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Modifications in Lethal Tolerance of Zebrafish, *Brachydanio rerio*, to Certain Heavy Metals Through Sublethal Metal Pre-exposure

Gonum Reddy

A Thesis

in

The Department

of

Biology

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ABSTRACT

Modifications in Lethal Tolerance of Zebrafish, Brachydanio rerio, to Certain Heavy Metals through Sublethal Metal Pre-exposure

Gonum Reddy

Experiments were conducted to examine the modifying effects of sublethal pre-exposure to either cadmium, copper, silver or nickel for 7 d, on subsequent lethal tolerance of zebrafish (Brachydanio rerio) to these metals. The results demonstrate that pre-exposure to 2.0 mg Cd/L resulted in enhanced tolerance to lethal levels of cadmium. Similarly, pre-exposure to 76 ug Cu/L resulted in enhanced tolerance to lethal levels of copper. In contrast, pre-exposure to either 0.5, 1.0 or 5.0 mg Ni/L rendered fish less tolerant to lethal levels of that metal. For silver, sublethal pre-exposure resulted in either increased or decreased tolerance, depending on the pre-exposure concentration. Pre-exposure to 1.3 ug Ag/L increased subsequent lethal tolerance, whereas pre-exposure to 2.2 ug Ag/L resulted in subsequent decreased tolerance.

Furthermore, tolerance modifications were not apparently metal-specific. Pre-exposure of zebrafish to 52 ug Cu/L resulted in enhanced tolerance to lethal levels of cadmium and pre-exposure to 40 and 70 ug Cu/L resulted in enhanced tolerance to lethal

levels of nickel. Finally, pre-exposure to 1.4 and 6.0 mg Ni/L resulted in decreased tolerance to lethal levels of copper. The results may indicate the existence of upper and lower pre-exposure concentration limits to tolerance modifications.

Some of the factors which may be responsible for these shifts in tolerance, as well as their possible impact on the environment are considered.

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INTRODUCTION

Natural sources of heavy metals in the environment have probably exerted a significant influence on the ecology and evolution of life. Certain heavy metals are essential for normal biological function. Most of these, however, have the potential to cause toxic effects if present at higher than physiological levels. Others are not identifiable as serving any beneficial function, and are therefore potentially toxic if they contact biological systems (Schroeder, 1965; Brown, 1976). For this reason, there is great concern over the increasing release of heavy metals into the environment as a result of anthropogenic activities. Ultimately, these contaminants may enter natural waters, where they can pose a threat to the aquatic biota. Hence, the discharge of heavy metals into waterways presents a serious pollution problem.

Nevertheless, it is interesting to note that some aquatic organisms are able to acclimate to gradual increases of heavy metals in their environment. Since 1937, when this phenomenon was first documented (King, 1937), there have been numerous reports of species from metal-contaminated sites which are more tolerant to the associated metals than conspecifics from uncontaminated sites (Paul, 1952, Stokes et al., 1973; Bryan, 1974; Antonovics, 1975; Ernst, 1975).

The term acclimation refers to any compensatory physiological adjustments made in an organism in response to a change in the level of an environmental stressor which consequently result in the

return of the organism to a steady-state or homeostasis (Fry, 1971). Hence, acclimation results in increased tolerance of individuals, and allows for their survival under conditions where this would otherwise be impossible. It is well known that aquatic organisms can acclimate to different levels of natural variables in their environment, including temperature, salinity, oxygen and carbon dioxide (Shepard, 1955; Mcleese, 1956; Saunders, 1962; Fry, 1971). However, detailed quantified information on the environmental tolerance of organisms to pollutants is also necessary for the conservation of aquatic life. Furthermore, in the laboratory most studies on the toxicity of pollutants are conducted on organisms maintained in "clean" water. Results from these experiments could be misleading when applied to the field situation where there is the potential for acclimation. Thus, the study of physiological acclimation to toxicants is also of primary importance in research designed expressly for the purpose of setting water quality criteria.

Environmental acclimation of fish to heavy metals was first noticed in hatchery fish (Paul, 1952; Schofield, 1965). Paul (1952) observed that the Sacramento River was polluted with high levels of copper and zinc, to which the resident population was apparently adapted, but which prevented the introduction of fish from a local hatchery. Schofield (1965) clearly demonstrated that this enhanced tolerance of indigenous fish was a result of prior exposure to the heavy metals present in the water. He found that brook trout (Salvelinus fontinalis) stocked into zinc polluted Honnedaga Lake from a Cornell hatchery also contaminated with zinc,

had greater survival ratios than trout introduced from a second uncontaminated hatchery. He concluded that the previous exposure of the trout to zinc from the contaminated hatchery resulted in their subsequent enhanced tolerance to the higher zinc concentration of the lake. This hypothesis was further reinforced when he exposed the fish from the second hatchery to the Cornell water and found that their survival ratios in the lake also increased. Subsequent studies also indicated that fish survive in waters polluted with heavy metals at levels which are considered lethal on the basis of laboratory toxicity tests (EIFAC, 1977; Van Loon & Beamish, 1977; Roch et al., 1982).

More comprehensive laboratory studies on the acclimation of fish to heavy metals have also been carried out. Recently, Dixon & Sprague (1981a) explored the possible modifications in lethal tolerance of rainbow trout (Salmo gairdneri) to copper following previous sublethal exposure for either one, two or three weeks. They found that the lethal tolerance of pre-exposed trout was significantly increased relative to trout without prior exposure. These authors measured lethal tolerance by calculating LC50s, the concentration lethal to 50% of the test population, after 144 h of lethal exposure. Subsequent investigators observed that the lethal tolerance of rainbow trout to zinc (Bradley et al., 1985), aluminium (Orr et al., 1986) and arsenic (Dixon & Sprague, 1981b), and of white suckers (Catastomus commersoni) to cadmium (Duncan & Klaverkamp, 1983) were also increased following sublethal pre-exposure to the respective metal.

In natural waters today, the wide variety of chemicals which

are introduced into the water increases the likelihood that organisms will face exposures to pollutant mixtures. The biological repercussions of such events may be difficult to predict. However, there have been few studies to examine the possibility of increased lethal tolerance to one metal as as result of sublethal pre-exposure to a different metal, or cross-tolerance. Kim et al. (1977) and Heisinger et al. (1979) both demonstrated that the toxic effects of mercury were mitigated by sublethal pre-exposure to selenium for both the northern creek chub (Semotilus atromaculatus) and the goldfish (Carassius auratus). Duncan & Klaverkamp (1983) showed that the tolerance of white suckers to lethal levels of cadmium was increased following sublethal pre-exposure to not only cadmium, but also to mercury and zinc. However, pre-exposure to selenium did not protect white suckers against lethal levels of cadmium, suggesting that there may be some metal-specificity in the ability of a pre-exposure metal to protect organisms against subsequent lethal levels of a different metal.

Interestingly, Duncan & Klaverkamp's (1983) study also demonstrated that the duration of lethal exposure may influence the tolerance of pre-exposed fish relative to control fish. They pre-exposed white suckers to sublethal levels of zinc and found that the 12- and 24-h cadmium LC50s of pre-exposed fish were significantly higher than the corresponding cadmium LC50s of the control fish, but that the 48-, 72-, and 96-h LC50s were no different from control fish.

The present study was designed to further examine any possible

modifications in the lethal tolerance of fish to heavy metals, as a result of prior sublethal exposure. There were four main objectives. The first objective was to examine the possible modifications in lethal tolerance to a metal as a result of previous exposure to the same metal. The second objective was to examine any modifications in lethal tolerance to a metal, following sublethal pre-exposure to a different metal. Thirdly, to assess the possible effects of pre-exposure concentration on lethal tolerance modifications. Finally, to assess the relative lethal tolerance of pre-exposed fish at different lethal exposure times.

Four metals were selected for this investigation: cadmium, copper, silver and nickel. Cadmium and copper are both common aquatic pollutants which are known to be highly toxic to aquatic organisms. Levels of copper in natural waters do not exceed 5 ug Cu/L (Nriagu, 1979). However, in polluted waters, levels of copper can reach 300 to 500 ug Cu/L (Van Loon & Beamish, 1977). Effects of pre-exposure to nickel or silver on fish have not previously been examined. In natural waters, levels of nickel are generally very low, ranging from less than 2 to 10 ug Ni/L (Nriagu, 1980). However, nickel is a common pollutant particularly in the vicinity of certain smelting and mining industries where levels can reach up to 3 to 6 mg Ni/L (Stokes et al., 1973; Rehwoldt, 1973). Natural levels of silver in the aquatic environment are usually extremely low level ranging from 2 to 3 ug Ag/L (Stokes et al., 1973), and is not thought to be as common a contaminant as certain other heavy metals. However, the apparent absence of silver in natural waters may be due to the previous detection limitations of the analytical

instrumentation (Davies et al., 1973). Nevertheless, for aquatic organisms, silver is one of the most toxic of heavy metals, ranking second only to mercury (Davies et al., 1973; Lima et al., 1982).

MATERIALS AND METHODS

2.1 Test Organism

Adult zebrafish (Brachydanio rerio) were selected as the test organism. This species has been proposed as a standard test species for toxicity testing by the International Standards Organization (Fogels & Sprague, 1977). These fish were also chosen for this study because they are small, readily available throughout the year, and easy to maintain in the laboratory. Although zebrafish are a tropical species, information from bioassays using this species are still applicable to many of Canada's indigenous and economically important species, such as the salmonids. Their tolerance to toxicants can often be correlated with that of rainbow trout by a factor of 2.6 (Fogels & Sprague, 1977). This difference in tolerance falls within the range of variability reported for rainbow trout in the same or between labs (Fogels & Sprague, 1977; Brown, 1968).

2.2 Holding Conditions

Fish were purchased from Tropicarium, St. Bruno, Quebec, when required. Upon arrival in the laboratory, fish were housed in glass holding tanks, each with a water volume of 36L, at a loading density of approximately 1.4g fish/L. Montreal City water was used as the source water. From the City mains, the water was passed through a charcoal filter, heated to 25°C, and then supplied to a headbox. This headbox was installed to eliminate possible fluctuations in pressure in the source water. Hence, within the

headbox, a constant head was maintained by means of a standpipe. The water in the headbox was also degassed by means of airstones. The flow rate into each of the holding tanks was adjusted to 2L/min which provided a 99% replacement time of less than 2 h. This exchange rate was equivalent to 58L/g fish/d, and is considered to be within acceptable limits for fish of this size according to the guidelines set out by Sprague (1969).

In the experimental tanks used in the pre-exposure phase and the toxicity testing phase (Sections 2.3 & 2.4), water was passed from the headbox to a distribution chamber before entering the tanks. From this chamber, an equal flow of water was supplied to each tank by means of adjustable glass faucets. The glass faucet system of ensuring a constant and highly accurate rate of water flow is described in detail by Hewitt (1979). Using this system, the flow rates in the experimental tanks never varied by more than 0.5ml/min.

Fish were allowed to acclimate to laboratory conditions for a minimum of 28 d prior to use. During acclimation, fish were closely examined, and only those which were visibly in good health were used for experimentation. A diurnal photoperiod of 12 h light (8AM - 8PM) was maintained by overhead fluorescent fixtures which were controlled by a time switch. Fish were fed once daily ad libitum with Tetramin Tropical Fish food. Excess food and fecal wastes were siphoned out of each tank once daily. Certain characteristics of the laboratory water are given in Table 1.

2.3 Pre-exposure Phase

The pre-exposure phase was designed for sublethal exposure of the test fish to one of the heavy metals before subsequent determination of lethal tolerance. Thus, during the pre-exposure phase, zebrafish were exposed to three different sublethal concentrations of the metal under consideration.

The following procedure was carried out. Preliminary static 144-h LC50s were conducted to obtain an estimate of the lethal concentration range for each metal. Based on these data, three nominal concentrations were selected for each experiment. These nominal concentrations ranged from approximately 0.01 to 0.50 times the static 144-h LC50 for each metal, and hopefully would be non-lethal during the pre-exposure phase. In subsequent definitive tests, no mortality was observed during the pre-exposure phase. The measured pre-exposure concentrations for each experiment are given in Table 2.

Prior to each experiment, fish were individually weighed by the water displacement method. This method involves introducing each fish into a beaker of water of known weight and re-weighing after addition of the fish. The difference between the two weights is the weight of the fish. Those within the selected size range of 0.3-0.8g were randomly distributed among 24 specially designed cages. These cages comprised four pre-exposure sets, each set having six cages (Figure 1). All 24 cages had been divided among four glass tanks, prior to weighing the fish, so that there were six cages per tank. Each cage was a rectangular container, with dimensions of 8.5 x 10 x 18cm. Two opposite sides and the bottom

were made of 5mm thick, solid plexiglass. The remaining two sides were covered in plastic mesh screening, with a mesh size of 2.5 x 4.0mm. This mesh size was small enough to prevent fish from escaping through the mesh, yet large enough to permit adequate circulation of water between the outside and inside of the cages. The cages sat on the floor of the pre-exposure tanks, and the sides of the cages projected above the water line of the tank. The top of the cage was open, allowing for introduction and inspection of the test organisms. Approximately 15-18 fish were housed in each cage (see Appendix). The meshed sides of the cages were aligned with the direction of water flow to facilitate circulation of water. The water volume of each pre-exposure tank was 27L. The flow rate of water supplied to each tank was 500ml/min which provided a 99% replacement time of 4.2 h. As the loading density of fish in each tank was approximately 2g fish/L, this flow rate provided approximately 13L/g fish/d. Circulation and aeration was promoted by air stones suspended within each tank, immediately outside each cage. This was to ensure that oxygen and toxicant would not become depleted within the cages. Water quality tests performed throughout experimentation showed no differences in the oxygen or toxicant concentration between the inside and outside of the cages. Fish were permitted to acclimate to the cage environments for 7 d prior to toxicant exposure.

After acclimation to the cages, test lots in three of the four pre-exposure tanks were exposed to one of the metals under study. Three different nominal sublethal concentrations of the same metal were selected, one for each of three of the pre-exposure tanks.

Test lots in the fourth tank were not exposed to toxicant. This latter tank served as a control in the subsequent lethal exposure phase to which the lethal tolerance of pre-exposed fish could be compared. Seven experiments were conducted in this study in total. The first four experiments were conducted to examine the effects of pre-exposure to a metal on relative tolerance to lethal levels of the same metal. These four experiments were carried out using cadmium, copper, silver and nickel, respectively. The next three experiments were conducted to examine the effects of pre-exposure to one metal on relative tolerance to lethal levels of a different metal. The metal combinations used for these latter three experiments were based on the findings of the first four experiments, and these selections are discussed in the Results (pg 58). The combinations, as well as their measured pre-exposure and lethal exposure concentrations are given in Table 2.

Pre-exposure to toxicant was continued for 7 d. This time period was chosen because previous studies have shown that maximum increases in tolerance are usually attained within 7 d of pre-exposure (Dixon & Sprague, 1981a). Constant monitoring showed that the desired level of toxicant in each tank was attained by 7 h of the initial exposure and then remained constant for the rest of the 7 d. This toxicant-adjustment period was equivalent to approximately 4% of the total exposure period. Test fish were fed once daily during the first 5 d of the pre-exposure period. The fish were not fed during the latter 2 d of the pre-exposure phase, nor during the subsequent lethal exposure phase. This was to decrease the possibility of toxicant assimilation from ingested

food.

2.4 Lethal Exposure Phase

The lethal exposure phase was designed to assess the modifying effects of sublethal pre-exposure on subsequent lethal tolerance. The following procedure was carried out. After the 7 d pre-exposure phase, the 24 cages representing six sets of three sublethal pre-exposure test lots and one control test lot were quickly redistributed to six lethal exposure tanks. The cages were transported to the lethal exposure phase area in buckets containing test tank water to minimize the shock of the transfer. Five of these latter tanks contained a series of five potentially lethal concentrations of the metal under study. The sixth tank served as a control, and was supplied with diluent water only. Each tank had a water volume of 27L, and was supplied with a flow rate of 450ml/min. This provided a 99% replacement time of 4.4h. As the loading density was 1.3g fish/L, the flow rate provided 18L/g fish/d. Cages from each of the four pre-exposure regimes were selected randomly in composing the six lethal exposure tanks. Hence, in the lethal exposure phase, each of the six tanks contained four cages, one from each of the pre-exposure tanks. Figure 1 diagrams the toxicant exposure system and the distribution of the cages from the pre-exposure tanks to the lethal exposure tanks. The advantage of this experimental design is that each of the three pre-exposure regimes can be simultaneously compared to the control response, thus eliminating any possible temporal effects of consecutive testing. However, a shortcoming in this

design is that there were no replicates. This lack of replication prevented a measure of the variance within each experimental regime as may have occurred during either the pre-exposure and/or the lethal exposure phase of the experiments. It was not possible, therefore, to use multivariate analysis to identify differences amongst experimental regimes. However, the lethal exposure phases of certain experiments were repeated during the overall study, providing a measure of repeatability. Further information on data treatment and statistical analysis is provided in Section 2.8.

Mortality was recorded in each bioassay tank at frequent intervals of once every 2-4 h, depending on the mortality rate. Death was acknowledged when fish showed no signs of respiration and failed to respond to touch.

The lethal exposure phase lasted for 144 h. This time period was chosen in an attempt to obtain an incipient lethal level for each metal. The incipient lethal level is defined as the concentration beyond which 50% of the test population cannot survive indefinitely (Sprague, 1969). Thus, the incipient lethal level marks the boundary between sublethal and lethal effects. Ideally, experiments on the lethal toxicity testing of toxicants should be continued until this threshold to lethality is reached, however, Sprague (1969), reviewing a number of articles, concluded that a 96 h exposure period is generally sufficient to demonstrate an incipient lethal level. Therefore, it was decided to run the lethal exposure phase for 144 h, a time period which allowed an extra 48 h of exposure.

2.5 Toxicity Curves

Incipient lethal levels for each metal were estimated by constructing toxicity curves according to the U.S. Standard Method described in Sprague (1969). This type of toxicity curve is constructed by plotting the LC50 against the corresponding lethal exposure time on a logarithmic-logarithmic scale. Toxicity curves are a reflection of the change in lethal tolerance with time and the point at which these curves (which are traditionally fitted by eye) asymptote to the time axis corresponds to the incipient lethal level.

A sublethal concentration can be related to the incipient lethal level by a Toxic Unit (TU). A TU is the fraction of this concentration to the incipient lethal level (Sprague, 1970). Hence, in the present study, pre-exposure concentrations were converted to TU using the following equation:

$$\text{Toxic Unit (TU)} = \frac{\text{Measured Pre-exposure Conc.}}{\text{Incipient Lethal Level}}$$

2.6 Toxicant Delivery System

Concentrated toxicant stock solutions of each metal were prepared by adding either nickel sulphate ($NiSO_4.6H_2O$), copper sulphate ($CuSO_4.5H_2O$), cadmium chloride ($CdCl_2.2.5H_2O$) or silver nitrate ($AgNO_3$) to distilled water. All metals were purchased as reagent grade quality (Fisher Scientific), except for nickel sulphate which was industrial grade (Canadian Industries Ltd). Stock solutions of silver nitrate were covered in black plastic to prevent photo-oxidation. Toxicant was introduced into each of the

experimental tanks employing a method similar to that outlined by Grenier (1960). Stock solutions were held in 20L Mariotte bottles, and one stock solution was made up per experimental tank. Toxicant was dripped from each Mariotte bottle via a feeder tube (Intramedic tubing, Fisher) into a collecting funnel suspended above its respective tank where it mixed with inflowing water from the distribution chamber. The flow of toxicant was started 24 h before a bioassay commenced. This time period was sufficient to allow the required toxicant concentration to be established in each exposure tank.

2.7 Analytical Methods

The concentration of heavy metal was measured in each experimental tank. Duplicate water samples were collected daily during the pre-exposure and lethal exposure phases. Samples were stored in sealed polyethylene vials, and acidified with 10 uL of concentrated nitric acid to approximately pH < 2. Acidification was carried out to minimize precipitation and possible adsorption of metal to the walls of the container. Samples were analyzed for their heavy metal content using a Perkin Elmer 503 atomic absorption spectrophotometer. Three readings were taken from each sample giving a total of 42 readings per exposure phase. Concentrations given in this report are the mean of these 42 readings. Standard deviations of each concentration are given in the Appendix. Samples whose concentration of heavy metal were relatively low were analyzed using carbon-rod (flameless) atomization. Higher concentrations were analyzed by volatilization

using an atomic absorption spectrophotometer equipped with a flame furnace. The detection limits and the precision of both these methods are given in Table 3. Levels of copper, cadmium, nickel and silver in the control (uncontaminated) water, were all below detectable limits when analyzed using the carbon-rod atomization method (Table 1).

2.8 Statistical Methods

The experimental design permitted an evaluation of lethal dose-response relationships as affected by the immediately preceding metal pre-exposure. The concentration lethal to 50% (LC50) was used as a measure of tolerance. The LC50 value for each pre-exposure regime was calculated according to the nomographic method of Litchfield & Wilcoxon (1949). This method, which utilizes nomographs, permits rapid but accurate estimation of the LC50 and its 95% confidence limits. The method involves plotting, on a logarithmic-probability scale, the percentage of organisms reacting (i.e. the percentage mortality), within a specified time interval, against the potentially lethal concentration to which they were exposed. A regression line is then fitted by "eye" to the points. The complex mathematical procedures of Finney (1971) may also be used to estimate the LC50, in which the line is fitted by successive approximations based on maximum likelihood estimates, but Litchfield & Wilcoxon (1953) have shown that logarithmic-probability lines fitted by eye are highly accurate. However, with this method, no more than two consecutive 100% mortalities at the upper end of the dosage range, nor more than two

consecutive 0% mortalities at the lower end of the dosage range may be used in the computations. LC50s were not estimated for data sets in which these criteria were not met.

