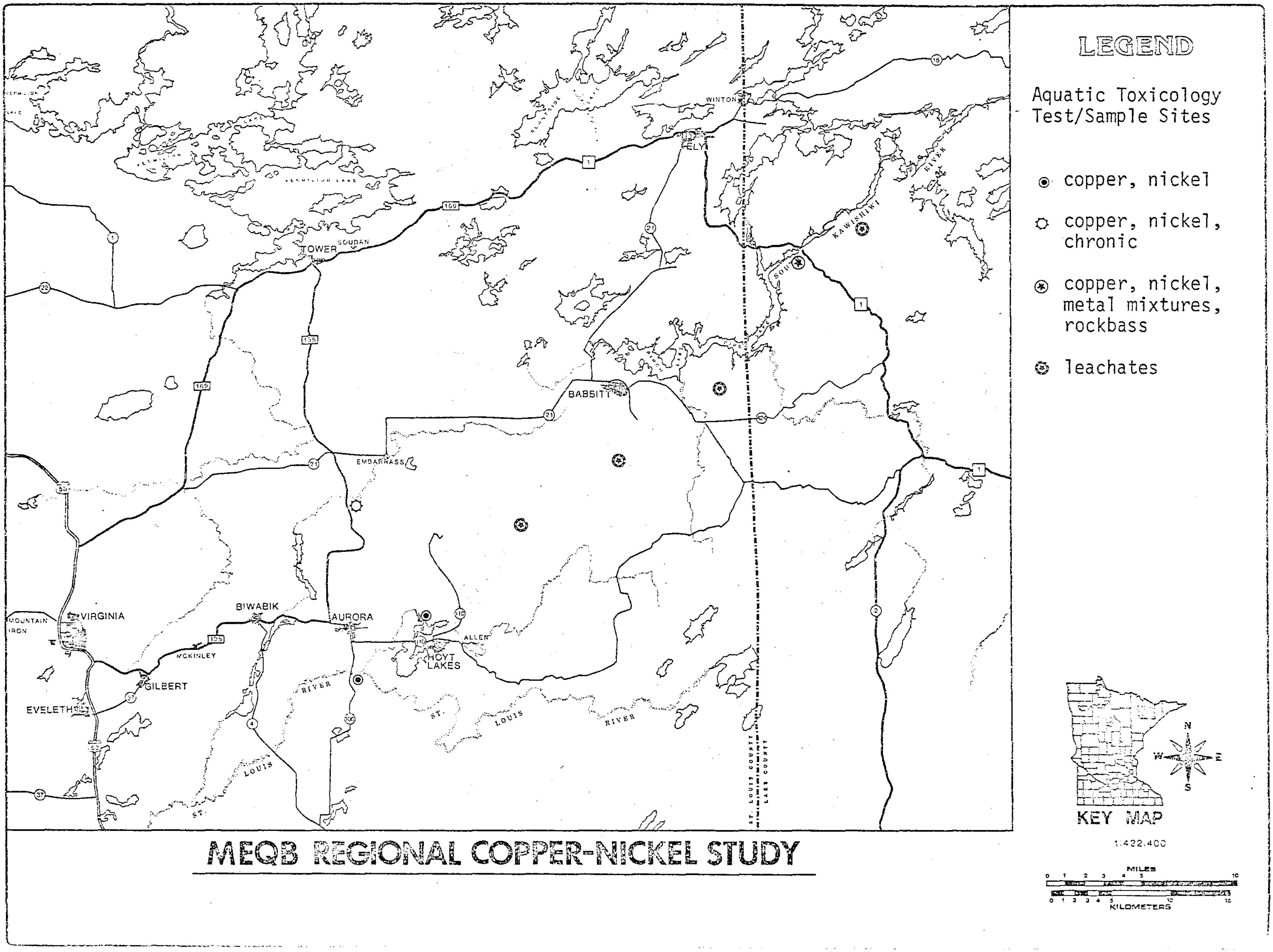
LEGEND
Aquatic Toxicology Test/Sample Sites
copper, nickel
copper, nickel, chronic
copper, nickel, metal mixtures, rockbass
leachates
WINTON
ELY
TOWER
SOUDAN
BABBITT
EMBARRASS
VIRGINIA
MOUNTAIN IRON
BIWABIK
AURORA
HOYT LAKES
ALLEN
MCKINLEY
GILBERT
EVELETH
VERMILION LAKE
KAWISHIWI RIVER
SOUTH
ST. LOUIS RIVER
ST. LOUIS COUNTY
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KEY MAP
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MILES
KILOMETERS
MEQB REGIONAL COPPER-NICKEL STUDY

Table 1. Toxicity of Copper, Nickel and Cobalt to the Fathead Minnow in Water from Lake Superior and the South Kawishiwi River.