Regressions were fitted to each data set, where available and complete, at 48, 60, 72, 84, 96, 120 and 144 h of lethal exposure. Thus, at each lethal exposure time, four LC50 estimates were obtained, one for each pre-exposure regime. The dosage-mortality data used for these calculations is given in the Appendix. A sample calculation of the method used to estimate an LC50 is given in the Results (Section 3.2).

An assumption to the dose-response format is that data are normally distributed on a logarithmic scale. This assumption was tested for by a using a Chi Square analysis for homogeneity of data about a linear regression which is incorporated into the method of Litchfield & Wilcoxon (1949). In the majority of data sets, the test for homogeneity was met. In two data sets, however, the data were heterogeneous. In this case, the method is modified slightly to give corrected confidence limits. Heterogenous data sets are indicated as such in the results (Section 3.5). Full details of the method, along with the nomographs, are given in Litchfield & Wilcoxon (1949).

Once LC50 estimates were obtained for each pre-exposure regime, each pre-exposure test lot LC50 was compared to its corresponding control LC50 for significance of difference ($p<0.05$). This is done by computing the standard error of the difference using the method of Litchfield & Wilcoxon (1949). This method compares the ratio of the two LC50s with a theoretical

reaction-ratio derived from the related standard errors. However, two LC50s may only be compared for significance of difference as long as the variances of the two dose response lines are not significantly different, that is, when the lines are parallel. Variance in dose-response relationships is reflected in the slope of the dose-response line. In the Litchfield & Wilcoxon (1949) test, a slope function is calculated from the predicted dose-response line and compared to the slope function of a second line for parallelism. When this condition is met, the two LC50s can then be compared for significance of difference. Slope functions and their 95% confidence limits for all estimated LC50s are given in the Results. A sample calculation for the test for parallelism between two slope functions and the significance of difference between two LC50s is given in Section 3.2.

The Litchfield & Wilcoxon (1949) test for the significance of difference permits one to identify whether two LC50s are significantly different but does not provide the assurance that the difference is a consequence of the pre-exposure regime. Only replication, or, to some degree, repetition would provide the latter insight. As mentioned previously, replicates were not incorporated into the experimental design. However, certain experiments did provide an opportunity to compare differences between similar tests run sequentially. The differences in the results of these repeated tests are given in the Results (Section 3.9), and the similarity between corresponding data sets for a particular regime is a measure of confidence in these results.

2.9. Relative Tolerance Factor.

Modifications in lethal tolerance as a result of prior sublethal exposure may be interpreted in a number of ways. One approach, used in this thesis, may be to compare the tolerance of pre-exposed fish to that of fish without prior sublethal exposure, at selected lethal exposure times. This relationship was depicted graphically in this thesis, by standardizing the control response, giving their tolerance measurements a value of 1.0, and presenting pre-exposure responses as a ratio of the control response. Although they may both be varying through time, the control can be viewed as the normal response, and the pre-exposure modification in relation to this normal response. The ratio of the two LC50s can be thought of as a "relative tolerance factor" and converting the relative tolerance response to a common factor also allows for the comparison of relative tolerance modifications between different metals.

Table 1. Certain Chemical Characteristics of the Laboratory Water

*[1]Alkalinity	85 mg/L as $CaCO_3$
*Hardness	128 mg/L as $CaCO_3$
Temperature	25 ± 1°C
Dissolved Oxygen	90 to 95% saturation
pH	7.8
Silver	< 0.4 ug/L
Cadmium	< 0.2 ug/L
Copper	< 2.5 ug/L
Nickel	< 7.5 ug/L

[1] * Values supplied by the City of Montreal Municipal Water Works

Table 2. Measured Concentrations of Cadmium, Copper, Silver and Nickel used for Pre-exposure and Lethal exposure in each of the seven experiments.

Expt.	Pre-exposure Concentration	Lethal Exposure Concentration
Cd-Cd[2]	0.2, 0.75, 2.0 mg Cd/L	3.3, 4.7, 6.8, 10.1, 11.3 mg Cd/L
Cu-Cu	16, 30, 76 ug Cu/L	239, 245, 253, 258, 271 ug Cu/L
Ag-Ag	0.5, 1.3, 2.2 ug Ag/L	2.7, 3.3, 4.8, 7.2, 10.0 ug Ag/L
Ni-Ni	0.5, 1.0, 5.0 mg Ni/L	24.4, 28.2, 41.1, 46.3, 51.4 mg Ni/L
Cu-Cd	20, 30, 52 ug Cu/L	1.2, 2.2, 4.1, 6.0, 8.5 mg Cd/L
Cu-Ni	16, 40, 70 ug Cu/L	19.7, 29.8, 33.5, 42.3, 54.5 mg Ni/L
Ni-Cu	0.6, 1.4, 6.0 mg Ni/L	254, 268, 276, 281, 293 ug Cu/L

[2] The Pre-exposure Metal followed by the Lethal Exposure Metal

Table 3. Detection Limits (ug/L) of the Carbon-Rod Atomization (flameless) and Volatilization (flame) methods of Atomic Absorption Spectrophotometry for Cadmium, Copper, Silver and Nickel. The Precision of each method is enclosed in parentheses (Coefficient of Variation for N=10).

	carbon-rod atomization	volatilization
cadmium	0.2 (1.3%)	1 (2.1%)
copper	2.5 (1.9%)	2 (3.6%)
silver	0.4 (0.3%)	2 (1.0%)
nickel	7.5 (2.2%)	10 (4.7%)

Figure 1. Schematic of Toxicant Exposure System, where:

A - Head Box

B - Water Distribution Chamber

C - Mariotte Bottle with Toxicant, shown for two tanks only

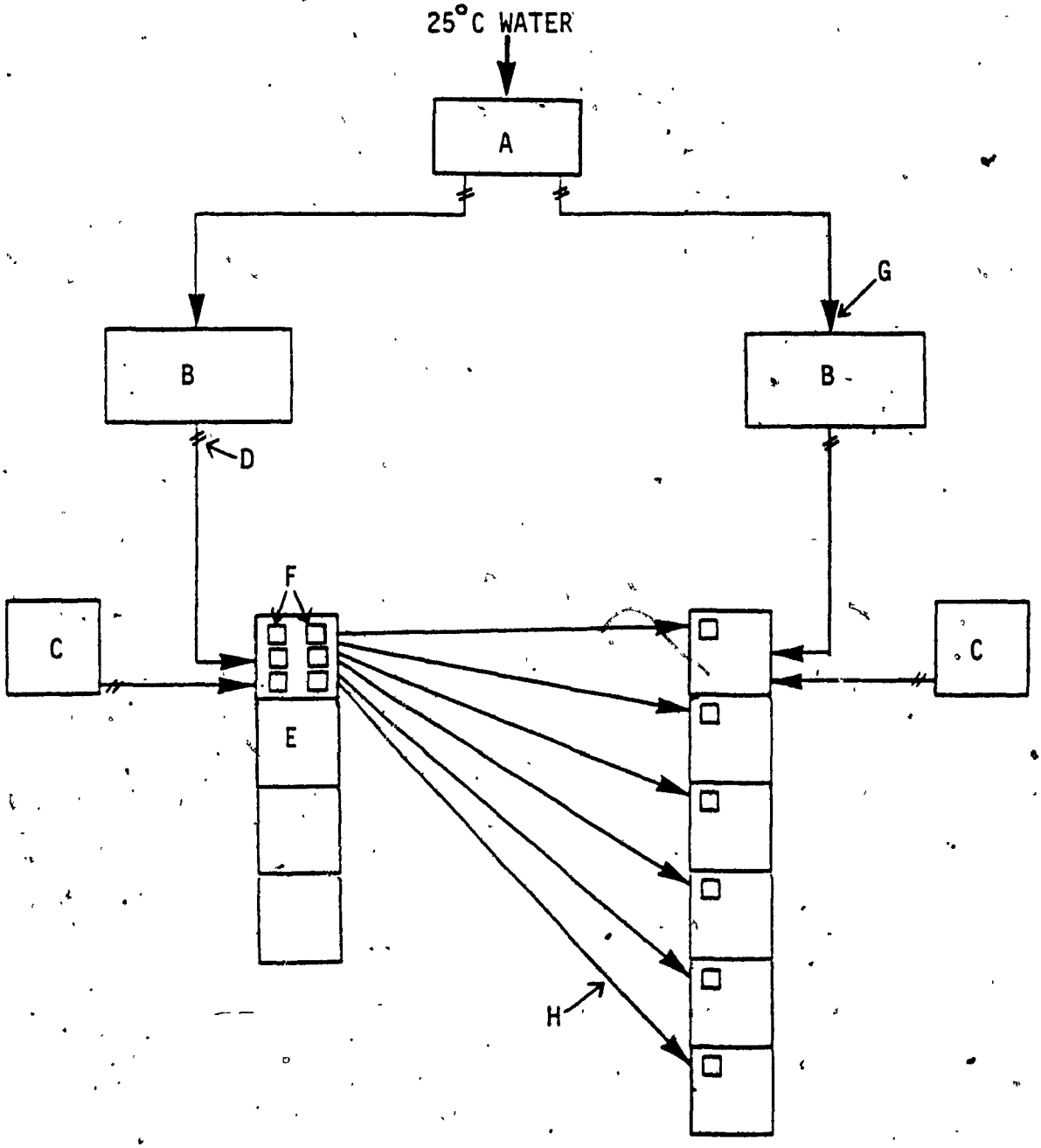
D - Flow Control

E - Bioassay Tank

F - Cages for housing test fish; shown for one pre-exposure regime only

G - Direction of Water Flow

H - Distribution of cages from Pre-exposure to Lethal Exposure tanks


25°C WATER
A
B
B
G
D
F
C
C
E
H
PRE-EXPOSURE PHASE
LETHAL EXPOSURE PHASE

RESULTS

In the present study, seven experiments were conducted to examine the modifying effects of sublethal pre-exposure to either cadmium, copper, silver or nickel, on subsequent lethal tolerance. However, before these experiments are discussed, the toxicity curves of these four metals will first be presented.

3.1 Toxicity Curves

Toxicity curves for cadmium, copper, silver and nickel were constructed by using the control LC50s for each metal. Cadmium, copper and nickel each had two separate experiments in which control LC50s were estimated. For these metals, the control LC50s from both experiments were used to plot the toxicity curve. Thus, data for the cadmium toxicity curve were taken from the control pre-exposure test lots of experiments cadmium-cadmium and copper-cadmium (Tables 4 & 14); data for the copper toxicity curve were taken from the copper-copper and nickel-copper experiments (Tables 8 & 18); data for the nickel toxicity curve were taken from the nickel-nickel and copper-nickel experiments (Tables 12 & 16). Data for the silver toxicity curve were taken from one experiment only (Table 10).

Toxicity curves for cadmium, copper, silver and nickel are depicted in Figures 2, 3, 4 and 5. The concentration at the asymptote for cadmium and for copper, that is, the incipient lethal level is estimated to be 3.2 mg Cd/L and 261 ug Cu/L. For silver and for nickel, there is no apparent asymptote within the duration

Figure 2. Cadmium Toxicity Curve.

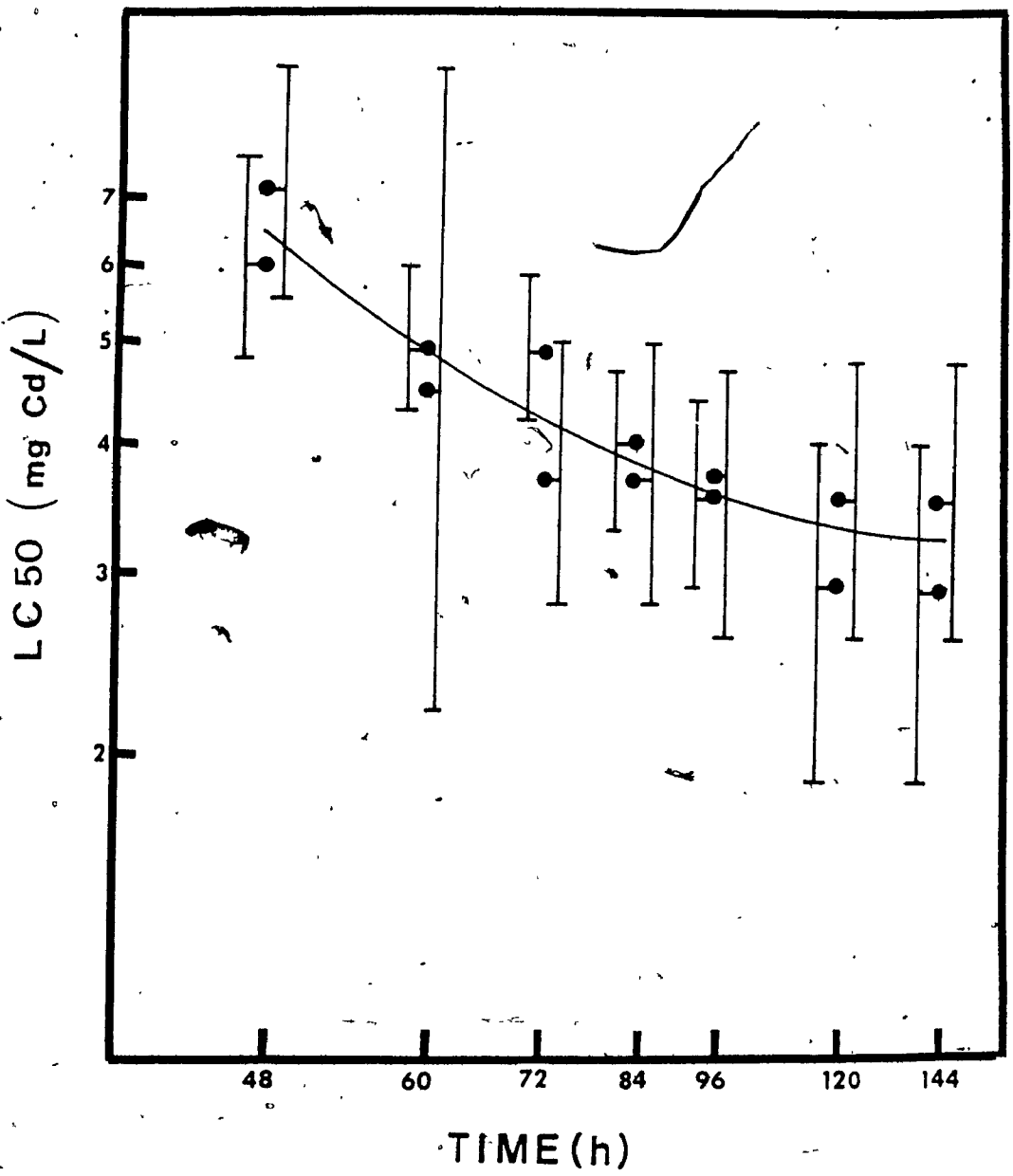

LC 50 (mg Cd/L)
7
6
5
4
3
2
48
60
72
84
96
120
144
TIME (h)

Figure 3. Copper Toxicity Curve

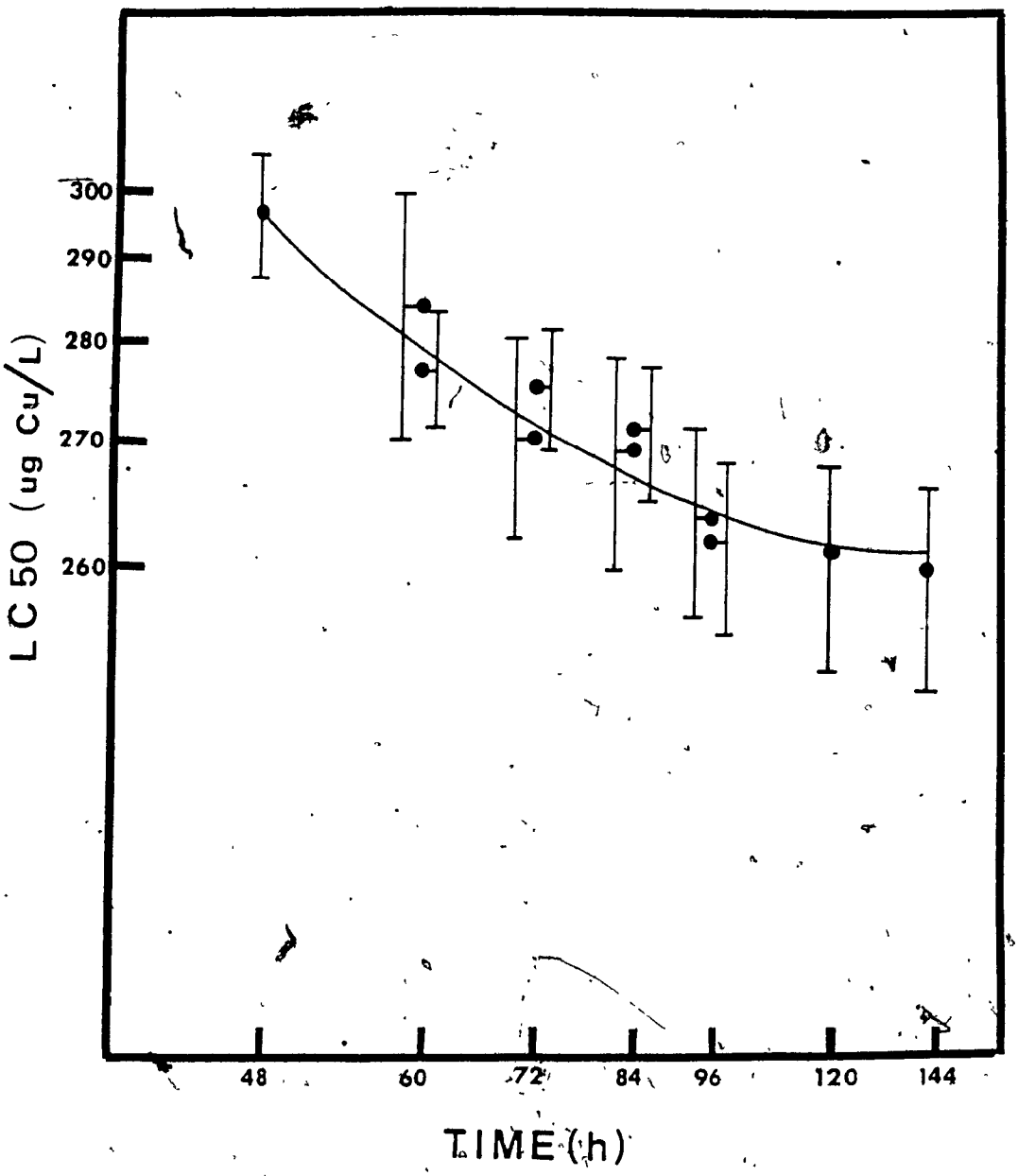

300
290
280
270
260
LC 50 (ug Cu/L)
48
60
72
84
96
120
144
TIME (h)

Figure 4. Silver Toxicity Curve.

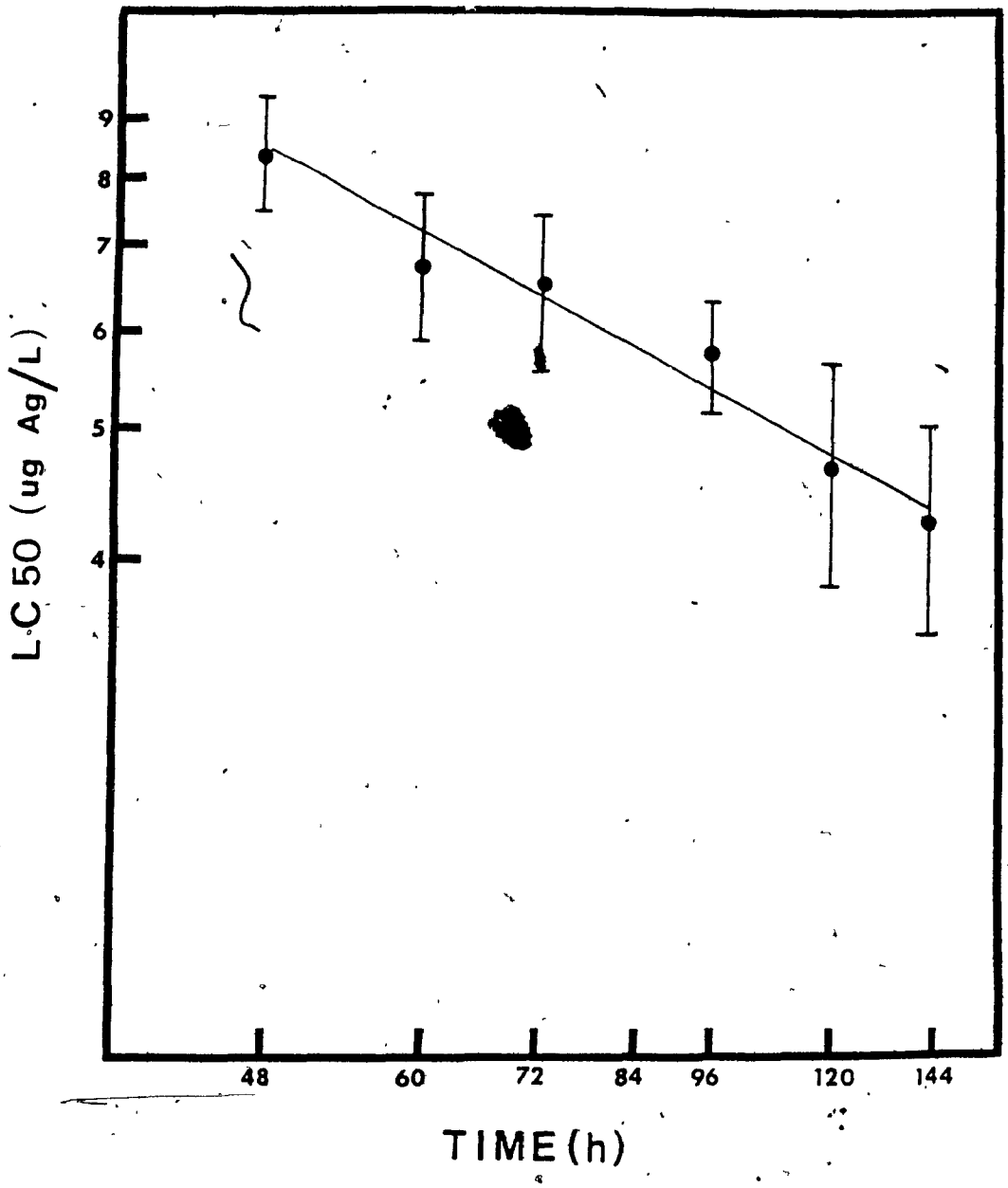
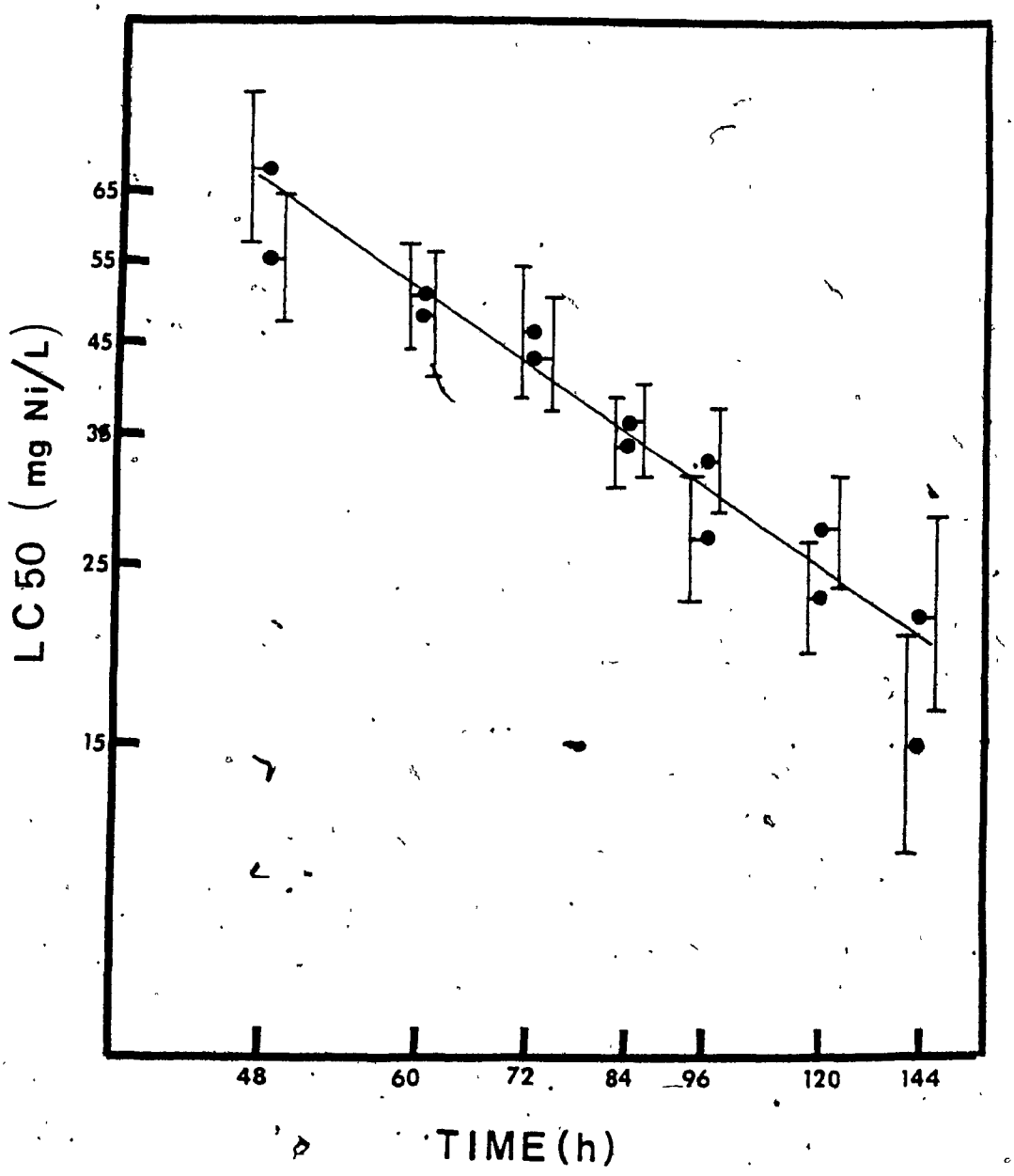

LC 50 (ug Ag/L)
9
8
7
6
5
4
48
60
72
84
96
120
144
TIME (h)

Figure 5. Nickel Toxicity Curve.


LC 50 (mg Ni/L)
65
55
45
35
25
15
48
60
72
84
96
120
144
TIME (h)

of the experiments. Therefore, the 144-h LC50s of 4.4 ug Ag/L and 21 mg Ni/L (Figures 4 & 5) are used as approximations of the incipient lethal level.

The first four experiments examined the effects of pre-exposure and lethal exposure to the same metal.

3.2 Effects of Sublethal Pre-exposure to Cadmium on Relative Tolerance to Lethal Levels of Cadmium

In this experiment, zebrafish were pre-exposed to either 0.2, 0.75 or 2.0 mg Cd/L, before lethal exposure to 3.3, 4.7, 6.8, 10.1 and 11.3 mg Cd/L. As the incipient lethal level for cadmium was estimated from the toxicity curve of this metal to be 3.2 mg Cd/L, the pre-exposure concentrations are equivalent to 0.06, 0.23 and 0.63 TU respectively. The LC50s and their 95% confidence limits are given in Table 4. The control LC50s at 48, 60, 72, 84, 96, 120 and 144 h are 6.0, 5.0, 4.9, 4.0, 3.6, 2.9 and 2.9 mg Cd/L respectively (Table 4). A sample calculation of the estimation of the LC50, the slope function of the LC50 and their 95% confidence limits, are given in Table 5 for the control test lot at 48 h. The dose-response line for this LC50 is depicted in Figure 6. Slope functions of the LC50 dose-response lines and their 95% confidence limits are given in Table 6. A sample calculation for the significance of difference between the control and 2.0 mg Cd/L test lots at 96 h, which includes the test for parallelism between two slope functions is given in Table 7. The slope functions of any two LC50s being compared for significance of difference do not

Table 4. Cadmium Concentration (mg Cd/L) Lethal to 50% (LC50) of test fish, following a 7 d Pre-exposure to either 0.2, 0.75, 2.0 mg Cd/L, or diluent water alone (Control). Parentheses enclose the 95% Confidence Limits of the LC50.

Lethal Exposure Time (h)	Cadmium Pre-exposure Regime			
	Control	0.2 (mg Cd/L)	0.75 (mg Cd/L)	2.0 (mg Cd/L)
	Cadmium LC50 (mg Cd/L)			
48	6.0 (4.8 - 7.5)	6.4 (5.0 - 8.1)	7.6 (6.2 - 9.2)	7.4 (6.0 - 9.2)
60	5.0 (4.3 - 5.9)	5.1 (4.3 - 6.2)	5.9 (4.8 - 7.4)	6.7 (5.5 - 8.1)
72	4.9 (4.2 - 5.8)	4.3 (3.5 - 5.1)	4.8 (3.8 - 6.0)	6.4 (5.2 - 8.0)
84	4.0 (3.3 - 4.7)	3.9 (3.2 - 4.8)	4.4 (3.5 - 5.4)	5.3 (4.2 - 6.6)
96	3.6 (2.9 - 4.4)	3.6 (3.0 - 4.3)	4.3 (3.5 - 5.2)	4.9*[3] (4.0 - 6.1)
120	2.9 (2.1 - 4.0)	3.3 (2.7 - 4.0)	3.9 (3.1 - 4.9)	4.7* (4.0 - 5.6)
144	2.9 (2.1 - 4.0)	3.3 (2.7 - 4.0)	3.9 (3.1 - 4.9)	4.7* (4.0 - 5.6)

[3] * Indicates that the LC50 deviates significantly ($p<0.05$) from the corresponding control LC50.

Figure 6: Dose-Response Line for Control test lot from the Cadmium-Cadmium experiment, at 48 h of lethal exposure.

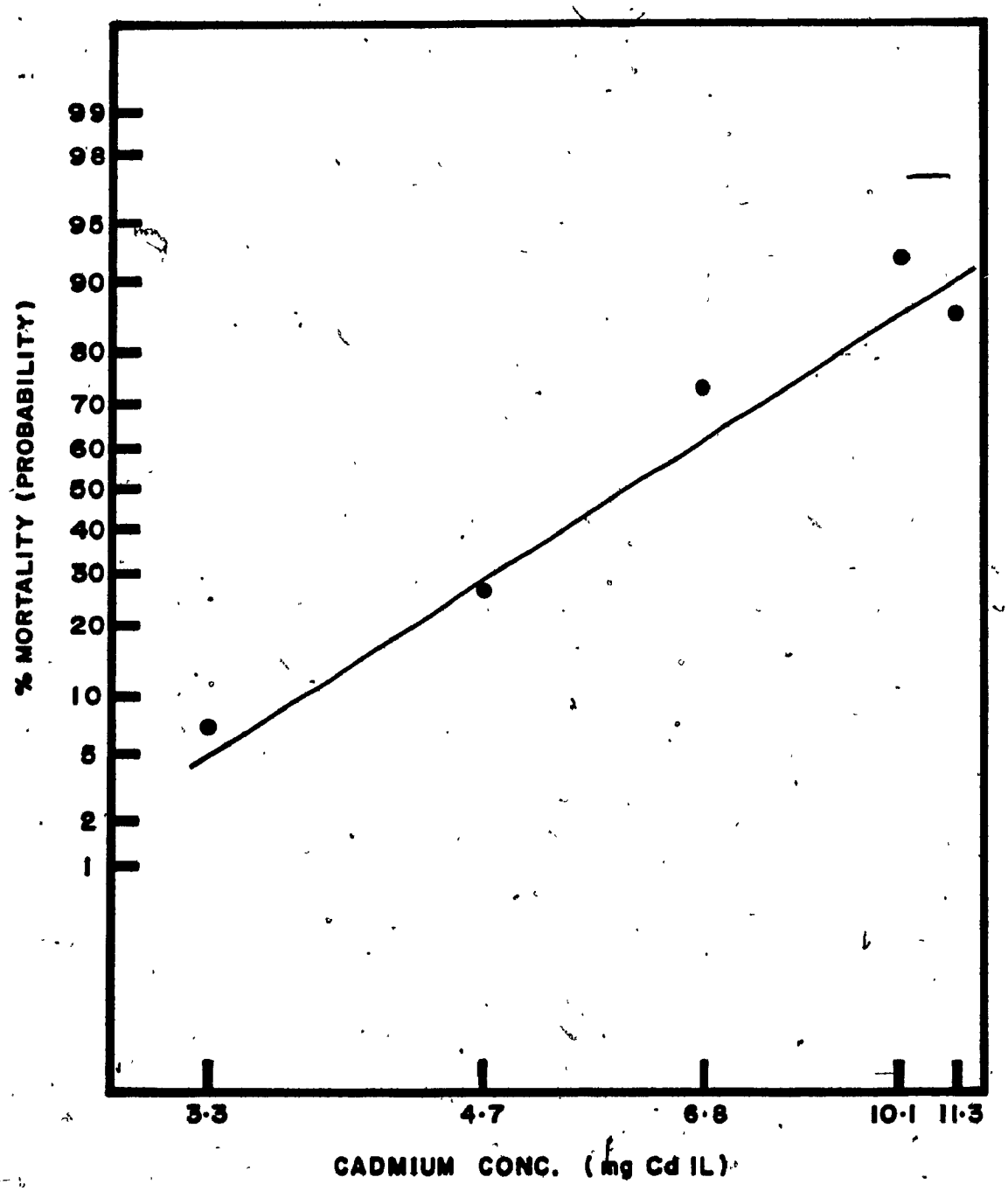

% MORTALITY (PROBABILITY)
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
3·3
4·7
6·8
10·1
11·3
CADMIUM CONC. (mg Cd /L)

Table 5. Sample calculation of the estimation of the LC50, the Slope Function, and their 95% Confidence Limits, for homogeneous data. Data are taken from the Control test lot (No. of fish = 72) of the Cadmium-Cadmium experiment at 48 h. See Litchfield & Wilcoxon (1949) for full details of the method

Exposure Conc. (mg Cd/L)	Observed % Mortality	Expected % Mortality[4]	Observed minus Expected	Chi^2
3.3	6.7	5.0	1.7	0.006
4.7	26.7	28	1.3	0
6.8	73.3	61	12.3	0.06
10.1	92.3	86	6.3	0.035
11.3	85.7	90	4.3	0.02

$\Sigma Chi^2 = 0.121$

1) Contributions to $Chi^2 = 0.121$
2) Average No. of Fish per Dose = Total No. of Fish ÷ k, the No. of Doses
   $= 14.4$
3) Chi^2 of the line = 0.121 x 14.4 = 1.7424
4) df = k - 2 = 3
5) From Litchfield & Wilcoxon (1949),

   $Chi^2[3] = 7.82$ ($p<0.05$)

6) Chi^2 of the line < 7.82, therefore, the data are not significantly heterogeneous
7) From Figure 6,
   LC50 = 6.0 LC16 = 4.1 LC84 = 9.7
8) The Slope Function, S,
   = [LC84÷LC50 + LC50÷LC16]÷2
   = 1.55
9) N′ = No. of fish whose expected effects are between 16 and 84%
   = 30
10) to calculate the factor of the LC50, $fLC50 = S^{2.77 \div \sqrt{N'}}$
    = 1.25
11) 95% Confidence Limits of the LC50,
    LC50 x fLC50 = upper limit
    LC50 ÷ fLC50 = lower limit
12) LC50 = 6.0 (4.8 - 7.5)
13) to calculate the factor of S, fS,
    Dosage Range, R = 11.3÷3.3 = 3.4242

/cont...

[4] From the predicted line of Figure 6.

$A = \text{Antilog}\, 1.1\ (\log S)^2 \div \log R$

$= 1.1873$

$fS = A^{10(k-1) \div k\sqrt{N}}$

$= 1.2850$

14) 95% Confidence Limits of the S,

$S \times fS$ = upper limit

$S \div fS$ = lower limit

15) S = 1.55 (1.21 - 1.99)

Table 6. Slope Functions of the Cadmium LC50s following Pre-exposure to either 0.2, 0.75, 2.0 mg Cd/L, or diluent water alone (Control). Parentheses enclose the 95% Confidence Limits of the Slope Function.

Lethal Exposure Time (h)	Cadmium Pre-exposure Regime			
	Control	0.2 (mg Cd/L)	0.75 (mg Cd/L)	2.0 (mg Cd/L)
	Slope Function of Cd LC50			
48	1.55 (1.21 - 1.99)	1.56 (1.25 - 1.95)	1.92 (1.28 - 2.87)	1.83 (1.30 - 2.58)
60	1.31 (1.15 - 1.50)	1.61 (1.18 - 2.20)	1.76 (1.25 - 2.48)	1.71 (1.31 - 2.24)
72	1.33 (1.14 - 1.55)	1.36 (1.17 - 1.58)	1.58 (1.26 - 1.98)	1.70 (1.31 - 2.22)
84	1.37 (1.20 - 1.56)	1.28 (1.18 - 1.38)	1.60 (1.26 - 2.04)	1.58 (1.20 - 2.10)
96	1.51 (1.21 - 1.88)	1.22 (1.13 - 1.30)	1.58 (1.26 - 2.23)	1.51 (1.21 - 1.89)
120	1.55 (1.08 - 2.22)	1.48 (1.21 - 1.82)	1.67 (1.18 - 2.36)	1.49 (1.25 - 1.78)
144	1.55 (1.08 - 2.22)	1.48 (1.21 - 1.82)	1.67 (1.18 - 2.36)	1.49 (1.25 - 1.78)

Table 7. Sample calculation of the test for Parallelism between two Slope Functions and for the Significance of Difference ($p<0.05$) between two LC50s. Data are taken from the Cadmium-Cadmium experiment at 144 h, and compares the Control and 2.0 mg Cd/L pre-exposure test lots. See Litchfield & Wilcoxon (1949) for full details of the method.

Pre-exposure Regime	LC50	fLC50	S	fS
Control	2.9	1.37	1.55	1.44
2.0 mg Cd/L	4.7	1.19	1.49	1.20

1) Test for parallelism:
   Calculate the Slope Function Ratio, SR,
   SR = 1.55÷1.49
   = 1.04
2) Using fs values, read fSR from Nomograph 4 (Litchfield & Wilcoxon, 1949)
   fSR = 1.50
3) SR < fSR, therefore the curves do not deviate significantly ($p<0.05$) and can be considered parallel

4) Test for Significance of Difference between the two LC50s
   Compute the Potency Ratio, PR,
   PR = 4.7÷2.9
   = 1.62
5) Using the fLC50 values read fPR from Nomograph 4
   fPR = 1.43
6) PR > fPR, therefore the two LC50s are significantly different ($p<0.05$)

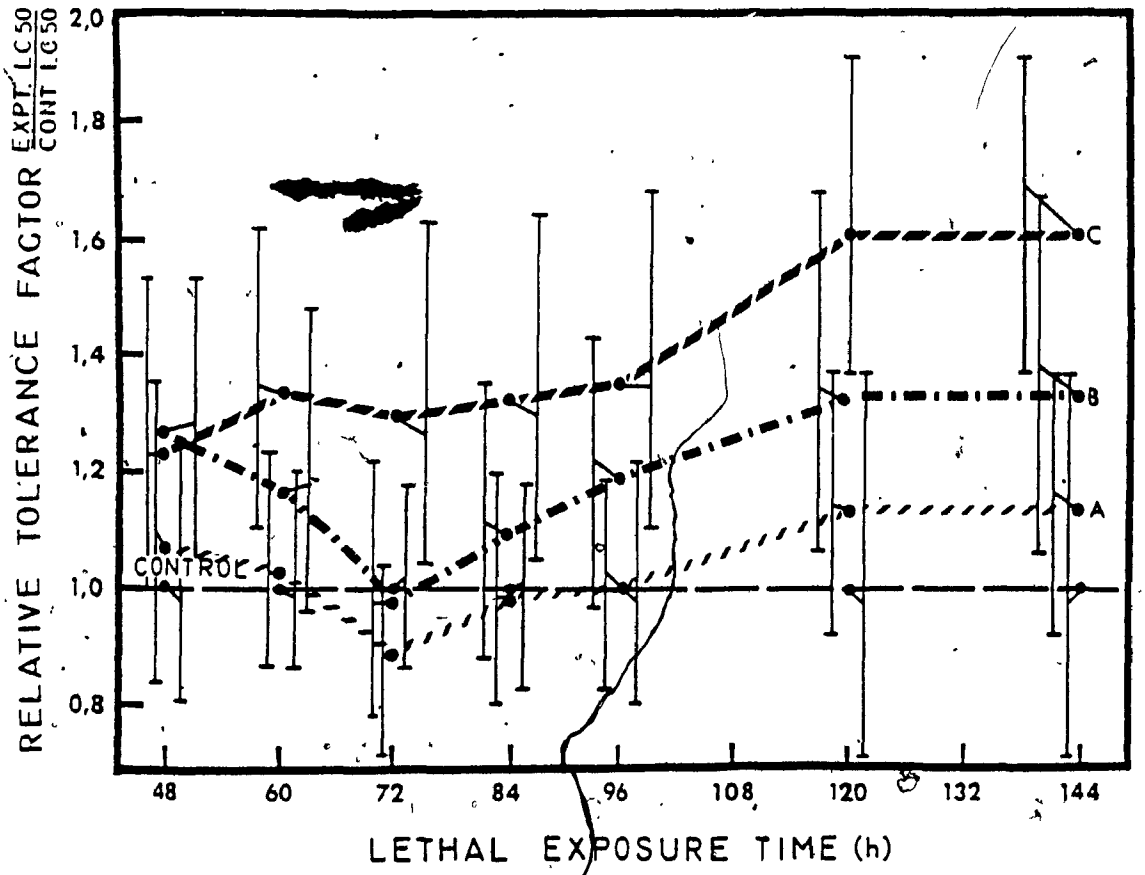
deviate significantly from parallelism.