Test Water	Toxicant	Test Date	96-hr LC50 (μg/1)	Weighted mean LC50 (μg/1)
Lake Superior	copper	2/14/77	114(98,132)*	108
	copper	3/7/77	121(105,139)	
	copper	3/21/77	88.5(76,103)	
	nickel	2/28/77	5209(4504,6025)	5186
	nickel	3/14/77	5163(4471,5962)	
	cobalt	1/15/78	689(618,769)	531
	cobalt	1/15/78	397(360,437)	
	cobalt	1/23/78	524(470,584)	
South Kawishiwi River	copper	7/12/77	436(387,490)	478
	copper	8/1/77	516(463,575)	
	nickel	7/18/77	2916(2594) (3279)	2920
	nickel	7/25/77	2923(2625) (3256)	

*95% confidence limits on LC50.

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	Cu conc. / Ni conc.[a]	96-hr LC50 as Cu(μg/1)	Mixture LC50 as Cu / weighted mean Cu LC50
Lake Superior	4/4/77	.0255	66.0 (59.4,73.4)[b]	.610
Lake Superior	4/21/77	.0313	46.3 (41.1,52.2)	.428
Lake Superior	5/9/77	.0280	45.5 (38.5,53.9)	.421
So. Kawishiwi R.	8/29.77	.156	229 (165,316)	.478
So. Kawishiwi R.	9/5/77	.124	241 (223,260)	.503

[a]potency ratio = .0209 (Lake Superior)

.164 (So. Kawishiwi R.)

[b]95% confidence limits on LC50.

Table 3. Toxicity of Copper, Nickel and Cobalt to Daphnia pulicaria in Water from Lake Superior and the South Kawishiwi River.

Test Water	Toxicant	Test Date	48-hr LC50 (μg/l)	Weighted Mean LCSG (μg/l)
Lake Superior	copper	1/26/77	11.4(9.7,13.3)*	9.29
	copper	1/26/77	9.06(8.31,9.87)	
	copper	2/16.77	7.24(6.64,7.89)	
	copper	3/16/77	10.8(9.6,12.0)	
	nickel	1/26/77	2182(1421,3353)	1901
	nickel	2/16/77	1813(1462,2247)	
	nickel	3/16/77	1836(1382,2437)	
	cobalt	12/21/77	2380(1962,2887)	2025
	cobalt	1/4/78	3037(2604,3542)	
	cobalt	1/11/78	1619(1449,1808)	
	cobalt	2/7/78	1765(1382,2255)	
	cobalt	4/13/78	1498(1299,1728)	
South Kawishiwi River	copper	7/6/77	55.5(41.4,74.2)	54.5
	copper	7/13/77	55.3(48.1,63.4)	
	copper	7/26/77	53.3(46.8,60.7)	
	nickel	7/13/77	697(562,863)	987
	nickel	7/26/77	1140(985,1321)	
	nickel	8/2/77	1034(882,1212)	

*95% confidence limits on LC50.

Table 4. Toxicity of Copper-Nickel Mixtures to *Daphnia pulicaria* in Water from Lake Superior and the South Kawishiwi River.