Figure 7 depicts the modifications in lethal tolerance following pre-exposure to either 0.2, 0.75 or 2.0 mg Cd/L. Pre-exposure to 2.0 mg Cd/L apparently results in a trend of enhanced relative tolerance at all lethal exposure times. Relative tolerance is also apparently increased after pre-exposure to 0.2 and 0.75 mg Cd/L, except at 72 and 84 h when there appears to be an apparent decrease in tolerance compared to the control (Figure 7). However, when LC50s are statistically compared using the Litchfield & Wilcoxon test for significance of difference (1949), pre-exposure to 0.2 and 0.75 does not result in any significant ($p<0.05$) difference in tolerance, relative to the controls, at any lethal exposure time (Table 4). However, pre-exposure to 2.0 mg Cd/L does result in a significant increase in relative tolerance, but only at certain lethal exposure times. The LC50s for this test lot are significantly greater than the corresponding control test lot at 96, 120 and 144 h. At these times, the 2.0 mg Cd/L test lot LC50s are 4.9, 4.7 and 4.7 mg Cd/L (Table 4), which are increases in tolerance of 1.36, 1.62 and 1.62 times the respective control LC50s (Figure 7).

3.3 Effects of Sublethal Pre-exposure to Copper on Relative Tolerance to Lethal Levels of Copper

Zebrafish were pre-exposed to either 16, 30 or 76 ug Cu/L, prior to lethal exposure to 239, 245, 253, 258 and 271 ug Cu/L. The incipient lethal level for copper was estimated from the toxicity curve of this metal to be 261 ug Cu/L (Figure 3). Thus,

Figure 7. Cadmium LC50s for Zebrafish Pre-exposed to either A) 0.2, B) 0.75 or C) 2.0 mg Cd/L, expressed relative to the LC50s of Control fish.


RELATIVE TOLERANCE FACTOR EXPT. LC50 / CONT LC50
2,0
1,8
1,6
1,4
1,2
1,0
0,8
CONTROL
C
B
A
48
60
72
84
96
108
120
132
144
LETHAL EXPOSURE TIME (h)

the pre-exposure concentrations of 16, 30 and 76 ug Cu/L are equivalent to 0.06, 0.11 and 0.29 TU respectively.

The LC50s and their 95% confidence limits are given in Table 8. At 48 h, mortality was too low in all pre-exposure test lots to permit estimation of LC50s (Table 8). At 60, 72, 84, 96, 120 and 144 h, the control LC50s are 284, 271, 269, 264, 261 and 260 ug Cu/L (Table 8). The slope functions and their 95% confidence limits are given in Table 9. The slope functions of any two LC50s being compared for significance of difference do not deviate significantly from parallelism.

Pre-exposure to sublethal levels of copper results in an apparent trend of enhanced tolerance to lethal levels of this metal, relative to control fish (Figure 8). However, despite this trend, relative tolerance to lethal levels of copper does not appear to be significantly ($p<0.05$) modified by pre-exposure to either 16 or 30 ug Cu/L (Table 8). Pre-exposure to 76 ug Cu/L, however, results in a significant ($p<0.05$) increase in tolerance relative to the controls at 144 h of lethal exposure. At this time, the LC50 for this test lot is 314 ug Cu/L, which is an increase in tolerance of 1.21 times the control LC50 of 260 ug Cu/L (Figure 8). Prior to 144 h, the mortality in this test lot was too low to permit estimation of LC50 values.

3.4 Effects of Sublethal Pre-exposure to Silver on Relative Tolerance to Lethal Levels of Silver

In this experiment, zebrafish were pre-exposed to either 0.5, 1.3 or 2.2 ug Ag/L, before lethal exposure to 2.7, 3.3, 4.8, 7.2

Table 8. Copper Concentration (ug Cu/L) Lethal to 50% (LC50) of test fish, following a 7 d Pre-exposure to either 16, 30, 76 ug Cu/L, or diluent water alone (Control). Parentheses enclose the 95% Confidence Limits of the LC50.

Lethal Exposure Time (h)	Copper Pre-exposure Regime			
	Control	16 (ug Cu/L)	30 (ug Cu/L)	76 (ug Cu/L)
	Copper LC50 (ug Cu/L)			
48	-[5]	-	-	-
60	284 (270 - 299)	283 (266 - 301)	-	-
72	271 (262 - 280)	277 (266 - 288)	281 (271 - 292)	-
84	269 (260 - 278)	274 (264 - 283)	275 (267 - 283)	-
96	264 (257 - 271)	270 (261 - 278)	278 (267 - 289)	-
120	261 (254 - 268)	267 (258 - 277)	272 (262 - 281)	-
144	260 (253 - 266)	263 (255 - 271)	273 (262 - 285)	314*[6] (275 - 360)

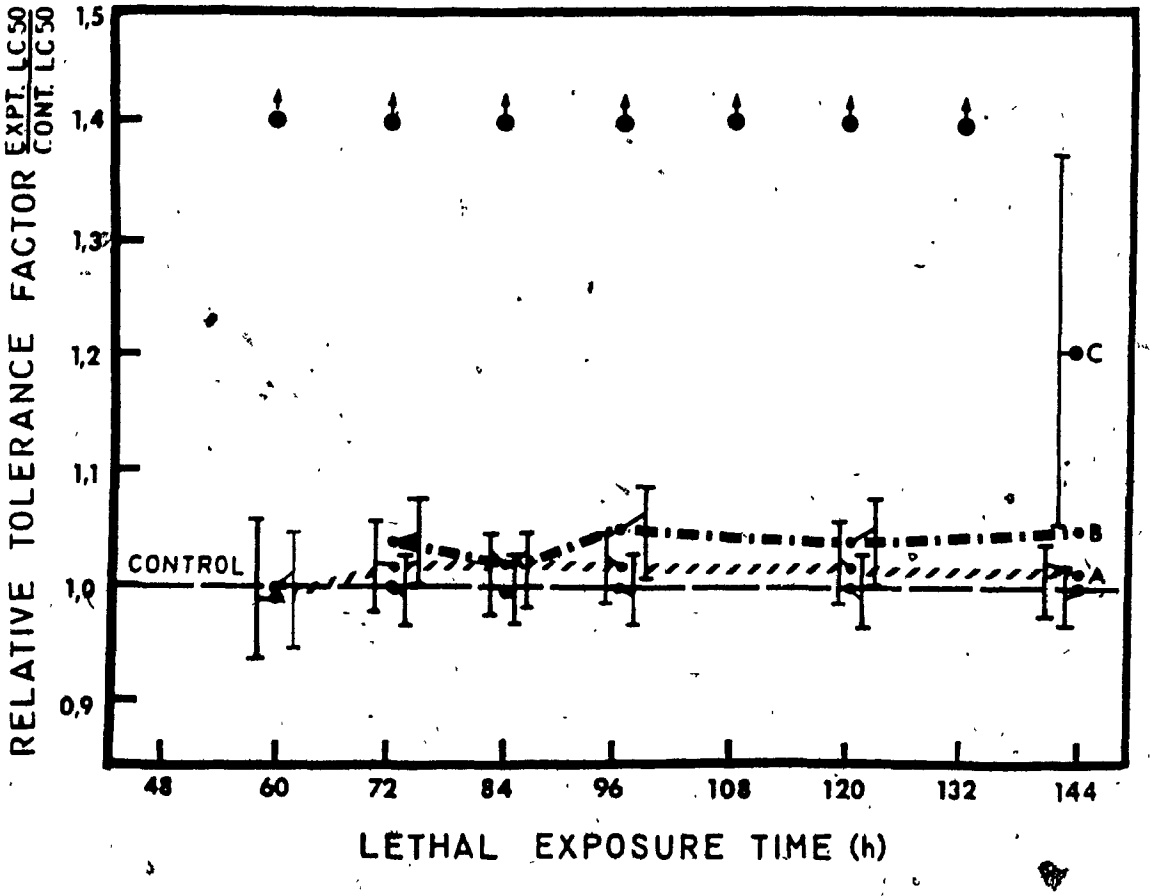
[5] - Indicates that the mortality is too low to permit estimation of LC50

[6] * Indicates that the LC50 deviates significantly ($p<0.05$) from the corresponding control LC50

Table 9. Slope Functions of the Copper LC50s following Pre-exposure to either 16, 30, 76 ug Cu/L, or diluent water alone (Control). Parentheses enclose the 95% Confidence Limits of the Slope Function.

Lethal Exposure Time (h)	Copper Pre-exposure Regime			
	Control	16 (ug Cu/L)	30 (ug Cu/L)	76 (ug Cu/L)
	Slope Function of Cu LC50			
48	-	-	-	-
60	1.12 (0.85 - 1.46)	1.12 (0.94 - 1.35)	-	-
72	1.06 (1.01 - 1.12)	1.10 (0.98 - 1.22)	1.07 (0.97 - 1.17)	-
84	1.09 (1.01 - 1.18)	1.11 (0.98 - 1.26)	1.08 (1.00 - 1.17)	-
96	1.07 (1.02 - 1.13)	1.10 (1.00 - 1.20)	1.10 (0.97 - 1.28)	
120	1.11 (1.00 - 1.24)	1.12 (0.99 - 1.26)	1.11 (0.93 - 1.32)	
144	1.10 (1.01 - 1.21)	1.10 (1.01 - 1.19)	1.14 (0.95 - 1.37)	1.17 (0.69 - 1.99)

Figure 8. Copper LC50s for Zebrafish Pre-exposed to either A) 16, B) 30 or C) 76 ug Cu/L, expressed relative to LC50s of Control fish.


RELATIVE TOLERANCE FACTOR EXPT. LC50 / CONT. LC50
1,5
1,4
1,3
1,2
1,1
1,0
0,9
CONTROL
C
B
A
48
60
72
84
96
108
120
132
144
LETHAL EXPOSURE TIME (h)

and 10.0 ug Ag/L. No apparent incipient lethal level was evident in the toxicity curve for this metal within 144 h, hence, the 144-h LC50 of 4.4 ug Ag/L was used as an approximation of the incipient lethal level (Figure 4). Thus, the pre-exposure concentrations of 0.5, 1.3 and 2.2 ug Ag/L are equivalent to 0.11, 0.30 and 0.50 TU respectively.

The LC50s and their 95% confidence limits are given in Table 10. The control LC50s at 48, 60, 72, 84, 96, 120 and 144 h, are 8.4, 6.8, 6.5, 6.2, 5.8, 4.7 and 4.3 ug Ag/L, respectively (Table 10). Slope functions and their 95% confidence limits are given in Table 11. The slope functions of any two LC50s being compared for significance of difference do not deviate significantly from parallelism.

Pre-exposure to 0.5 and 1.3 ug Ag/l results in an apparent trend of enhanced relative tolerance to lethal levels of this metal (Figure 9). Despite this trend, pre-exposure to 0.5 ug Ag/L does not result in any significant ($p<0.05$) increase in relative tolerance (Table 10). Pre-exposure to 1.3 ug Ag/l results in significant increases in relative tolerance to lethal levels of silver at 48, 60, 72 and 84 h (Table 10). The LC50s of this test lot at these times are 14.6, 12.9, 9.6 and 9.3 ug Ag/L. These are increases of 1.74, 1.90, 1.48 and 1.50 times the corresponding control LC50s (Figure 9). At 96, 120 and 144 h, however, pre-exposure to 1.3 ug Ag/L results in no significant modifications in relative tolerance. In contrast, pre-exposure to 2.2 ug Ag/L results in an apparent trend of decreased tolerance (sensitization) to lethal levels of this metal, compared to the tolerance of

Table 10. Silver Concentration (ug Ag/L) Lethal to 50% (LC50) of test fish, following a 7 d Pre-exposure to either 0.5, 1.3, 2.2 ug Ag/L, or diluent water alone (Control). Parentheses enclose the 95% Confidence Limits of the LC50.

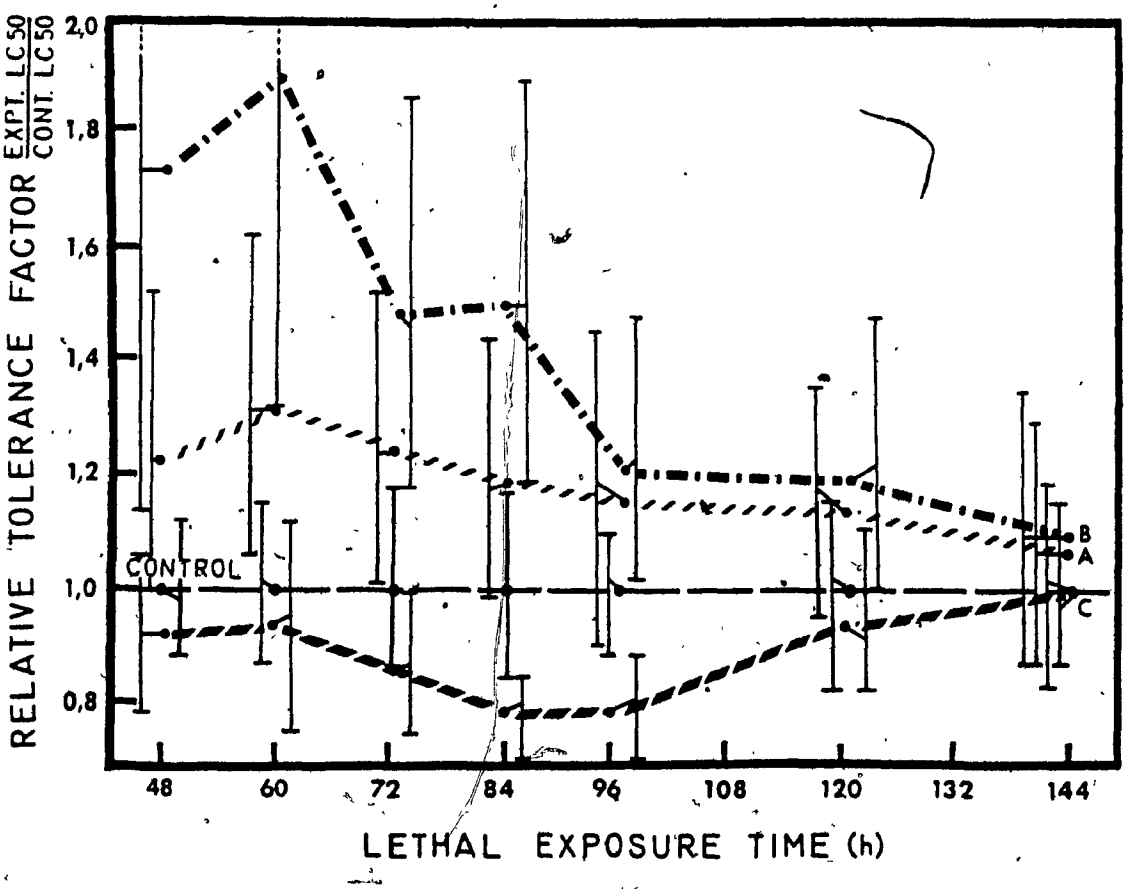
Lethal Exposure Time (h)	Silver Pre-exposure Regime			
	Control	0.5 (ug Ag/L)	1.3 (ug Ag/L)	2.2 (ug Ag/L)
	Silver LC50 (ug Ag/L)			
48	8.4 (7.5 - 9.4)	10.3 (8.4 - 12.6)	14.6*[7] (9.6 - 22.2)	7.7 (6.6 - 8.9)
60	6.8 (5.9 - 7.8)	8.9 (7.2 - 11.0)	12.9* (8.8 - 18.8)	6.3 (5.2 - 7.6)
72	6.5 (5.6 - 7.5)	8.1 (6.6 - 9.9)	9.6* (7.6 - 12.1)	5.6 (4.9 - 6.5)
84	6.2 (5.3 - 7.3)	7.4 (6.1 - 8.9)	9.3* (7.4 - 11.7)	4.9* (4.4 - 5.3)
96	5.8 (5.2 - 6.4)	6.7 (5.3 - 8.4)	7.1 (5.9 - 8.6)	4.6* (4.1 - 5.2)
120	4.7 (3.9 - 5.7)	5.4 (4.5 - 6.4)	5.7 (4.7 - 6.9)	4.4 (3.9 - 5.0)
144	4.3 (3.6 - 5.1)	4.6 (3.8 - 5.6)	4.7 (3.8 - 5.8)	4.3 (3.8 - 5.0)

[7] * Indicates that the LC50 deviates significantly ($p<0.05$) from the corresponding control LC50

Table 11. Slope Functions of the Silver LC50s following Pre-exposure to either 0.5, 1.3, 2.2 ug Ag/L, or diluent water alone (Control). Parentheses enclose the 95% Confidence Limits of the Slope Function.

Lethal Exposure Time (h)	Silver Pre-exposure Regime			
	Control	0.5 (ug Ag/L)	1.3 (ug Ag/L)	2.2 (ug Ag/L)
	Slope Function of Ag LC50			
48	1.26 (1.14 - 1.39)	1.31 (1.08 - 1.59)	1.85 (0.89 - 3.83)	1.23 (1.10 - 1.37)
60	1.21 (1.10 - 1.33)	1.51 (1.19 - 1.92)	2.12 (0.73 - 6.16)	1.47 (1.20 - 1.80)
72	1.23 (1.10 - 1.37)	1.50 (1.19 - 1.89)	1.72 (1.14 - 2.60)	1.47 (1.22 - 1.77)
84	1.22 (1.13 - 1.32)	1.49 (1.22 - 1.82)	1.68 (1.19 - 2.36)	1.33 (1.15 - 1.53)
96	1.26 (1.18 - 1.34)	1.36 (1.14 - 1.62)	1.56 (1.28 - 1.90)	1.35 (1.15 - 1.59)
120	1.42 (1.15 - 1.75)	1.45 (1.22 - 1.73)	1.44 (1.22 - 1.70)	1.31 (1.15 - 1.49)
144	1.36 (1.22 - 1.52)	1.33 (1.15 - 1.53)	1.35 (1.17 - 1.56)	1.17 (1.11 - 1.24)

Figure 9. Silver LC50s for Zebrafish Pre-exposed to either A) 0.5, B) 1.3 or C) 2.2 ug Ag/L, expressed relative to the LC50s of Control fish.


RELATIVE TOLERANCE FACTOR EXPT. LC50 / CONT. LC50
2,0
1,8
1,6
1,4
1,2
1,0
0,8
CONTROL
B
A
C
48
60
72
84
96
108
120
132
144
LETHAL EXPOSURE TIME (h)

control fish (Figure 9). These apparent reductions in relative tolerance are significantly ($p<0.05$) different from the control tolerance level at 84 and 96 h of lethal exposure (Table 10). At these two times, the LC50s of this test lot are 4.9 and 4.6 ug Ag/L, which is 0.79 times the corresponding control LC50 values (Figure 9).

3.5 Effects of Sublethal Pre-exposure to Nickel of Relative Tolerance to Lethal Levels of Nickel

In this experiment, zebrafish were pre-exposed to either 0.5, 1.0 or 5.0 mg Ni/L, before lethal exposure to 24.4, 28.2, 41.1, 46.3 and 51.4 mg Ni/L. The toxicity curve for this metal shows that no apparent incipient lethal level was evident within 144 h (Figure 5). Therefore, the 144-h LC50 of 21 mg Ni/L was used as an approximation of the incipient lethal level. Thus, the pre-exposure concentrations of 0.5, 1.0 and 5.0 mg Ni/L are equivalent to 0.02, 0.05 and 0.24 TU respectively.

The LC50s and their 95% confidence limits are shown in Table 12. The control LC50s at 48, 60, 72, 84, 96, 120 and 144 h, are 69, 50, 46, 35, 27, 23 and 15 mg Ni/L respectively (Table 12). Mortality was too high at 144 h of lethal exposure in the 0.5, 1.0 and 5.0 mg Ni/L test lots, and at 120 h of lethal exposure in the 1.0 and 5.0 mg Ni/L test lots to permit estimation of LC50s. Two data sets are heterogeneous. These are for the 0.5 mg Ni/L test lot at 48 and 72 h and corrected confidence limits are given (Table 12). Slope functions and their 95% confidence limits are given in Table 13. The slope functions of any two LC50s being compared for

Table 12. Nickel Concentration (mg Ni/L) Lethal to 50% (LC50) of test fish, following a 7 d Pre-exposure to either 0.5, 1.0, 5.0 mg Ni/L, or diluent water alone (Control). Parentheses enclose the 95% Confidence Limits of the LC50.

	Nickel Pre-exposure Regime			
Lethal Exposure Time (h)	Control	0.5 (mg Ni/L)	1.0 (mg Ni/L)	5.0 (mg Ni/L)
	Nickel LC50 (mg Ni/L)			
48	69 (57 - 82)	52 (43 - 63)	53 (42 - 67)	44* (40 - 48)
60	50 (44 - 57)	39*[8] (34 - 44)	34* (30 - 39)	36* (33 - 40)
72	46 (39 - 55)	33 (24 - 45)	32* (28 - 37)	30* (26 - 34)
84	35 (31 - 39)	31 (28 - 35)	30 (27 - 34)	27* (24 - 31)
96	27 (23 - 32)	28 (24 - 32)	27 (23 - 31)	21 (17 - 26)
120	23 (20 - 27)	22 (20 - 25)	-[9]	-
144	15 (11 - 21)	-	-	-

[8] * Indicates that the LC50 deviates significantly ($p<0.05$) from the corresponding control LC50

[9] - Indicates that the mortality is too high to permit estimation of LC50

Table 13. Slope Functions of the Nickel LC50s following Pre-exposure to either 0.5, 1.0 or 5.0 mg Ni/L, or diluent water alone (Control). Parentheses enclose the 95% Confidence Limits of the Slope Function.

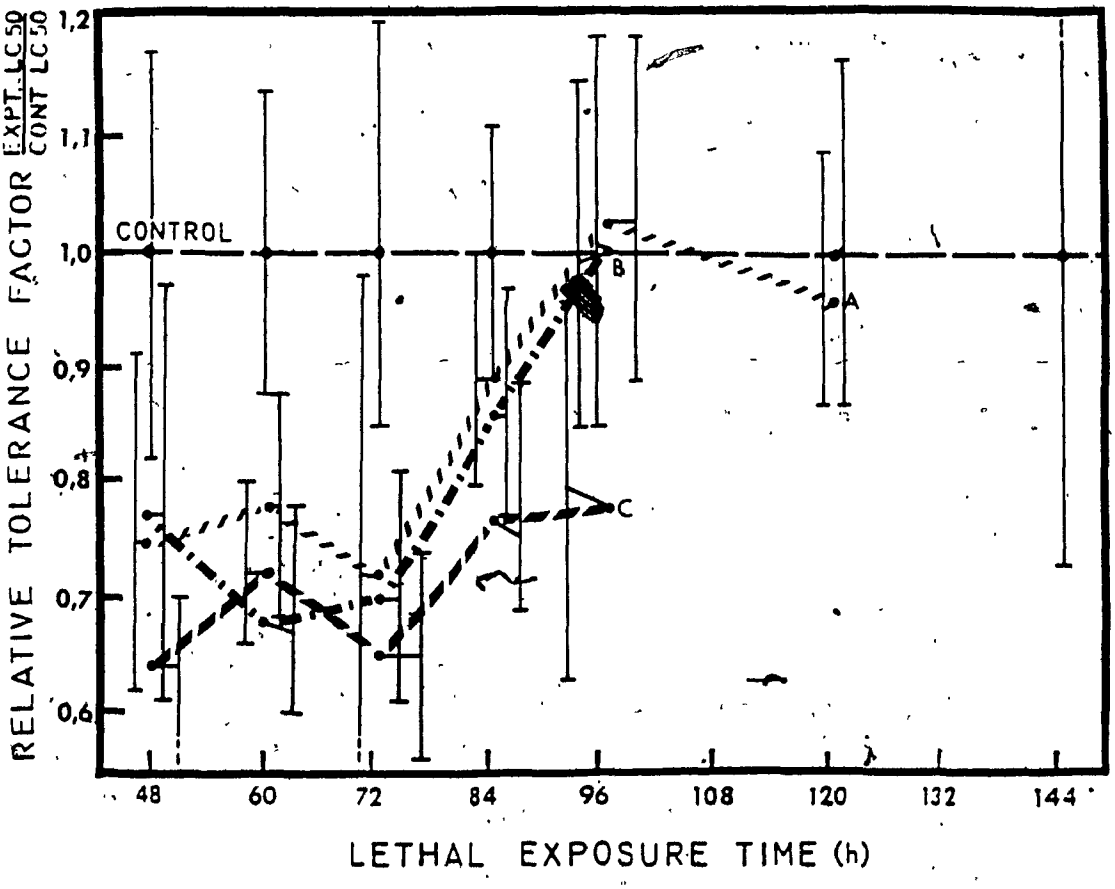
Lethal Exposure Time (h)	Nickel Pre-exposure Regime			
	Control	0.5 (mg Ni/L)	1.0 (mg Ni/L)	5.0 (mg Ni/L)
	Slope Function of Ni LC50			
48	1.50 (1.09 - 2.06)	1.70 (1.12 - 2.58)	1.84 (1.01 - 3.36)	1.39 (1.18 - 1.64)
60	1.46 (1.17 - 1.82)	1.46 (1.22 - 1.75)	1.48 (1.23 - 1.79)	1.47 (1.20 - 1.80)
72	1.63 (1.13 - 2.35)	1.44 (0.89 - 2.33)	1.42 (1.20 - 1.68)	1.40 (1.17 - 1.67)
84	1.78 (1.20 - 2.64)	1.46 (1.22 - 1.75)	1.36 (1.17 - 1.58)	1.29 (1.13 - 1.47)
96	1.57 (1.15 - 2.14)	1.42 (1.18 - 1.71)	1.37 (1.14 - 1.65)	1.74 (0.89 - 3.39)
120	1.59 (1.06 - 2.38)	1.47 (1.10 - 1.96)	-	-
144	1.80 (0.74 - 4.40)	-	-	-

significance of difference do not deviate significantly from parallelism.

Figure 10 shows that pre-exposure to either 0.5 or 1.0 mg Ni/L results in an apparent trend of sensitization to lethal levels of nickel, relative to the controls, from approximately 48 to 72 h. From 72 h on, however, sensitization apparently diminishes compared to the control. However, according to the statistical test for significance of difference ($p<0.05$), pre-exposure to 0.5 mg Ni/L results in a significant decrease in relative tolerance at 60 h of lethal exposure only (Table 12). At this time, the LC50 of this test lot is 39 mg Ni/L, which is 0.78 times the corresponding control LC50 of 50 mg Ni/L (Figure 10). Likewise, pre-exposure to 1.0 mg Ni/L results in a significant reduction in relative tolerance at 60 and 72 h only. The LC50s for this test lot are 34 and 32 mg Ni/L at 60 and 72 h, which are 0.68 and 0.70 times the corresponding control LC50s (Figure 10). Pre-exposure to 5.0 mg Ni/L results in apparent sensitization from 48 to 96 h, the only times at which LC50s were estimated (Figure 10). However, sensitization, relative to the control, is significant from 48 to 84 h of lethal exposure. The LC50s of this test lot at 48, 60, 72 and 84 h are 44, 36, 30 and 27 mg Ni/L, which are 0.64, 0.72, 0.65 and 0.77 times the corresponding control LC50s (Figure 10). At 96 h of lethal exposure, there is no significant difference in relative tolerance between the control and the 5.0 mg Ni/L pre-exposure test lot.

The next phase of the study was designed to examine whether

Figure 10. Nickel LC50s for Zebrafish Pre-exposed to either A) 0.5, B) 1.0 or C) 5.0 mg Ni/L, expressed relative to the LC50s of Control fish.


RELATIVE TOLERANCE FACTOR EXPT. LC50 / CONT LC50
1,2
1,1
1,0
0,9
0,8
0,7
0,6
CONTROL
A
B
C
48
60
72
84
96
108
120
132
144
LETHAL EXPOSURE TIME (h)

tolerance modifications are metal specific, that is, can enhanced tolerance or sensitization be conferred towards lethal levels of metals which are different from the metal of pre-exposure.

Three further experiments were conducted to address this issue. The first two experiments were conducted using, for pre-exposure, one of the metals which resulted in subsequent enhanced relative tolerance. Copper was selected as the metal of pre-exposure, followed by lethal exposure to either cadmium or nickel. The third experiment was conducted using, for pre-exposure, one of the metals which resulted in sensitization. Nickel was selected as the pre-exposure metal, followed by lethal exposure to copper.

3.6 Effects of Sublethal Pre-exposure to Copper on Relative Tolerance to Lethal Levels of Cadmium

Zebrafish were pre-exposed to either 20, 30 or 52 ug Cu/L (equivalent to 0.08, 0.11 and 0.20 TU), before subsequent lethal exposure to 1.2, 2.2, 4.1, 6.0 and 8.5 mg Cd/L.

The LC50s and their 95% confidence limits are given in Table 14. The control cadmium LC50s at 48, 60, 72, 84, 96, 120 and 144 h, are 7.1, 4.5, 3.7, 3.7, 3.5, 3.5 and 3.5 mg Cd/L. The slope functions and their 95% confidence limits are given in Table 15. The slope functions of any two LC50s being compared for significance of difference do not deviate significantly from parallelism.

Figure 11 suggests that pre-exposure to either 20, 30 or 52 ug Cu/L results in an apparent trend of enhanced tolerance, relative

Table 14. Cadmium Concentration (mg Cd/L) Lethal to 50% (LC50) of test fish, following a 7 d Pre-exposure to either 20, 30, 52 ug Cu/L, or diluent water alone (Control). Parentheses enclose the 95% Confidence Limits of the LC50.

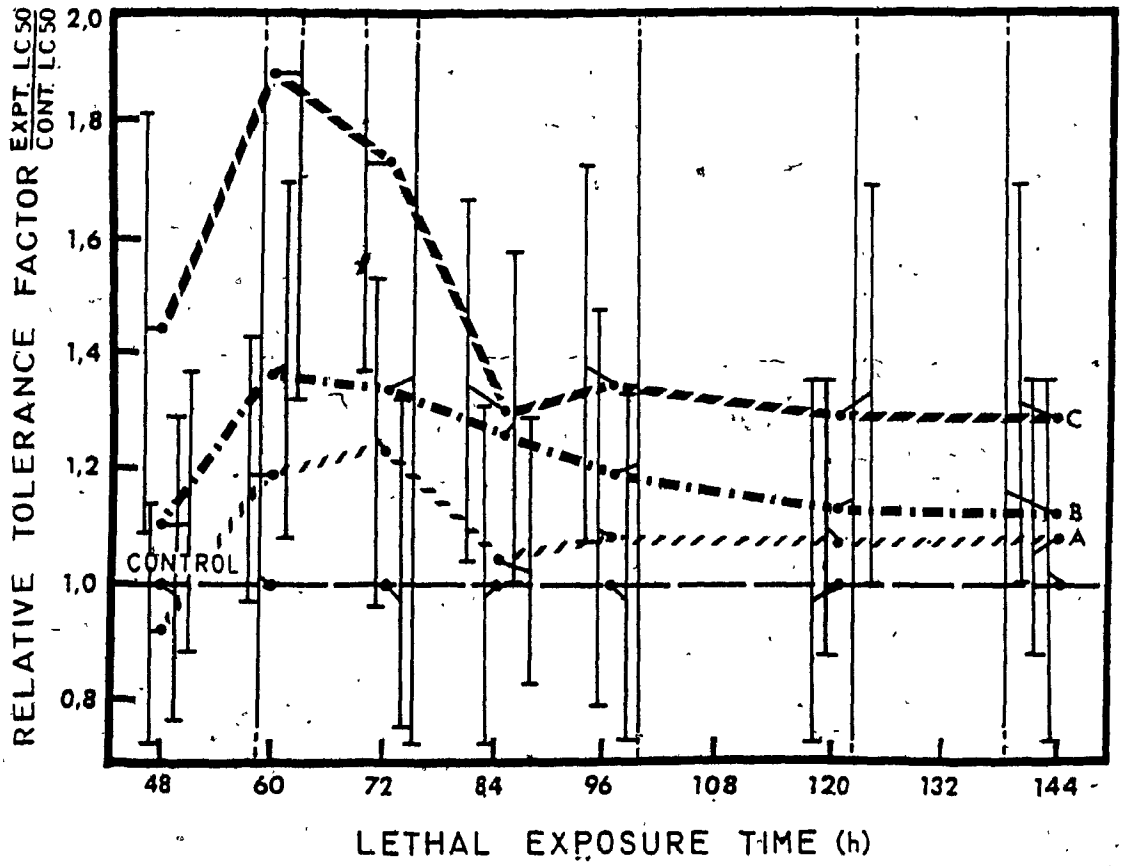
Lethal Exposure Time (h)	Copper Pre-exposure Regime			
	Control	20 (ug Cu/L)	30 (ug Cu/L)	52 (ug Cu/L)
	Cadmium LC50 (mg Cd/L)			
48	7.1 (5.5 - 9.2)	6.5 (5.2 - 8.2)	7.9 (6.3 - 9.8)	10.3 (7.8 - 13.0)
60	4.5 (2.2 - 9.2)	5.4 (4.4 - 6.5)	6.2 (4.9 - 7.7)	8.6 (6.0 - 12.2)
72	3.7 (2.8 - 5.0)	4.6 (3.6 - 5.7)	5.0 (2.7 - 9.2)	6.5*[10] (5.1 - 8.2)
84	3.7 (2.7 - 4.9)	3.9 (3.1 - 4.8)	4.7 (3.7 - 5.9)	4.9 (3.9 - 6.2)
96	3.5 (2.6 - 4.7)	3.8 (2.8 - 5.2)	4.2 (1.6 - 10.9)	4.8 (3.8 - 6.1)
120	3.5 (2.6 - 4.8)	3.8 (3.1 - 4.8)	4.0 (1.7 - 9.7)	4.6 (3.5 - 6.0)
144	3.5 (2.6 - 4.8)	3.8 (3.1 - 4.8)	4.0 (1.7 - 9.7)	4.6 (3.5 - 6.0)

[10] * Indicates that the LC50 deviates significantly ($p<0.05$) from the corresponding control LC50

Table 15. Slope Functions of the Cadmium LC50s following Pre-exposure to either 20, 30, 52 ug Cu/L, or diluent water alone (Control). Parentheses enclose the 95% Confidence Limits of the Slope Function.

Lethal Exposure Time (h)	Copper Pre-exposure Regime			
	Control	20 (ug Cu/L)	30 (ug Cu/L)	52 (ug Cu/L)
	Slope Function of Cd LC50			
48	1.44 (1.24 - 1.67)	1.34 (1.17 - 1.53)	1.38 (1.23 - 1.55)	1.68 (1.25 - 2.12)
60	1.58 (1.00 - 2.50)	1.32 (1.21 - 1.44)	1.33 (1.17 - 1.51)	2.96 (1.41 - 6.21)
72	1.40 (1.23 - 1.60)	1.33 (1.22 - 1.45)	1.32 (1.17 - 1.48)	1.52 (1.25 - 1.84)
84	1.40 (1.23 - 1.60)	1.50 (1.31 - 1.71)	1.60 (1.38 - 1.85)	1.95 (1.47 - 2.58)
96	1.44 (1.23 - 1.68)	1.52 (1.45 - 1.60)	1.58 (1.33 - 1.88)	1.81 (1.45 - 2.26)
120	1.60 (1.39 - 1.83)	1.53 (1.32 - 1.77)	1.62 (1.34 - 1.96)	1.69 (1.37 - 2.07)
144	1.60 (1.39 - 1.83)	1.53 (1.32 - 1.77)	1.62 (1.34 - 1.96)	1.69 (1.37 - 2.07)

Figure 11. Cadmium LC50s for Zebrafish Pre-exposed to either A) 20, B) 30 or C) 52 ug Cu/L, expressed relative to the LC50s of Control fish.


RELATIVE TOLERANCE FACTOR EXPT. LC50 / CONT. LC50
2,0
1,8
1,6
1,4
1,2
1,0
0,8
CONTROL
C
B
A
48
60
72
84
96
108
120
132
144
LETHAL EXPOSURE TIME (h)

to the control fish. However, despite this apparent trend, pre-exposure to either 20 or 30 ug Cu/L results in no significant differences in LC50 compared to the corresponding control LC50s (Table 14). Furthermore, pre-exposure to 52 ug Cu/L only results in a significant increase in LC50, compared to the control, at 72 h. At this time, the LC50 of this test lot is 6.5 mg Cd/L, which is 1.76 times the corresponding control LC50 of 3.7 mg Cd/L (Figure 11).

3.7 Effects of Sublethal Pre-exposure to Copper on Relative Tolerance to Lethal Levels of Nickel

In this experiment, zebrafish were pre-exposed to either 16, 40 or 70 ug Cu/L (equivalent to 0.06, 0.15 and 0.27 TU respectively), before subsequent exposure to 19.7, 29.8, 33.5, 42.3, 54.5 mg Ni/L.

The LC50s and their 95% confidence limits are given in Table 16. The nickel LC50s for the control test lot at 48, 60, 72, 84, 96, 120 and 144 h are 55, 48, 43, 36, 33, 28 and 22 mg Ni/L (Table 16). At 48 h, mortality was too low in the 16, 40 and 70 ug Cu/L pre-exposure test lots to permit estimation of LC50s. At 60 h, mortality was too low in the 70 ug Cu/L pre-exposure test lot to permit estimation of an LC50 (Table 16). The slope functions and their 95% confidence limits are given in Table 17. The slope functions of any two LC50s being compared for significance of difference do not deviate significantly from parallelism.

The trend depicted in Figure 12 suggests that pre-exposure to 16, 40 and 70 ug Cu/L results in an apparent increase in relative

Table 16. Nickel Concentration (mg Ni/L) Lethal to 50% (LC50) of test fish, following a 7 d Pre-exposure to either 16, 40, 70 ug Cu/L, or diluent water alone (Control). Parentheses enclose the 95% Confidence Limits of the LC50.

	Copper Pre-exposure Regime			
Lethal Exposure Time (h)	Control	16 (ug Cu/L)	40 (ug Cu/L)	70 (ug Cu/L)
	Nickel LC50 (mg Ni/L)			
48	55 (47 - 65)	-[11]	-	-
60	48 (41 - 56)	55 (46 - 66)	84*[12] (58 - 121)	-
72	43 (38 - 50)	42 (38 - 47)	53 (43 - 64)	64* (52 - 78)
84	36 (32 - 41)	35 (32 - 38)	40 (35 - 45)	43 (38 - 49)
96	33 (29 - 38)	34 (31 - 37)	33 (29 - 37)	36 (32 - 40)
120	28 (24 - 32)	27 (23 - 31)	24 (21 - 28)	29 (27 - 32)
144	22 (17 - 29)	18 (13 - 24)	21 (18 - 25)	20 (16 - 25)

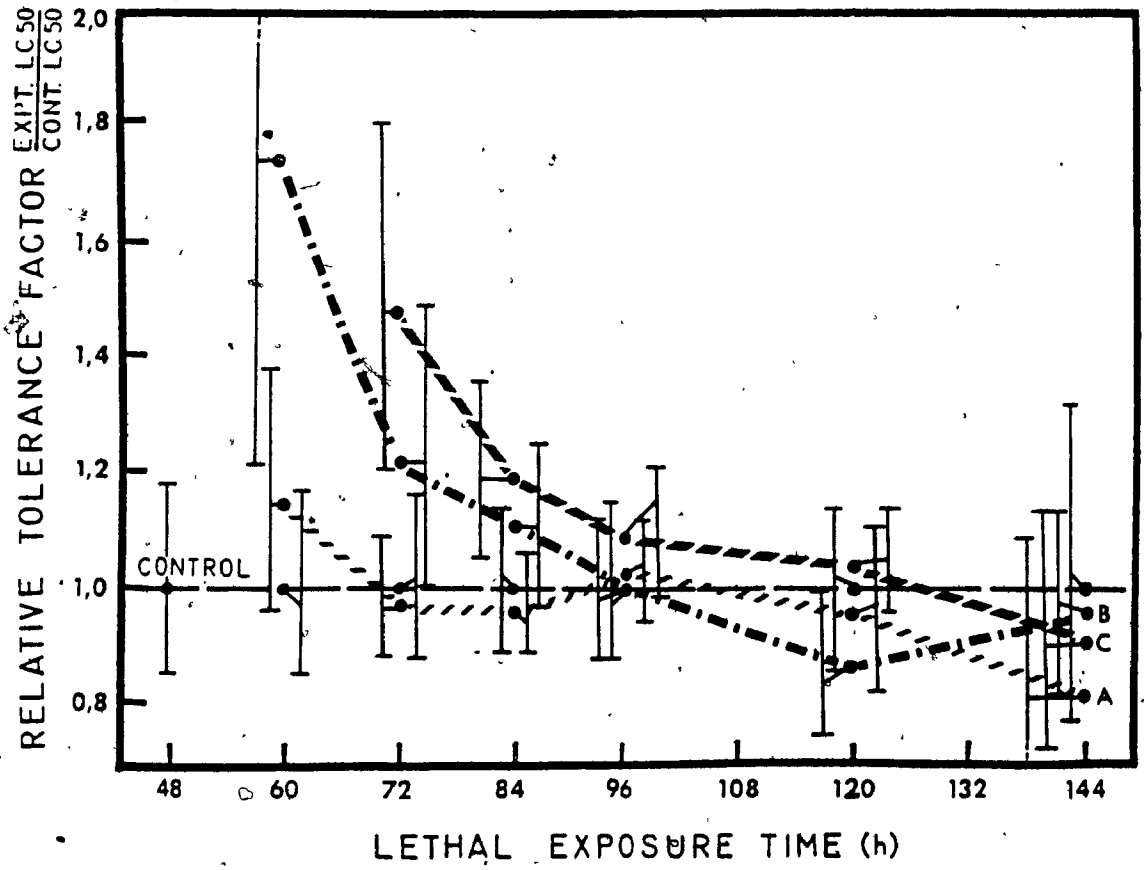
[11] - Indicates that the mortality is too low to permit estimation of the LC50

[12] * Indicates that the LC50 deviates significantly ($p<0.05$) from the corresponding control LC50

Table 17. Slope Functions of the Nickel LC50s following Pre-exposure to either 16, 40, 70 ug Cu/L, or diluent water alone (Control). Parentheses enclose the 95% Confidence Limits of the Slope Function.