Test Water	Test Date	Cu conc.[a] / Ni conc.	48-hr LC50 as Cu (μg/l)	Mixture LC50 as Cu / weighted mean Cu LC50
Lake Superior	3/30/77	.00697	7.77 (6.96,8.67)[b]	.836
Lake Superior	4/5/77	.00634	8.12 (7.39,8.93)	.874
Lake Superior	4/13/77	.00652	7.18 (6.70,7.69)	.773
S. Kawishiwi R.	8/2/77	.0883	65.0 (61.6,68.5)	1.193
S. Kawishiwi R.	8/9/77	.0628	58.6 (c)	1.077
S. Kawishiwi R.	8/9/77	.0617	60.0 (55.9,64.4)	1.101
S. Kawishiwi R.	8/16/77	.0245	67.0 (c)	1.230
S. Kawishiwi R.	8/23/77	.0308	57.1 (50.5,64.7)	1.049
S. Kawishiwi R.	8/23/77	.0256	68.2 (62.1,75.0)	1.252
S. Kawishiwi R.	8/30/77	.0284	74.7 (66.8,83.6)	1.372

[a] potency ration = .00489 (Lake Superior)
.05521 (S. Kawishiwi R.)

[b] 95% confidence limits on LC50.

[c] confidence limits could not be calculated.

Table 5. Toxicity of Copper-Cobalt Mixtures to the Fathead Minnow in Lake Superior Water

Test Date	Cu conc.[a] / Co conc.	96-hr LC50 as Cu(μg/l)	Mixture LC50 as Cu / Weighted Mean Cu LC50
1/30/78	.218	128 (116,141)[b]	1.18
2/6/78	.219	163 (144,186)	1.51
2/27/78	.152	149 (135,165)	1.38

[a] potency ratio = .204

[b] 95% confidence limits on LC50.

Table 6. Toxicity of Copper-Cobalt Mixtures to *Daphnia pulicaria* in Lake Superior Water.

Test Date	Cu conc.[a] / Co conc.	48-hr LC50 as Cu (μg/l)	Mixture LC50 as Cu / Weighted Mean Cu LC50
1/31/78	.00615	8.74 (7.88,9.68)[b]	.940 (.848,1.041)
2/7/78	.00642	13.0 (11.51.4,14.717)	1.40 (1.24,1.58)

[a] potency ratio = .00459

[b] 95% confidence limits on LC50

Table 7. Toxicity of Nickel-Cobalt Mixtures to the Fathead Minnow in Lake Superior Water

Test Date	Co conc.[a] / Ni conc.	96-hr LC50 as Co (μg/1)	Mixture LC50 as Co / Weighted Mean Co LC50
2/13/78	.125	619(563,680)[b]	1.17
2/20/78	.120	797(696,912)	1.50
3/6/78	.110	602(539,671)	1.13

[a] potency ratio = .102

[b] 95% confidence limits on LC50

Table 8. Toxicity of Nickel-Cobalt Mixtures to *Daphnia pulicaria* in Lake Superior Water

Test Date	Ni conc. / Co conc.[a]	48-hr LC50 as Ni(μg/l)	Mixture LC50 as Ni / Weighted Mean Ni LC50
4/12/78	.477	1962 (1760,2187)[b]	1.03
4/17/78	.954	1096 (858,1399)	.577
4/25/78	.949	1648 (961,2828)	.867

[a] potency ratio = .939

[b] 95% confidence limits on LC50

Page 9. Acute toxicity of copper to the fathead minnow in different surface waters.

Test water	Test date	96-hr LC50 (μg/l)	95% confidence interval on LC 50 (μg/l)	Hardness (mg/l as $CaCO_3$)	Alkalinit (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
S. Kawishiwi R.	8/1/77	516	111	37	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
Colby Lake	5/15/78	550	142	84	12	7.07	15
Colby Lake	6/5/78	1001	234	66	12	6.97	34
Embarrass R.	7/3/78	2050	535	117	41	7.29	30
Embarrass R.	7/10/78	2336	548	121	36	7.28	36

Table 10. Acute toxicity of copper 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	11.4	3.59	48	42	8.03*	2.6
Lake Superior	1/26/77	9.06	1.56	48	42	8.03*	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
S. Kawishiwi R.	7/6/66	55.4	32.8	31	27	6.66	14
S. Kawishiwi R.	7/13/77	55.3	15.3	29	27	6.97	13
S. Kawishiwi R.	7/26/77	53.3	13.9	28	22	7.20	13
St. Louis R.	9/6/77	97.2	30.7	88*	20*	7.01	28
St. Louis R.	9/22/77	199	46.3	100	20	7.55	34
St. Louis R.	9/27/77	627	169	86	22	7.25	34
St. Louis R.	10/5/77	213	114	82	18	6.99	32*
St. Louis R.	10/12/77	165	12.9	84	17	7.01	32
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
Cloquet 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
Colby Lake	5/16/77	84.7	47.9	84	13	7.08	13