Lethal Exposure Time (h)	Copper Pre-exposure Regime			
	Control	16 (ug Cu/L)	40 (ug Cu/L)	70 (ug Cu/L)
	Slope Function of Ni LC50			
48	1.28 (1.06 - 1.47)	-	-	-
60	1.48 (1.23 - 1.79)	1.47 (1.18 - 1.83)	1.70 (0.96 - 3.00)	-
72	1.57 (1.26 - 1.96)	1.32 (1.20 - 1.45)	1.57 (1.17 - 2.11)	1.67 (1.13 - 2.47)
84	1.31 (1.19 - 1.44)	1.20 (1.14 - 1.26)	1.34 (1.18 - 1.52)	1.40 (1.22 - 1.60)
96	1.38 (1.21 - 1.58)	1.14 (1.06 - 1.22)	1.38 (1.22 - 1.56)	1.31 (1.20 - 1.43)
120	1.25 (1.15 - 1.36)	1.37 (1.22 - 1.54)	1.27 (1.17 - 1.38)	1.17 (1.12 - 1.22)
144	1.25 (1.04 - 1.50)	1.50 (1.05 - 2.14)	1.37 (1.11 - 1.69)	1.28 (1.10 - 1.49)

Figure 12. Nickel LC50s for Zebrafish Pre-exposed to either A) 16, B) 40 or C) 70 ug Cu/L, expressed relative to the LC50s of Control fish.


RELATIVE TOLERANCE FACTOR EXP'T. LC50 / CONT. LC50
2,0
1,8
1,6
1,4
1,2
1,0
0,8
CONTROL
48
60
72
84
96
108
120
132
144
LETHAL EXPOSURE TIME (h)
A
B
C

tolerance to nickel during the initial hours of lethal exposure, but that this relative increase in nickel tolerance diminishes with time until by 144 h there is apparent sensitization to nickel, relative to the control. However, despite this trend, the apparent sensitization at 144 h is not significant ($p<0.05$) (Table 16). Furthermore, pre-exposure to 16 ug Cu/L results in no significant differences in relative tolerance to nickel at any lethal exposure time (Table 16). However, pre-exposure to 40 ug Cu/L results in a significant increase in relative tolerance to nickel at 60 h. At this time, the LC50 of this test lot is 84 mg Ni/L, which is an increase of 1.75 times the corresponding control LC50 (Figure 12). From 72 to 144 h, pre-exposure to 40 ug Cu/L results in no significant difference in tolerance to nickel compared to the control. Pre-exposure to 70 ug Cu/L results in a significant increase in relative tolerance to nickel at 72 h. The LC50 of this test lot at this time is 64 mg Ni/L, an increase of 1.49 times the corresponding control LC50 (Figure 12). After 72 h, pre-exposure to 70 ug Cu/L has no significant effect on relative tolerance (Table 16).

3.8 Effects of Sublethal Pre-exposure to Nickel on Relative Tolerance to Lethal Levels of Copper

Zebrafish were pre-exposed to either 0.6, 1.4 or 6.0 mg Ni/L (equivalent to 0.03, 0.07 and 0.29 TU respectively), before subsequent exposure to 254, 268, 276, 281 and 293 ug Cu/L.

In this experiment, the lethal exposure phase was conducted for 96 h only, due to technical problems with the apparatus after

this time.

The LC50s and their 95% confidence limits are given in Table 18. The copper LC50s for the control fish at 48, 60, 72, 84 and 96 h are 296, 277, 275, 271 and 262 ug Cu/L (Table 18). The slope functions and their 95% confidence limits are given in Table 19. The slope functions of any two LC50s being compared for significance of difference do not deviate significantly from parallelism.

Figure 13 suggests that pre-exposure to either 0.6, 1.4 or 6.0 mg Ni/L results in an apparent trend of increased sensitization relative to the control fish. Nevertheless, pre-exposure to 0.6 mg Ni/L results in no significant difference ($p<0.05$) in LC50 compared to the corresponding control LC50s (Table 18). However, pre-exposure to 1.4 mg Ni/L results in a significant decrease in copper LC50s compared to the corresponding control LC50s from 48 to 96 h. The LC50s for this test lot are 271, 262, 259, 255 and 250 ug Cu/L at 48, 60, 72, 84 and 96 h respectively. These LC50s are 0.92, 0.95, 0.94, 0.94 and 0.95 times the control LC50s respectively (Figure 13). For the 6.0 mg Ni/L pre-exposure test lot, tolerance is significantly reduced compared to the control at 48 h. At this time, the LC50 of this test lot is 252 ug Cu/L, which is 0.85 times the control LC50 of 296 ug Cu/L (Figure 13). After 48 h, however, mortality was too high in this pre-exposure test to permit estimation of the LC50s.

3.9 Duplicate Control LC50s for Cadmium, Copper and Nickel

Certain data sets were duplicated in the course of the overall

Table 18. Copper Concentration (ug Cu/L) Lethal to 50% (LC50) of test fish, following a 7 d Pre-exposure to either 0.6, 1.4, 6.0 mg Ni/L, or diluent water alone (Control). Parentheses enclose the 95% Confidence Limits of the LC50.

Lethal Exposure Time (h)	Nickel Pre-exposure Regime			
	Control	0.6 (mg Ni/L)	1.4 (mg Ni/L)	6.0 (mg Ni/L)
	Copper LC50 (ug Cu/L)			
48	296 (287 - 305)	285 (280 - 290)	271*[13] (267 - 275)	252* (243 - 261)
60	277 (271 - 283)	271* (266 - 275)	262* (258 - 266)	-[14]
72	275 (269 - 281)	266 (261 - 271)	259* (255 - 264)	-
84	271 (265 - 277)	262 (256 - 268)	255* (249 - 261)	-
96	262 (256 - 268)	253 (246 - 261)	250* (244 - 256)	-

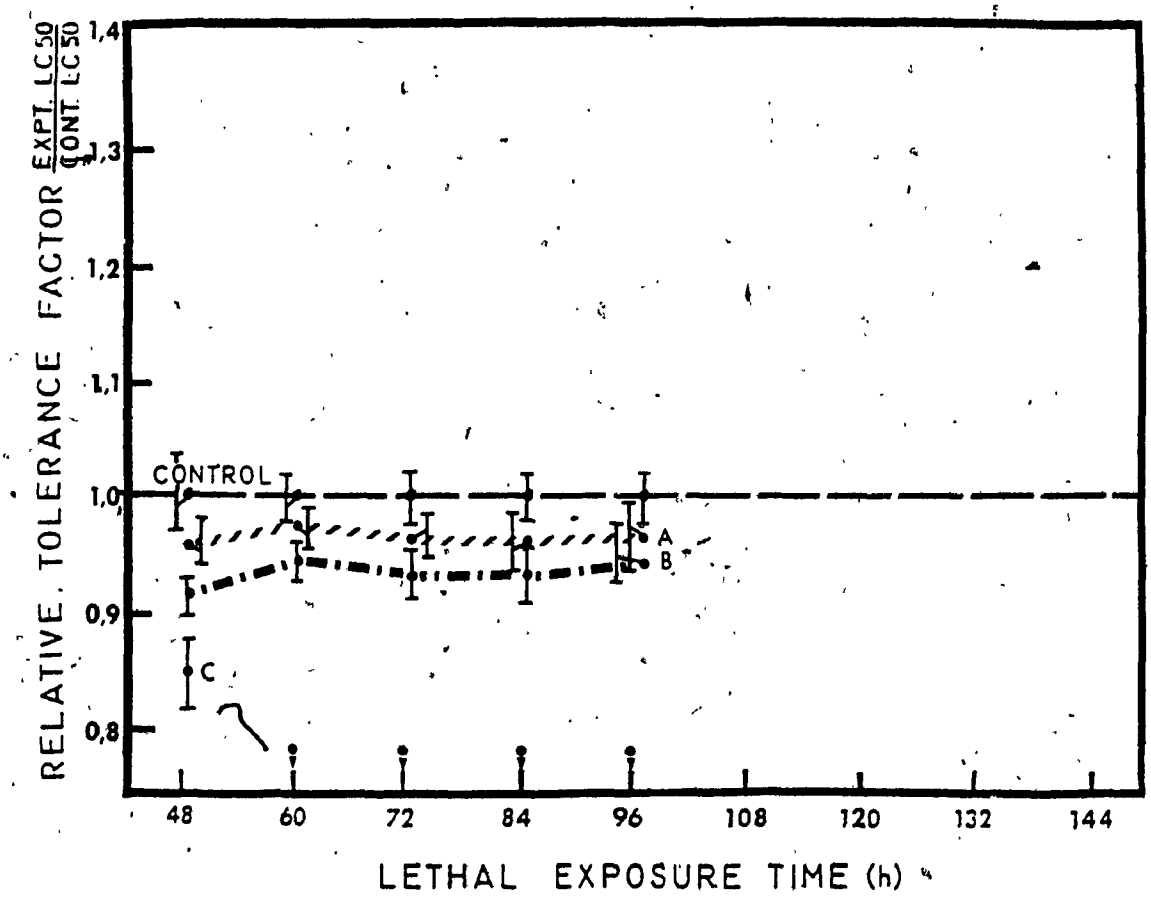
[13] * Indicates that the LC50 deviates significantly ($p<0.05$) from the corresponding control LC50

[14] - Indicates that the mortality is too high to permit estimation of LC50

Table 19. Slope Functions of the Copper LC50s following Pre-exposure to either 0.6, 1.4, 6.0 mg Ni/L, or diluent water alone (Control). Parentheses enclose the 95% Confidence Limits of the Slope Function.

	Nickel Pre-exposure Regime			
Lethal Exposure Time (h)	Control	0.6 (mg Ni/L)	1.4 (mg Ni/L)	6.0 (mg Ni/L)
	Slope Function of Cu LC50			
48	1.08 (1.01 - 1.15)	1.05 (1.03 - 1.07)	1.04 (1.02 - 1.06)	1.04 (1.02 - 1.06)
60	1.12 (1.02 - 1.23)	1.06 (1.03 - 1.09)	1.05 (1.03 - 1.07)	-
72	1.08 (1.03 - 1.13)	1.06 (1.03 - 1.09)	1.04 (1.02 - 1.06)	-
84	1.06 (1.03 - 1.09)	1.04 (1.02 - 1.06)	1.03 (1.01 - 1.05)	-
96	1.06 (1.03 - 1.09)	1.05 (1.02 - 1.08)	1.04 (1.01 - 1.07)	-

Figure 13. Copper LC50s for Zebrafish Pre-exposed to either A) 0.6, B) 1.4 or C) 6.0 mg Ni/L, expressed relative to the LC50s of Control fish.


RELATIVE TOLERANCE FACTOR EXPT. LC50 / CONT. LC50
1,4
1,3
1,2
1,1
1,0
0,9
0,8
CONTROL
A
B
C
48
60
72
84
96
108
120
132
144
LETHAL EXPOSURE TIME (h)

study, thereby allowing for some measure of repeatability despite the lack of replication. These duplicate data sets are the control LC50s for those experiments which used the same metal for lethal exposure and are given in Tables 20, 21 and 22. Duplicate control LC50s were obtained for cadmium from two experiments using cadmium as the lethal exposure metal, cadmium-cadmium and copper-cadmium, the data of which are in Tables 4 and 14. Similarly, duplicate control LC50s for copper were obtained from the copper-copper and nickel-copper experiments from Tables 8 and 18. Finally, duplicate control LC50s for nickel were obtained from the nickel-nickel and copper-nickel experiments, the data of which are in Tables 12 and 16. Duplicate control data sets were not available for silver. A comparison between the LC50s of duplicate data sets, at any given lethal exposure time, shows that they are not significantly different ($p<0.05$) from each other. This is a measure of confidence in these results.

Table 20. Duplicate Control LC50s for Cadmium (mg Cd/L). Control LC50s are taken from the Cadmium-Cadmium and Copper-Cadmium experiments (Sections 3.2 & 3.6). Parentheses enclose the 95% Confidence Limits of the LC50.

Lethal Exposure Time (h)	Cadmium-Cadmium Control LC50	Copper-Cadmium Control LC50
48	6.0 (4.8 - 7.5)	7.1 (5.5 - 9.2)
60	5.0 (4.3 - 5.9)	4.5 (2.2 - 9.2)
72	4.9 (4.2 - 5.8)	3.7 (2.8 - 5.0)
84	4.0 (3.3 - 4.9)	3.7 (2.7 - 4.9)
96	3.6 (3.0 - 4.4)	3.5 (2.6 - 4.7)
120	2.9 (2.0 - 4.0)	3.5 (2.6 - 4.8)
144	2.9 (2.0 - 4.0)	3.5 (2.6 - 4.8)

Table 21. Duplicate Control LC50s for Copper (ug Cu/L). Control LC50s are taken from the Copper-Copper and Nickel-Copper experiments (Sections 3.3 & 3.8). Parentheses enclose the 95% Confidence Limits of the LC50.

Lethal Exposure Time (h)	Copper-Copper Control LC50	Nickel-Copper Control LC50
48	-[15]	296 (287 - 305)
60	284 (270 - 299)	277 (271 - 283)
72	271 (262 - 280)	275 (269 - 281)
84	269 (260 - 278)	271 (265 - 277)
96	264 (257 - 271)	262 (256 - 268)
120	261 (254 - 268)	-
144	260 (253 - 266)	-

[15] - Indicates that the mortality is too low to permit estimation of the LC50

Table 22. Duplicate Control LC50s for Nickel (mg Ni/L). Control LC50s are taken from the Nickel-Nickel and Copper-Nickel experiments (Sections 3.5 & 3.7). Parentheses enclose the 95% Confidence Limits of the LC50.

Lethal Exposure Time (h)	Nickel-Nickel Control LC50	Copper-Nickel Control LC50
48	69 (57 - 82)	55 (47 - 65)
60	50 (44 - 57)	48 (41 - 56)
72	46 (39 - 55)	43 (38 - 50)
84	35 (31 - 39)	36 (32 - 41)
96	27 (23 - 32)	33 (29 - 38)
120	23 (20 - 27)	28 (24 - 32)
144	15 (11 - 21)	22 (17 - 29)

DISCUSSION

The present study provides further insight into the modifications in lethal tolerance to heavy metals following prior sublethal exposure. The results suggest that lethal tolerance may be influenced by a number of factors, including the metal selected for pre-exposure and its concentration, as well as the duration of the lethal exposure phase. Some of the trends observed in this study, as well as their possible environmental implications are discussed in the following sections.

4.1 Effects of Sublethal Pre-exposure to a Metal on Relative Tolerance to Lethal Levels of the Same Metal.

4.1.1 Modifying Effects of Pre-exposure Concentration on Relative Tolerance - Apparent Limits to Relative Tolerance Modifications.

The results obtained with cadmium, copper, silver and nickel suggest that relative tolerance to lethal levels is dependent on the concentration of the metal used for pre-exposure. The discussion examines the results obtained with cadmium, copper and silver first. For cadmium, the concentrations used for pre-exposure were 0.2, 0.75 and 2.0 mg Cd/L. However, only pre-exposure to 2.0 mg Cd/L resulted in any significant ($p<0.05$) increases in tolerance to lethal levels of this metal, compared to the control (Table 4). Similarly, for copper, the concentrations used for pre-exposure were 16, 30 and 76 ug Cu/L, but only

pre-exposure to 76 ug Cu/L was found to significantly increase relative tolerance to subsequent lethal levels (Table 8). For silver, the concentrations used for pre-exposure were 0.5, 1.3 and 2.2 ug Ag/L. However, only pre-exposure to 1.3 ug Ag/L resulted in any significant increases in tolerance relative tolerance at certain lethal exposure times (Table 10). Furthermore, these increases were dependent on the duration of lethal exposure (see Section 4.1.2).

The increases in relative tolerance attained by pre-exposure to either 2.0 mg Cd/L, 76 ug Cu/L or 1.3 ug Ag/L were approximately 1.6, 1.2 and 1.9 times, respectively, the corresponding control LC50s (Figures 7, 8 & 9). These pre-exposure concentrations are equivalent to 0.63, 0.29 and 0.30 TU. These increases in relative tolerance are similar to those reported in the literature for other teleosts. For example, coho salmon (Oncorhynchus kisutch) showed increases in copper tolerance of 1.6, 1.7, 1.9 & 2.2 times the control tolerance at pre-exposure concentrations equivalent to 0.38, 0.50, 0.55 & 0.75 TU (McCarter & Roch, 1983). The tolerance of rainbow trout to copper increased by 1.9 times relative to the control tolerance, following pre-exposure to copper at concentrations equivalent to 0.58 TU (Dixon & Sprague, 1981a). Similarly, the tolerance of white suckers to cadmium increased by 1.8 times the control tolerance at pre-exposure concentrations of 0.60 TU (Duncan & Klaverkamp, 1983). Similar increases in tolerance (of up to 1.5 to 2.5 times the control tolerance) have been obtained in fish for other metals, including aluminium (Orr et al., 1985) and zinc (Bradley, et al., 1985). The fact that

tolerance can be similarly increased by different metals and by different species suggests that there is a common mechanism.

These findings also suggest that protective mechanisms may only be functional within specific pre-exposure concentrations. There may be lower and upper pre-exposure limits beyond which the mechanisms which are responsible may be incapable of inducing enhanced relative tolerance.

Evidence of a lower limit to tolerance modifications is shown by the apparent lack of increased tolerance elicited following pre-exposure to either 0.2 and 0.75 mg Cd/L, 16 and 30 ug Cu/L or 0.5 ug Ag/L (Tables 4, 8 & 10), for which there were no significant ($p<0.05$) increases in tolerance, whereas pre-exposure to higher concentrations did elicit a significant increase in relative tolerance. This would suggest that pre-exposure to concentrations above a certain threshold "triggers" the protective mechanism, and in response to this mechanism, tolerance is enhanced. The threshold for the point of onset of enhanced relative tolerance is evidently within the pre-exposure concentration ranges selected for these three metals. Hence, this threshold is apparently between 0.75 and 2.0 mg Cd/L, between 30 and 76 ug Cu/L and between 0.5 and 1.3 ug Ag/L.

Other investigators have also reported evidence of a lower limit to tolerance modification. Dixon & Sprague (1981a) observed that pre-exposure of rainbow trout to 58 ug Cu/L did not significantly alter their tolerance to lethal levels of copper, although higher levels of 94 to 194 ug Cu/L significantly increased tolerance. Presumably, 58 to 94 ug Cu/L marks the threshold to

tolerance modification for rainbow trout in their study. They also proposed that this lower limit could potentially be used as a criterion for establishing a safe level for aquatic pollutants (Dixon, 1979; Dixon & Sprague, 1981a). They reasoned that the ratio of this threshold value to the LC50 of control fish may be a good estimate of the application factor for the pollutant. This would be a relatively fast and inexpensive method for estimating application factors to replace the present methods of measuring chronic toxicity which are often time-consuming and expensive. They derived a copper application factor of 0.11 for rainbow trout, which compares favourably with the value of 0.10 widely accepted in the literature (Dixon, 1979). This is also consistent with the threshold value obtained for copper in the present study, using zebrafish, which was estimated to be between 30 and 76 ug Cu/L. This is equivalent to an application factor of between 0.11 and 0.29. However, the experiments of Dixon & Sprague (1981a) also reveal that pre-exposure of rainbow trout to levels below the threshold resulted in sensitization to subsequent lethal levels (Dixon 1979). This suggests that at levels below the threshold, if not sublethally toxic in themselves may be capable of deleterious interactions with subsequent lethal levels, and may be considered hazardous in the aquatic environment on this basis alone.

The results of this study also demonstrate that an upper limit to tolerance modification may exist. This aspect is addressed first with respect to the results obtained with the highest pre-exposure concentration of silver and then with respect to the results obtained following pre-exposure to nickel.

Although zebrafish pre-exposed to 1.3 ug Ag/L were significantly more tolerant to lethal levels of silver compared to fish without prior exposure to silver, those fish pre-exposed to 2.2 ug Ag/L were apparently less tolerant to lethal levels of silver (Table 10), although this was dependent on the time of lethal exposure (see Section 4.1.2). This suggests that as the silver pre-exposure concentration increased, it reached a level which was beyond the adaptive capacity of the protective system, or at which the system was not invoked. Apparently, the upper limit for enhanced relative tolerance to lethal levels of silver is located between 1.3 and 2.2 ug/L, or 0.30 to 0.51 TU. This upper limit for silver is apparently lower than that of other metals which have been studied so far, when pre-exposure concentrations are compared on the basis of toxic units. For example, pre-exposure to copper, cadmium, zinc or aluminium at approximately 0.50 TU increases relative tolerance to subsequent lethal levels of the respective metal, while pre-exposure to silver at the same level results in sensitization. There are no specific reports in the literature of pre-exposure levels exceeding the upper limit of tolerance. However, Chapman (1985) in his review on metal tolerance, suggests (based on unpublished data) that it may lie near 0.8 - 0.9 TU.