Table 10. Acute toxicity of copper to *Daphnia pulicaria* in different surface waters (continued)

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)
Colby Lake	5/31/78	184	39.3	92	19	7.22	21
Embarrass River	7/15/78	240	60.0	106	36	7.44	34

*mean of known values substituted for missing datum.

Table 11. Acute toxicity of nickel to the fathead minnow in different surface water

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
S. Kawishiwi R.	7/18/77	2916	685	29	20	6.50	12
S. 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
Colby Lake	5/22/78	5383	1186	86	18	7.16	15

*mean of known values substituted for missing datum.

Tabel 12. Acute toxicity of nickel to <u>Daphnia pulicaria</u> 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
S. Kawishiwi R.	7/13/77	697	301	29	26	6.77	13
S. Kawishiwi R.	7/26/77	1140	336	28	22	7.23	15
S. 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.	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
Colby Lake	5/23/78	2042	431	89	14	7.41	18
Colby Lake	6/7/78	2717	503	89	14	7.09	34
Embarrass R.	6/27/78	3156	564	114	43	7.43	27
Embarrass R.	7/11/78	3607	666	120	37	7.47	33

*mean of known values substituted for missing datum.

Table 13. Effect of added calcium and magnesium sulfates on the toxicity of copper to *Daphnia pulicaria* in Lake Superior Water

Treatment	Test Date (weighted mean)	Added Hardness (mg/1 as $CaCO_3$)	48-hr. LC50 (µg/1)
none		0	9.3
Ca	3/8/78	50	17.8
Ca	3/8/78	100	23.7
Ca	3/8/78	200	27.3
Mg	3/21/78	50	25.2
Mg	3/21/78	100	25.1
Mg	3/8/78	200	25.1

Table 14. Effect of added calcium and magnesium sulfates on the toxicity of nickel to *Daphnia pulicaria* in Lake Superior water.

Treatment	Test Date (weighted mean)	Added Hardness (mg/l as $CaCO_3$)	48-hr LC50 (µg/l)
none		0	1901
Ca	6/8/78	50	3162
Ca	6/8/78	100	3826
Ca	6/20/78	200	3304
Mg	4/4/78	50	2470
Mg	4/4/78	100	2470
Mg	3/15/78	200	2409

Table 15. Acute toxicity of copper-nickel leachates to *Daphnia pulicaria*

Leachate	Dilution Water	Test Date	Effect	% Leachate Having Effect	Chemical Conditions in Listed Percentage of Leachate: 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 Ni LC50	Cu conc./pred. Cu LC50 + Ni conc/Pred Ni LC5
Seep 3	——	4/6/77	no mortality	100%	T270[a] F52[b]	T3200 F930	T73	T440	T2740 F840	446	T20 F18	8.30	98	8866	3.1 (total metal) 0.64 (filtered metal)
Seep 3	Oil Discharge	9/14/77	48-hr LC50	2.4% (1.5,3.7)[c]	T46	T720	T17	T40	T238	267	F7.3	8.09	27	6159	1.8
Seep 3	Oil Dishcarge	6/24/78	48-hr LC50	8.0% (5.9,10.9)	T68	T1438	T45	T34	T511	309	T8.1	8.23	31	6833	2.4
U.S. Steel	EM-6	4/6/77	complete mortality	1.7%	T210	T294	T5.3	T13	T171	164	T7.9	8.32	30	4360	7.1
U.S. Steel	S. Kawishiwi R.	4/6/77	complete mortality	1.7%	T210	T294	T3.8	T12	T109	31	T11	7.45	46	1320	4.8
U.S. Steel	Lake Superior	4/26/77	complete mortality	0.1%	T11	T22	T1.3	T1.4	T6.0	45	T3.0	7.99	8.9	1751	1.2
U.S. Steel	EM-6	10/26/77	48-hr LC50	1.0%	T150	T130	T6.7	T11	T67	118	F9.4	8.20	38	3439	4.0
U.S. Steel	S. Kawishiwi R.	10/27/77	48-hr LC50	0.7% (0.6,0.8)	T114	T101	T4.1	T7.7	T77	23	T13	7.04	57	1064	2.1
U.S. Steel	Partridge R	7/25/78	48-hr LC50	19.9% (7.2,13.7)	T408	T643	T12	T33	T244	86	T32	7.80	178	2756	2.5
INCO	——	7/28/77	no mortality	100%	T96	T65	T2.7	T17	—	765	F19	8.31	92	13,002	1.0
INCO	——	11/29/77	no mortality	100%	T23 F9.9	T4500 F3500	T13	T250 F201	—	490	F12	8.35	51	9478	0.93 (total metal) 0.56 (filtered metal)
EM-8	Oil Discharge	6/25/78	48-hr LC50	84% (71,100	T54	T7408	T135	T152	T1439	831	T9.0	8.04	36	13,791	2.0
Minnamax	——	7/26/78	20% mortality	100%	T21	T310	T100	T22	T220	699	T8.3	7.89	32	12,197	0.68

[a]Total concentration [b]Filtered concentration [c]95% confidence limits on LC50

Table 16. Chronic toxicity of copper to fathead minnow embryos and larvae in Lake Superior water[a]

Mean Cu^{++} concentration (µg/l)	Fraction of 96-hr LC50[b]	Mean Cu Body Accumulation (µg/g dry wt)	Mean Percentage embryo survival to hatch[c]	Mean percentage fish survival after hatch[c]	Mean fish weight after 30 days (mg)[d]
<5	--	5.14	93	99	107
5.0	.0462	11.6	89	99	112
9.0	.0832	19.8[e]	97	97	96
13.1	.121	25.2[e]	91	88	14[e]
26.2	.242		92	62[e]	3[e]
52.1	.482		90	15[e]	2[e]

[a] experiment began on 4/4/77

[b] Acute tests run in Lake Superior water using 8-week-old fathead minnows. Weighted mean 96-hr LC50 = 108 µgCu^{++}/l.

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

[d] log x transformation used on weights

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

Table 17. Chronic toxicity of nickel to fathead minnow embryos and larvae in Lake Superior water[a]

Mean Ni^{++} Concentration (μg/l)	Fraction of 96-hr LC50[b]	Mean Ni Body Accumulation (μg/g dry wt)	Mean Percentage embryo survival to hatch	Mean Percentage fish survival after hatch[c]	Mean Fish weight after 30 days (mg)[d]
<6	--	2.72	98	97	105
21.0	.00405	11.13	93	95	97
44.4	.00857	17.60[e]	92	100	97
108.9	.0210	25.48[e]	92	99	98
433.5	.0836	--	71[e]	9[e]	123
1532.1	.295	--	0[e]	--	--

[a]Experiment began on 4/12/77

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

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

[d]log x transformation used on weights

[e]significantly different from control at = 0.05 (Dunnett)

Table 18. Chronic toxicity of cobalt to fathead minnow embryos and larvae in Lake Superior water[a]

Mean Co^{++} concentration (μg/l)	Fraction of 96-hr LC50[b]	Mean Co Body Accumulation (μg/g dry wt)	Mean Percentage embryo survival to hatch[c]	Mean Percentage fish survival after hatch[c]	Mean Fish weight after 30 days (mg)[d]
<1[d]	--	--	78[e]	21[e]	71[e]
14.7	.0277	9.3	94	96	82
29.5	.0556	14.3	88	85	78
48.7	.0918	21.3[f]	91	86	77
112.5	.212	37.9[f]	91	78[f]	66[f]
223.2	.421	76.1[f]	81	61[f]	65[f]

[a] Experiment began on 1/11/78

[b] Acute tests run in Lake Superior water using 8-week-old fathead minnows. Weighted mean 96-hr LC50 = 531 μg Co/l.

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

[d] log x transformation used on weights

[e] control data were not included in statistical analysis

[f] significantly different from lowest teatment at $\alpha = 0.05$.