However, since there was no incipient lethal level for silver in this experiment within 144 h of lethal exposure (Figure 4), the toxic units calculated for silver may in fact be an underestimation. This indicates that the lethal exposure phase of this experiment should have been extended for a longer period of

time.

The pre-exposure level of 2.2 ug Ag/L, which resulted in subsequent sensitization to lethal levels, is within the range presently considered to be "safe" for aquatic environments. The Environmental Protection Agency (1980a) sets a limit of silver at 4.1 ug Ag/L. This study emphasizes that even relatively low levels of silver may in fact be hazardous to fish.

Pre-exposure to 0.5 - 5.0 mg Ni/L resulted in apparent sensitization to subsequent lethal levels of nickel, although this was dependent on the time of lethal exposure (Section 4.1.2). Sensitization to nickel may be due, as in the case of silver sensitization, to pre-exposure to levels which are above the upper limit for tolerance modification. If this is so, then pre-exposure to levels of nickel which are lower than those used in this study may result in enhanced relative tolerance, rather than sensitization. The pre-exposure concentration range was equivalent to 0.02 - 0.24 TU, well within the toxic unit range that induces tolerance in other metals. But, as was seen with silver, there was no incipient lethal level for nickel within 144 hours (Figure 5). Thus, the 144 hour LC50 of 21 mg Ni/L may not be a good approximation of the incipient lethal level. The true incipient lethal level may be lower, which would make the pre-exposure concentrations higher toxic units than reported above. On the other hand, nickel sensitization may not be a result of pre-exposure to concentrations above the upper limit to tolerance modification, but may be a response unique to nickel. It is interesting to note that other investigators have found similar

findings with this metal, when working with mammals. Gabbioni et al. (1967) found that sublethal pre-exposure of rats to either cadmium or cobalt inhibited sensory ganglia lesion induced by cadmium, whereas pretreatment with either nickel or iron had no such protective effect. Yoshikawa (1970) demonstrated that pretreatment of mice with sublethal levels of cadmium or mercury produced enhanced tolerance against their own toxic action, but pretreatment with nickel or iron did not.

Nickel has been assessed as one of the less toxic of the heavy metals to fish. It has been shown to be moderately toxic to fish and other aquatic organisms when compared to many other metals (Pickering & Henderson, 1966; Pickering, 1974; EPA, 1980). However, this study shows that previous exposure to this metal causes significantly increased sensitivity in fish exposed to levels which are apparently below lethal levels and which are known to be found in the environment. Levels in Canadian waters have been reported as high as 3 - 6 mg Ni/L (Rehwoldt et al, 1973; Stokes et al, 1973). Results reported here show that pre-exposure to levels as low as 0.5 mg Ni/L nickel are sufficient to cause a significant decrease in relative tolerance after only 72 hours of lethal exposure to that metal (Table 12). This concentration is below the criteria proposed by the EPA (1980) as a safe level for nickel in aquatic environments. The EPA states that levels of nickel should not exceed 1.8 mg Ni/L, at any time, in water of equivalent hardness to that used in this study (100mg/L $CaCO_3$). Also, the guideline recommended by Environment Canada for nickel is a maximum of 0.25 mg/L in water of hardness $\geq$150 mg/L $CaCO_3$ (NRCC,

1981); this is relatively close to the levels used in this study.

Furthermore, the lack of an incipient lethal level by 144 hours suggests that nickel is more toxic than presently believed. Application factors for nickel are based on 96 or 144 hour LC50s on the assumption that an incipient lethal levels is reached by this time (Pickering, 1974; EPA, 1980). This study shows that this may not be so. Further studies are evidently needed to determine not only the incipient lethal level for this metal using longer periods of time, but also the effects of pre-exposure to lower levels than used here.

The actual upper and lower limits for pre-exposure concentrations that can induce tolerance may also vary with such factors as the type of metal, the length of pre-exposure and the species.

4.1.2 Effect of Duration of Lethal Exposure on Relative Tolerance to Lethal Levels

Relative tolerance was apparently dependent on the duration of the lethal exposure period. Pre-exposure to 2.0 mg Cd/L resulted in a significant increase in relative tolerance at 60, 96, 120 and 144 h of lethal exposure only (Table 4). At the other lethal exposure times at which tolerance was estimated for this test lot, there was no significant difference between the tolerance of the control and the pre-exposed fish. Likewise, pre-exposure to 1.3 ug Ag/L only resulted in significant increases in relative tolerance at 48, 60, 72, 84 and 96 h of lethal exposure. At 120 and 144 h, there was no significant difference in tolerance between

that of the control and of the pre-exposed fish (Table 10). It was not possible to determine if this effect also occurred following copper pre-exposure, as an LC_{50} could only be estimated at 144 h of lethal exposure for the highest pre-exposure concentration of 76 ug Cu/L. Nevertheless, the results obtained with cadmium and silver suggest that the time of lethal exposure could be an important factor in determining relative tolerance. Similarly, sensitization following either silver or nickel pre-exposure was also seen to be dependent on the lethal exposure time. Pre-exposure to 2.2 ug Ag/L resulted in significant sensitization at 84 and 96 h only (Table 10). For nickel, pre-exposure to either 0.5, 1.0 and 5.0 mg Ni/L resulted in significant sensitization at the times indicated in Table 12. In the environment, levels of a pollutant may frequently fluctuate through time. This could be due, for example, to episodic discharges of effluent. Consequently, the fact that relative tolerance to lethal levels may vary with the lethal exposure time could have ecological implications as far as acclimation is concerned. For example, one can speculate that pre-exposure to 2.0 mg Cd/L would only be an acclimatory advantage in situations in which fish are exposed to lethal levels of cadmium for relatively longer periods, after 84 h (Table 4).

4.2 Possible Mechanisms for Relative Tolerance Modifications.

The precise mechanisms for metal tolerance enhancement are not known. However, the ability of many organisms to synthesize a specific low molecular weight protein (approx. 10,000 daltons) in direct response to heavy metal exposure, is presently believed to

be a key factor in organismal metal detoxification (Kagi & Nordberg, 1978; Pascoe & Beattie, 1979). Isolation and characterization of this protein has revealed that it has a high cysteine content (Margoshes & Vallee, 1957). Due to the high amounts of cysteine, this protein has an abundance of -SH groups and so has been termed "metallothionein", although it is recognized that in many cases, metal-binding proteins which are biochemically distinct from metallothionein, but which are functionally similar, have been isolated (e.g. Roesijadi & Hall, 1981). Cysteine has a high binding affinity for heavy metals (Williams, 1981), and thus, metallothionein is thought to function in the detoxification of heavy metals by binding to the cations and thus diverting them from more essential and sensitive metabolic components (Kagi & Nordberg, 1978). Metallothionein is reported to be present in a number of distantly related organisms, including mammals (Leber & Miya, 1976), fish (Buckley et al., 1982; Thomas et al., 1983; Noel-Lambot et al., 1978), molluscs (Wiedow et al., 1982) and blue-green algae (McLean et al., 1972). It is believed to function in the normal metabolism of copper and zinc (Kagi & Nordberg, 1978). However, it is also induced in vivo following administration of cadmium, mercury, silver and nickel (Winge et al., 1975; Sunderman et al., 1983; Sabbioni & Marafante, 1975; Webb, 1972; Noel-Lambot et al., 1978).

Cherian & Nordberg (1983) proposed a model for metallothionein detoxification of heavy metals which is supported by the general findings on pre-exposure and enhanced tolerance. They proposed that the increased translation of

metallothionein/mRNA is inhibited in some way in unexposed organisms, perhaps by means of a repressor molecule. On pre-exposure to sufficient levels of metal, the inhibitor is somehow removed and so releases the metallothionein/mRNA for increased metallothionein synthesis which continues during subsequent exposure to the challenge metal. Thus, according to this model, within the pre-exposure range which results in enhanced tolerance, metallothionein would be induced in response to metal exposure, resulting in subsequent detoxification of lethal levels of metal.

At certain pre-exposure concentrations, metal accumulation within the organism may not occur sufficiently for induction of metallothionein (Roesijadi & Fellingham, 1987). This may account for the apparant lack of increased tolerance following pre-exposure to either 0.2, 0.75 mg Cd/L, 16, 30 ug Cu/L or 0.5 ug Ag/L, in the present study, resulting in an apparent lower limit to tolerance modification. However, sensitization may also result from pre-exposure to levels below the lower limit if some deleterious effect of pre-exposure was carried over to combine with the impact of lethal exposure. This may account for the responses reported by Dixon & Sprague (1981a). Above a certain pre-exposure concentration, the metal may exceed the binding capacity of the induced metallothionein and may become a significant factor in toxicity itself. This is consistent with the spillover hypothesis of Winge et al. (1973). They proposed that the binding of cations to metallothionein protects other proteins, and that an increase in the levels of metal beyond the binding capacity of the

metallothionein results in the "spilling over" of cations onto more sensitive proteins, with concurrent enhanced toxiciy. This may explain the sensitization observed in the present study following pre-exposure to 2.2 ug Ag/L, the highest pre-exposure concentration used for that metal, resulting in an apparent upper limit to tolerance modification, and following pre-exposure to nickel. In this case, increased toxicity could have been due to the additive effects of the pre-exposure plus lethal exposure, or simply a latent lethal effect of pre-exposure. However, as mentioned previously, the response obtained with nickel may be unique to that metal. Evidence to support this latter argument may be found in the functioning of nickel metallothionein, which does not appear to have the same inductive and binding capacities as other metals such as cadmium and copper. For example, studies with rats have shown that nickel induces hepatic and renal metallothionein, but to a far lesser extent than either cadmium, mercury or zinc (Eaton et al., 1980; Piotrowski et al., 1976; Oskarsson et al., 1979; Mathur et al., 1979; Webb, 1972). Nevertheless, this might still be expected to result in slight, although much reduced, protection. However, several investigators have clearly shown in rats that although nickel has some capacity for metalloprotein induction, it does not appear to have the capacity to bind to the protein (Maitani & Suzuki, 1983; Suzuki & Yoshikawa, 1976; Sabbioni & Marafante, 1975; Sunderman et al, 1983). Hence, it follows that nickel pre-exposure would not confer any protective capacity against lethal levels. The presence and functioning of nickel-induced metallothioneins have not yet been established for fish. Judging from the findings

reported in this study, this would be an interesting aspect to explore.

However, mechanisms of enhanced tolerance in fish cannot be fully explained by this model as it does not account for all the empirical observations reported in the literature. Pre-exposure to metals which apparently do not induce synthesis of metallothionein can also confer protection to subsequent lethal levels. This appears to be the case for arsenic (Piotrowski et al., 1976; Dixon & Sprague, 1981b). Furthermore, in rainbow trout, enhanced tolerance to arsenic has been linked to enhanced excretion of that metal (Oladimeji, 1982). This suggests that pre-exposure induced alterations in the kinetics of metal uptake and excretion may also play an important role in tolerance modifications.

4.3. Effects of Pre-exposure to a Metal on Relative Tolerance to Lethal Levels of a Different Metal - Cross-Tolerance

The results suggest that pre-exposure to certain levels of copper can confer enhanced tolerance to lethal levels of cadmium. Although pre-exposure to 20 or 30 ug Cu/L did not result in any significant increase in relative tolerance (Table 14), despite the trend depicted in Figure 11, pre-exposure to 52 ug Cu/L did result in a significant increase in tolerance to cadmium at 72 h of lethal exposure (Table 14). Similarly, pre-exposure to certain levels of copper resulted in apparent increased tolerance to lethal levels of nickel, at least during the initial hours of lethal exposure. Although pre-exposure to 16 ug Cu/L did not result in any significant increases in tolerance, pre-exposure to 40 and 70 ug

Cu/L resulted in a significant increase at 60 and 72 h respectively. At later lethal exposure times, there was no significant difference in tolerance for these two latter test lots, compared to the control (Table 16). There are apparently no other studies conducted on copper-cadmium and copper-nickel cross-tolerance in fishes. However, cross-tolerance has been demonstrated between other metals. For the northern creek chub and for the goldfish, the toxic effects of mercury were found to be mitigated by previous exposure to selenium (Kim et al., 1977; Heisinger et al., 1979). The tolerance of white suckers to cadmium was increased by previous exposure to not only cadmium, but also by previous exposure to mercury and zinc (Duncan & Klaverkamp, 1983). However, pre-exposure to selenium did not protect white suckers against lethal levels of cadmium (Duncan & Klaverkamp, 1983). For rainbow trout, copper pre-exposure resulted in a short-term enhancement of tolerance to zinc (Dixon & Sprague, 1981a).

One plausible mechanism for the cross-tolerance demonstrated between certain metals could involve metallothionein binding behaviour. Studies suggest that the binding of certain cations to metallothionein is also non-specific to some extent. For example, following the de novo synthesis of a cadmium metallothionein in rat liver by injected cadmium, several metals, including copper, zinc, mercury, silver and tin were also incorporated in vivo into the metallothionein (Sabbioni & Marafante, 1975). In fish, there is evidence that cadmium can be sequestered by a zinc metallothionein in rainbow trout, as the protein is being newly formed following induction by zinc pre-exposure (Thomas et al., 1985).

It is interesting to note that the apparent lack of sustained cross-tolerance observed in the copper-nickel experiment, following pre-exposure to either 40 or 70 ug Cu/L (Table 16), has also been noted elsewhere. White suckers, following pre-exposure to either 195 or 890 ug Zn/L had 12- & 24-h cadmium LC50s that were significantly increased compared to controls, whereas the 48-, 72 & 96-h LC50s were not significantly different from the control (Duncan & Klaverkamp, 1983). Similarly, rainbow trout pre-exposed to 194 ug Cu/L (0.59 ILL), had increased relative tolerance to zinc during the first 60 h of lethal exposure to that metal only. From 60 h on, pre-exposed fish suffered increased mortality compared to control and by 144 h, tolerance was significantly reduced compared to the control (Dixon, 1980). Interestingly, this is similar to the trend depicted in the present study for copper-nickel (Figure 12), which also suggests that enhanced tolerance is followed by sensitization; however, this sensitization was not demonstrated in the present study to be significant. Dixon (1980) speculated that this response was due to the difference in the relative concentrations of copper and zinc used for lethal exposure. The zinc LC50 for rainbow trout is approximately one order of magnitude greater than that of copper; he hypothesized that this difference may be reflected in the cytosolic concentrations of the two ions. Thus, he suggested that copper-induced metallothionein subsequently bound zinc, resulting in initial enhanced tolerance to that metal. As zinc exposure continued, however, the relatively greater levels of zinc in the cytosol was presumed to displace copper from the metallothionein. The more cytotoxic copper was assumed to be

subsequently released into the system resulting in increased mortality. A similar situation could be envisaged in the experiment on copper-nickel in the present study. The nickel LC50 value for zebrafish is approximately two orders of magnitude greater than that of copper. On the other hand, it is possible that either zinc or nickel would rapidly accumulate to levels which exceed the binding capacity of the metallothionein and are themselves toxic. This is consistent with the spillover hypothesis of Winge et al. (1973). However, the hypothesis of Dixon (1980) is not supported by the findings of Duncan & Klaverkamp (1983) described above. In that experiment, the magnitude of the 96-h LC50s of cadmium and zinc is similar (1.1 mg Cd/L & 2.2 mg Zn/L respectively). Hence, these metals would presumably be present in the cytosol at similar levels.

The final cross-tolerance experiment was conducted using nickel as the pre-exposure metal, to determine if the apparent sensitization obtained with this metal is also metal-specific. Copper was used as the lethal exposure metal. The findings suggest that pre-exposure to certain levels of nickel can confer sensitization to lethal levels of copper. Pre-exposure to 0.6 mg Ni/L did not result in any significant sensitization to copper relative to the control (Table 18). However, pre-exposure to 1.4 mg Ni/L resulted in a significant decrease in relative tolerance to copper from 48 to 96 h (Table 18). Although the shifts would appear to be slight (at 48 h the LC50 decreased from 296 to 271 ug Cu/L, which is only 8% lower) they are apparently significant because of the narrow confidence limits. Pre-exposure to 6.0 mg

Ni/L resulted in significant sensitization to copper at 48 h. From 60 h on, mortality was too high in this test lot to permit estimation of LC50s. This high mortality, relative to the other test lots, may be due to the effects of nickel pre-exposure. As a pre-exposure to 0.6 mg Ni/L did not result in any significant decreases in tolerance, the threshold for the sensitization response may be between 0.6 and 1.4 mg Ni/L. This latter concentration is presently considered to be a "safe" level in natural waters (Environmental Protection Agency, 1980). Hence, fish exposed to this level may be at greater risk of mortality, if they subsequently encounter potentially lethal levels of copper. However, it is debatable whether such slight shifts in tolerance of only 8% would constitute a risk in the natural environment. Nevertheless, prior exposure to nickel could outweigh any possible acclimatory advantage of prior copper exposure. Conditions where nickel and copper co-exist have also been shown to present an unexpectedly high risk due to synergism (Anderson et al., 1979). Obviously, nickel and copper combinations are of particular concern in the environment.

4.4 Environmental Implications of Tolerance Modifications

In the environment, increased tolerance to heavy metals brought about by previous sublethal exposure can be viewed as beneficial for aquatic organisms. Increased survival resulting from chronic pre-exposure can subsequently protect against episodic discharges of lethal levels of metals (Benson & Birge, 1985). It is unlikely that organisms will be exposed to a constant level of

any contaminant, but more likely that levels will fluctuate. Certain conditions such as spring melts, effluents encountered during migrations or accidental spills could expose organisms to extremely high, possibly lethal levels. Acclimation could allow organisms to tolerate these higher levels better than non-acclimated organisms.

However, there are other more profound changes in an ecosystem which can result from increased tolerance to toxicant, and some of the more important are genetic changes. A great deal of variation exists in the response of organisms to pollutants (Sprague, 1970), and natural selection should favour those genetic combinations conferring tolerance (Rahel, 1981). Many of the species capable of tolerating toxicants are ecological opportunists (Luoma, 1977). Such species are characterized by a greater variety of genotypes available for colonizing broader ecological niches, than species that occupy more specialized niches. Therefore, when a toxicant is introduced into an ecosystem, the probability of an opportunist species developing a tolerant population and surviving, is higher than a more specialized species (Luoma, 1977). The resultant ecosystems will become more simplified because they lack toxicant sensitive species. Organisms at a higher trophic level appear to be especially susceptible, with top carnivores in the community being most commonly absent (Luoma, 1977).

Furthermore, the enhanced biomagnification of toxicants in tolerant organisms in heavily contaminated sites can be a potential hazard to consumers in higher trophic levels, including man (Duncan & Klaverkamp, 1983).

Also, it may be unclear whether the physiological changes during sublethal exposure which lead to increased tolerance are indeed compensatory, or are deleterious effects of the toxicant. For example, one side-effect of pre-exposure to sublethal levels of copper is a transitory decrease in growth rate (Dixon, 1980). This suggests that a metabolic cost may be incurred by the demands of a tolerance mechanism. Tolerance to lethal levels may therefore be accompanied by chronic toxicity. Such changes could also ultimately reduce the "fitness" of a population, that is, its probable genetic contribution to future generations. This could take place if, for example, enhanced tolerance were accompanied by a reduction in reproductive capacity (Roesijadi & Fellingham, 1987).

Increased sensitivity to a toxicant, resulting from previous exposure to low levels, as demonstrated in this study for nickel and silver, must be viewed as detrimental in every sense, resulting in the possible elimination of entire species or communities. In view of their environmental impact, both nickel and silver should now be considered as far greater risks to the environment, due to possible sequential interactions, than is presently believed. This especially applies in those environments where fish are likely to encounter repeated exposures to heavy metals, such as would occur near smelting and metallurgical plants.

REFERENCES

Anderson, P.D., H. Horovitch, N. Weinstein. 1979. Pollutant mixtures in the aquatic environment. In Proc. Fifth Annual Toxicity Workshop. Fish. Mar. Serv. Tech. Rep. No. 862

Antonovics, J. 1975. Metal tolerance in plants: perfecting an evolutionary paradigm. In International Conference on Heavy Metals in the Environment. 2(1):169-186.

Benson, W.H. and W.J. Birge, 1985. Heavy metal tolerance and metallothionein induction in fathead minnows: results from field and laboratory investigations. Environ. Toxicol. Chem. 4: 209-217.

Bradley, R.W., C. DuQuesnay and J.B. Sprague. 1985. Acclimation of rainbow trout, Salmo gaidneri Richardson, to zinc: kinetics and mechanism of enhanced tolerance induction. J. Fish Biol. 22: 367-379.

Brown, V.M. 1968. The calculation of the acute toxicity of mixtures of poisons to rainbow trout. Water Research. 2: 723-733.

Brown, V.M. 1976. Aspects of heavy metals toxicity in fresh waters. In Toxicity to biota of metal forms in natural waters. pp 59-75. Edited by R.W. Andrew, P.V. Hodson and D.E. Konasewich. Great Lakes International Joint Commission's Research Advisory Board, Windsor, Ontario.

Bryan, G.W. 1974. Adaptation of an estuarine polychaete to

sediments containing high concentrations of heavy metals. In Pollution and Physiology of Marine Organisms. pp 123-135. Edited by F.J. Vernberg and W.B. Vernberg. Academic Press, New York, N.Y. 492 pp.