(Duncans new multiple range test)

Table 19. Chronic toxicity of copper to fathead minnow embryos and larvae in Embarrass River water

Mean Cu Concentration (μg/l)	Fraction of 96-hr LC50[b]	Mean Percentage embryo survival to hatch[c]	Mean Percentage fish survival after hatch[c]	Mean Fish weight after 30 days (mg)[d]
6.5	.00295	70[e]	41	31
49.9	.0227	93	28	55
70.4	.0320	83	20	77
97.6	.0443	81	11	71
140.0	.0636	93[e]	10	61
221.8	.101	83	16	63

[a] Experiment began on 6/21/78.

[b] Acute tests run in Embarrass River water using 8-week-old fathead minnows. Weighted mean 96-hr LC50 = 220 μg Cu/l.

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

[d] log x transformation used on weights

[e] one duplicate was lost from this treatment

Table 20. Acute toxicity of copper and nickel to the rockbass in South Kawishiwi River water.

Toxicant	Test Date	96-hr LC50 (μg/l)	Hardness (mg/l as $CaCO_3$)	Alkalinity (mg/l as $CaCO_3$)	pH	TOC (mg/l)
nickel	8/22/77	2480(2059,2986)*	26	19	7.17	15
copper	8/30/77	1432(1290,1589)	24	14	7.55	15

*95% confidence limits on LC50

Table 15. Acute toxicity of copper-nickel leachates to Daphnia pulicaria

Leachate	Dilution Water	Test Date	Effect	% Leachate Having Effect	Chemical Conditions in Listed Percentage of Leachate: 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 Ni LC50	Cu conc./pred. Cu LC50 + Ni conc/Pred Ni LC50
Seep 3	----	4/6/77	no mortality	100%	T270[a] F52[b]	T3200 F930	T73	T440	T2740 F840	446	T20 F18	8.30	98	8866	3.1 (total metal) 0.64 (filtered metal)
Seep 3	011 Discharge	9/14/77	48-hr LC50	2.4% (1.5,3.7)[c]	T46	T720	T17	T40	T238	267	F7.3	8.09	27	6159	1.8
Seep 3	011 Dishcharge	6/24/78	48-hr LC50	8.0% (5.9,10.9)	T68	T1438	T45	T34	T511	309	T8.1	8.23	31	6833	2.4
U.S. Steel	EM-6	4/6/77	complete mortality	1.7%	T210	T294	T5.3	T13	T171	164	T7.9	8.32	30	4360	7.1
U.S. Steel	S. Kawishiwi R.	4/6/77	complete mortality	1.7%	T210	T294	T3.8	T12	T109	31	T11	7.45	46	1320	4.8
U.S. Steel	Lake Superior	4/26/77	complete mortality	0.1%	T11	T22	T1.3	T1.4	T6.0	45	T3.0	7.99	8.9	1751	1.2
U.S. Steel	EM-6	10/26/77	48-hr LC50	1.0%	T150	T130	T6.7	T11	T67	118	F9.4	8.20	38	3439	4.0
U.S. Steel	S. Kawishiwi R.	10/27/77	48-hr LC50	0.7% (0.6,0.8)	T114	T101	T4.1	T7.7	T77	23	T13	7.04	57	1064	2.1
U.S. Steel	Partridge R	7/25/78	48-hr LC50	19.9% (7.2,13.7)	T408	T643	T12	T33	T244	86	T32	7.80	178	2756	2.5
INCO	----	7/28/77	no mortality	100%	T96	T65	T2.7	T17	--	765	F19	8.31	92	13,002	1.0
INCO	----	11/29/77	no mortality	100%	T23 F9.9	T4500 F3500	T13	T250 F201	--	490	F12	8.35	51	9478	0.93 (total metal) 0.56 (filtered metal)
EM-8	011 Discharge	6/25/78	48-hr LC50	84% (71,100	T54	T7408	T135	T152	T1439	831	T9.0	8.04	36	13,791	2.0
Minnamax	----	7/26/78	20% mortality	100%	T21	T310	T100	T22	T220	699	T8.3	7.89	32	12,197	0.68

[a]Total concentration [b]Filtered concentration [c]95% confidence limits on LC50