Buckley, J.T., M. Roch, J.A. McCarter, C.A. Rendell and A.T. Matheson. 1982. Chronic exposure of coho salmon to sublethal concentrations of copper - I. Effect on growth, on accumulation and distribution of copper, and on copper tolerance. Comp. Biochem. Physiol. 72C (1): 15-19.

Chapman, G.A. 1985. Acclimation as a factor influencing metal criteria. In Aquatic Toxicology and Hazard Assessment: Eighth Symposium. ASTM STP 891. pp 119-136. Edited by R.C. Bahner and D.J. Hansen. American Society for Testing and Materials, Philadelphia.

Cherian, M.G. and M. Nordberg. 1983. Cellular adaptation in metal toxicology and metallothionein. Toxicology. 28: 1-15.

Davies, P.H., J.P. Goettl, Jr. and J.R. Sinley. 1978. Toxicity of silver to rainbow trout (Salmo gairdneri). Water Research. 12: 113-117.

Dixon, D.G. 1979. Acclimation to toxicants by rainbow trout and its potential use in predicting safe levels. In Proc. Fifth Annual Aquatic Toxicity Workshop. pp 162-166. Fish. Environ. Canada Fish. Marine Services. Tech. Rep. No. 862.

Dixon, D.G. 1980. Studies of tolerance induction in rainbow trout by pre-exposure to copper, arsenic or cyanide. Ph.D. Thesis. University of Guelph. 151pp.

Dixon, D.G. and J.B. Sprague. 1981a. Acclimation to copper by rainbow trout (Salmo gairdneri) - a modifying factor in toxicity. Can. J. Fish. Aquat. Sci. 38: 880-888.

Dixon, D.G. and J.B. Sprague. 1981b. Acclimation-induced changes in toxicity of arsenic and cyanide to rainbow trout, Salmo gairdneri Richardson. J. Fish Biol. 18: 579-589.

Duncan, D.A. and J.F. Klaverkamp. 1983. Tolerance and resistance to cadmium in white suckers (Catostomus commersoni) previously exposed to cadmium, mercury, zinc or selenium. Can. J. Fish. Aquat. Sci. 40: 128-138.

Eaton, D.L., N.H. Stacey, K-L. Wong and C.D. Klaassen. 1980. Dose-response effects of various metal ions on rat liver metallothionein, glutathione, heme oxygenase and cytochrome P-450. Toxicol. Appl. Pharmacol. 55: 393-402.

EIFAC. 1977. Report on the effect of zinc and copper pollution on the salmonid fisheries in a river and lake system in central Norway. FAO European Inland Fisheries Advisory Commission. Tech. Pap. 29: 1-34.

EPA. 1980a. Ambient water quality criteria for silver. Environmental Protection Agency. National Technical Information Service. PB 81-117822.

EPA. 1980b. Ambient water quality criteria for nickel. EPA-440/5-80-060. U.S. Environmental Protection Agency, Washington, D.C.

Ernst, W.H.O. 1975. Physiology of heavy metal resistance in plants. International Conference on Heavy Metals in the Environment. 2(1): 121-135.

Finney, D.J. 1971. Probit Analysis. 4th Ed. Cambridge University Press. 348 pp.

Fogels, A. and J.B. Sprague. 1977. Comparative short-term tolerance of zebrafish, flagfish, and rainbow trout to five poisons including potential reference toxicants. Water Research. 11: 811-817.

Fry, F.E.J. 1971. The effect of environmental factors on the physiology of fish. In Fish Physiology. Vol. 6. pp 1-98. Edited by W.S. Hoar and D.J. Randall. Academic Press, N.Y.

Gabbioni, G., D. Baic and C. Deziel. 1967. Studies on tolerance and ionic antagonism for cadmium and mercury. Can. J. Physiol. Pharmac. 45: 443-450.

Grenier, F. 1960. A constant flow apparatus for toxicity experiments on fish. J. Wat. Pollut. Control Fed. 32: 117-119

Heisinger, J.F., C.D. Hansen and J.H. Kim. 1979. Effect of selenium dioxide on the accumulatory and acute toxicity of mercuric chloride in goldfish. Arch. Environ. Contam. Tox. 8: 279-283.

Hewitt, L.A. 1980. Dose and time related response patterns

in test populations of Brachydanio rerio exposed to copper, cadmium and mercury in pure solutions and in binary mixtures. M.Sc. Thesis. Concordia University, Montreal, 122 pp.

Kagi, J.H.R. and M. Nordberg. 1978. Metallothionein. Birkhauser Verlag Basel 378 pp.

Kim, J.H., E. Birks and J.F. Heisinger. 1977. Protective action of selenium against mercury in northern creek chubbs. Bull. Environ. Contam. Toxicol. 17(2): 132-136.

King, W. 1937. Mortality of hatchery trout. Great Smoky Mountains National Park. Prog. Fish-Cult. 29: 132-136.

Leber, A.P. and T.S. Miya. 1976. A mechanism for cadmium- and zinc-induced tolerance to cadmium toxicity: involvement of metallothionein. Toxicol. Appl. Pharmac. 37: 403-414.

Lima, A.R., C. Curtis, D.E. Hammermeister, D.J. Call and T.A. Felhaber. 1982. Acute toxicity of silver to selected fish and invertebrates. Bull. Environ. Contam. Toxicol. 29: 184-189.

Litchfield, J.T. Jr. and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99-113.

Litchfield, J.T. Jr. and F. Wilcoxon. 1953. The reliability of graphic estimates of relative potency from dose-percent effect curves. J. Pharmac. exp. Ther. 108: 18-25.

Luoma, S.N. 1977. Detection of trace contaminant effects in aquatic ecosystems. J. Fish. Res. Board Can. 34: 436-439.

Maclean, F.I., O.J. Lucis, Z.A. Shaikh and E.R. Janz. 1972. The uptake and subcellular distribution of cadmium and zinc in microorganisms. Fed. Proc. Am. Soc. Exp. Bio. 31: 699-702.

Maitani, T. and K.T. Suzuki. 1983. Dose-dependent induction of metallothionein in kidneys of mice injected with indium and nickel ions. Chem. Pharm. Bull. 31(3): 979-984.

Margoshes, M. and B.L. Vallee. 1957. A cadmium protein from equine kidney cortex. J. Am. Chem. Soc. 79: 4813-4815

Mathur, A.K. and S.K. Tandon. 1979. Some biochemical alterations in early nickel toxicity. Chemosphere 11/12: 893-901.

McCarter, J.A. and M. Roch. 1983. Hepatic metallothionein and resistance to copper in juvenile coho salmon. Comp. Biochem. Physiol. 74C: 133-137.

McIntosh, A.W. 1975. Fate of copper in ponds. Pestic. Monit. J. 8:225-231.

McLeese, D.W. 1956. Effects of temperature, salinity and oxygen on the survival of the American Lobster. J. Fish. Res. Board Can. 13: 247-272.

Noel-Lambot, F., C.H. Gerday and A. Disteche. 1978. Distribution of cadmium, zinc and copper in the liver

and gills of the eel Anguilla anguilla with special reference to metallothioneins. Comp. Biochem. Physio. 61C: 177-187.

NRCC. 1981. National Research Council Canada. Effects of nickel in the Canadian environment. Associate Committee on Scientific Criteria for Environmental Quality. Publication No. NRCC 18568.

Nriagu, J.O. (Editor). 1979. Copper in the Environment. Part 2: Health Effects. Chap. 11: Toxicity of Copper to Aquatic Biota. J. Wiley & Sons. New York. 489 pp.

Nriagu, J.O. (Editor). 1980. Nickel in the Environment. J. Wiley & Sons. New York. 833 pp.

Odiai, E. 1977. Bioinorganic Chemistry: an Introduction. Allyn and Bacon Inc. Toronto, Ontario.

Oladimeji, A.A., S.U. Qadri and A.S.W. deFreitas. 1982. Effect of acclimation of rainbow trout (Salmo gairdneri) to arsenic on retention of a subsequent dose of arsenic. Ecotox. Environ. Safety. 6: 196-203.

Orr, P.L., R.W. Bradley, J.B. Sprague and N.J. Hutchinson. 1986. Acclimation-induced change in toxicity of aluminium to rainbow trout (Salmo gairdneri). Can. J. Fish. Aquat. Sci. 43: 243-246.

Oskarsson, A. and H. Tjalve. 1979. Binding of ^{63}Ni by cellular constituents in some tissues of mice after the administration of $^{63}NiCl_2$ and $^{63}Ni(CO)_4$. Acta. Pharmacol. et Toxicol. 45: 306-314.

Pascoe, D. and J.H. Beattie. 1979. Resistance to cadmium

by pre-treated rainbow trout alevins. J. Fish Biol. 14: 303-308.

Paul, R.M. 1952. Water pollution: a factor modifying fish populations in Pacific Coast streams. Scientific Monthly 74: 14-17.

Pickering, Q.H. 1974. Chronic toxicity of nickel to the fathead minnow. Wat. Pollut. Cont. Fed. 46: 760-765.

Pickering, Q.H. and C. Henderson. 1966. The acute toxicity of some heavy metals to different species of warmwater fishes. Air Water Pollut. Int. J. 10: 453-463.

Piotrowski, J.K. and J.A. Szymanska. 1976. Influence of certain metals on the level of metallothionein-like proteins in the liver and kidneys of rats. J. Toxicol. Environ. Health. 1: 991-1002

Rahel, F.J. 1981. Selection for zinc tolerance in fish: results from laboratory and wild populations. Trans. Amer. Fish. Soc. 110: 19-28.

Rehwoldt, R., G. Bida and B. Nerrie. 1971. Acute toxicity of copper, nickel and zinc ions to some Hudson River fish species. Bull. Environ. Contam. Toxicol. 6: 445-448.

Rehwoldt, R. 1973. Bull. Environ. Contam. Toxicol. 50: 291-294.

Roch, M., J.A. McCarter, A.T. Matheson, M.J.R. Clark and R.W. Olafson. 1982. Hepatic metallothionein in rainbow trout (Salmo gairdneri) as an indicator of metal pollution in the Campbell River system. Can. J.

Fish. Aquat. Sci. 39: 1596-1601.

Roesijadi, G. and J. Fellingham. 1987. Influence of copper, cadmium and zinc pre-exposure on mercury toxicity in the mussel. Can. J. Fish. Aquat. Sci. 44: 680-684.

Roesijadi, G. and R.E. Hall. 1981. Characterization of a mercury-binding protein from the gills of marine mussels exposed to mercury. Comp. Biochem. Physiol. 70C: 59-64.

Sabbioni, E. and E. Marafante. 1975. Heavy metals in rat liver cadmium binding protein. Environ. Physiol. Biochem. 5: 132-141.

Schofield, Jr. C.L. 1965. Water quality in relation to survival of brook trout, Salvelinus fontinalis (Mitchell). Trans. Amer. Fish. Soc. 94: 227-235.

Schroeder, H.A. 1965. The biological trace elements. J. Chron. Dis. 18: 217-228.

Shepard, M.P. 1955. Resistance and tolerance of young speckled trout (Salvelinus fontinalis) to oxygen lack, with special reference to low oxygen acclimation. J. Fish. Res. Board Can. 12: 387-446.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish I. Bioassay methods for acute toxicity. Water Research. 3: 793-821.

Sprague, J.B. 1970. Measurement of pollutant toxicity to fish II. Utilizing and applying bioassay results. Water Research. 4: 3-32.

Stokes, P.M., T.C. Hutchinson and K. Krauter. 1973. Heavy metal tolerance in algae isolated from polluted lakes near the Sudbury, Ontario smelters. Water Pollut. Res. Can. 8:178-187.

Sunderman, Jr. F.W. and C.B. Fraser. 1983. Effects of nickel chloride and diethyldithiocarbamate on metallothionein in rat liver and kidney. Ann. Clin. Lab. Sci. 13: 489-495.

Suzuki, Y. and H. Yoshikawa. 1976. Induction of hepatic zinc-binding proteins of rats by various metals. Ind. Health. 14: 25-31.

Thomas, D.G., A. Cryer, J.F. del G. Solbe and J. Kay. 1983. A comparison of the accumulation and protein binding of environmental cadmium in the gills, kidneys and liver of rainbow trout (Salmo gairdneri). Comp. Biochem. Physiol. 76C: 241-246.

Thomas, D.G., M.W. Brown, D. Shurben, J.F. del G. Solbe, A. Cryer and J. Kay. 1985. A comparison of the sequestration of cadmium and zinc in the tissues of rainbow trout (Salmo gairdneri) following exposure to the metals singly or in combination. Comp. Biochem. Physiol. 82C: 55-62.

Van Loon J.C. and R.J. Beamish. 1977. Heavy-metal contamination by atmospheric fallout of several Flin Flon area lakes and the relation to fish populations. J. Fish. Res. Board Can. 34: 899-906.

Webb, M. 1972. Binding of cadmium ions to rat liver and

kidney. Biochem. Pharmacol. 21: 2751-2765.

Wiedow, M.A., T.J. Kneip and S.J. Garte. 1982. Cadmium-binding proteins from blue crabs (Callinectes sapidus) environmentally exposed to cadmium. Environ. Research. 28: 164-170.

Williams, R.J.P. 1981. Physico-chemical aspects of inorganic element transfer through membranes. Phil. Trans. R. Soc. Lond. Ser. B. 294: 57-74.

Winge, D., J. Krasno and A.V. Colucci. 1973. Cadmium accumulation in rat liver: correlation between bound metal and pathology. In Trace Element Metabolism in Animals. Vol. 2. pp 500-501. Edited by W.G. Hoekstra, J.W. Suttie, H.E. Ganther and W. Mertz. University Park Press, Baltimore.

Winge, D.R., R. Premakumar and K.V. Rajagopalan. 1975. Metal-induced formation of metallothionein in rat liver. Arch. Biochem. Biophys. 170: 242-252.

Yoshikawa, H. 1970. Preventative effect of pretreatment with low doses of metals on the acute toxicity of metals in mice. Ind. Health. 8: 184-191.

APPENDIX

Dosage - Mortality Data for each experiment at selected Lethal Exposure times. Parentheses enclose the Standard Deviation (N=42) of each concentration. Asterisks denote those concentrations not used in the estimation of the LC50.

Cadmium - Cadmium

48 Hours

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	3.3 (0.3)	15	1	6.7
	4.7 (0.3)	15	4	26.7
	6.8 (0.4)	15	11	73.3
	10.1 (0.6)	13	12	92.3
	11.3 (0.6)	14	12	85.7

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.2 (0.0)	3.3	16	2	12.5
	4.7	14	2	14.3
	6.8	13	7	53.8
	10.1	13	10	76.9
	11.3	13	13	100

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.75 (0.0)	3.3	15	1	6.7
	4.7	15	4	26.7
	6.8	14	3	21.4
	10.1	15	12	80
	11.3	13	10	76.9

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
2.0 (0.1)	3.3	12	1	8.3
	4.7	14	3	21.4
	6.8	15	7	46.7
	10.1	14	9	64.3
	11.3	15	12	80

Cadmium - Cadmium

60 Hours

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	3.3	15	1	6.7
	4.7	15	7	46.7
	6.8	15	13	86.7
	10.1	13	13	100
	11.3	14	14	100

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.2	3.3	16	2	12.5
	4.7	14	7	50
	6.8	13	10	76.9
	10.1	13	11	84.6
	11.3	13	13	100

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.75	3.3	15	3	20
	4.7	15	4	26.7
	6.8	14	9	64.3
	10.1	15	12	80
	11.3	13	11	84.6

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
2.0	3.3	12	1	8.3
	4.7	14	3	21.4
	6.8	15	9	60
	10.1	14	10	71.4
	11.3	15	13	86.7

Cadmium - Cadmium

72 Hours

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	3.3	15	1	6.7
	4.7	15	8	53.3
	6.8	15	13	86.7
	10.1	13	13	100
	11.3	14	14	100

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.2	3.3	16	2	12.5
	4.7	14	11	78.6
	6.8	13	12	92.3
	10.1	13	12	92.3
	11.3	13	13	100

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.75	3.3	15	3	20
	4.7	15	8	53.3
	6.8	14	11	78.6
	10.1	15	14	93.3
	11.3	13	12	92.3

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
2.0	3.3	12	1	8.3
	4.7	14	4	28.6
	6.8	15	10	66.7
	10.1	14	10	71.4
	11.3	15	13	86.7

Cadmium - Cadmium

84 Hours

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	3.3	15	4	26.7
	4.7	15	10	66.7
	6.8	15	14	93.3
	10.1	13	13	100
	11.3	14	14	100

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.2	3.3	16	3	18.8
	4.7	14	12	85.7
	6.8	13	12	92.3
	10.1	13	13	100
	11.3	13	13	100

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.75	3.3	15	5	33.3
	4.7	15	8	53.3
	6.8	14	11	78.6
	10.1	15	14	93.3
	11.3	13	13	100

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
2.0	3.3	12	2	16.7
	4.7	14	5	35.7
	6.8	15	12	80
	10.1	14	12	85.7
	11.3	15	14	93.3

Cadmium - Cadmium

96 Hours

Pre-exp Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	3.3	15	5	33.3
	4.7	15	11	73.3
	6.8	15	15	100
	10.1	13	13	100
	11.3*	14	14	100

Pre-exp. Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.2	3.3	16	4	25
	4.7	14	12	85.7
	6.8	13	13	100
	10.1	13	13	100
	11.3*	13	13	100

Pre-exp. Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.75	3.3	15	5	33.3
	4.7	15	8	53.3
	6.8	14	12	85.7
	10.1	15	14	93.3
	11.3	13	13	100

Pre-exp. Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
2.0	3.3	12	2	16.7
	4.7	14	6	42.9
	6.8	15	12	80
	10.1	14	13	92.9
	11.3	15	15	100

Cadmium - Cadmium

120 Hours

Pre-exp. Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	3.3	15	8	53.3
	4.7	15	13	86.7
	6.8	15	15	100
	10.1	13	13	100
	11.3*	14	14	100

Pre-exp. Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.2	3.3	16	6	37.5
	4.7	14	12	85.7
	6.8	13	13	100
	10.1	13	13	100
	11.3*	13	13	100

Pre-exp. Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.75	3.3	15	6	40
	4.7	15	9	60
	6.8	14	12	85.7
	10.1	15	15	100
	11.3	13	13	100

Pre-exp. Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
2.0	3.3	12	2	16.7
	4.7	14	6	42.9
	6.8	15	15	100
	10.1	14	13	92.9
	11.3	15	15	100

Cadmium - Cadmium

144 Hours

Pre-exp. Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	3.3	15	8	53.3
	4.7	15	13	86.7
	6.8	15	15	100
	10.1	13	13	100
	11.3*	14	14	100

Pre-exp. Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.2	3.3	16	6	37.5
	4.7	14	12	85.7
	6.8	13	13	100
	10.1	13	13	100
	11.3*	13	13	100

Pre-exp. Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.75	3.3	15	6	40
	4.7	15	9	60
	6.8	14	12	85.7
	10.1	15	15	100
	11.3	13	13	100

Pre-exp. Conc. (mg Cd/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
2.0	3.3	12	2	16.7
	4.7	14	6	42.9
	6.8	15	15	100
	10.1	14	13	92.9
	11.3	15	15	100

Copper - Copper

60 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	239 (14)	15	0	0
	245 (15)	15	1	6.7
	253 (15)	15	1	6.7
	258 (16)	14	2	14.3
	271 (16)	15	5	33.3

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
16 (1)	239	15	0	0
	245	13	1	7.7
	253	14	0	0
	258	16	3	18.8
	271	12	4	33.3

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
30 (2)	239	15	0	0
	245	16	0	0
	253	16	0	0
	258	14	0	0
	271	15	3	20

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
76 (5)	239	15	0	0
	245	15	0	0
	253	15	0	0
	258	14	0	0
	271	10	0	0

Copper - Copper

72 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	239	15	0	0
	245	15	1	6.7
	253	15	1	6.7
	258	14	2	14.3
	271	15	8	53.3

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
16	239	15	0	0
	245	13	1	7.7
	253	14	0	0
	258	16	3	18.8
	271	12	5	41.6

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
30	239	15	0	0
	245	16	0	0
	253	16	0	0
	258	14	1	7.1
	271	15	4	26.7

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
76	239	15	0	0
	245	15	0	0
	253	15	0	0
	258	14	0	0
	271	10	0	0

Copper - Copper

84 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	239	15	0	0
	245	15	2	13.3
	253	15	2	13.3
	258	14	5	35.7
	271	15	8	53.3

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
16	239	15	0	0
	245	13	1	7.7
	253	14	2	14.3
	258	16	5	31.3
	271	12	5	41.7

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
30	239	15	0	0
	245	16	0	0
	253	16	2	12.5
	258	14	2	14.3
	271	15	6	40

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
76	239	15	0	0
	245	15	0	0
	253	15	0	0
	258	14	0	0
	271	10	1	10

Copper - Copper

96 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	239	15	1	6.7
	245	15	2	13.3
	253	15	2	13.3
	258	14	5	35.7
	271	15	10	66.7

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
16	239	15	0	0
	245	13	1	7.7
	253	14	3	21.4
	258	16	5	31.3
	271	12	6	50

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
30	239	15	0	0
	245	16	1	6.3
	253	16	2	12.5
	258	14	2	14.3
	271	15	6	40

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
76	239	15	0	0
	245	15	0	0
	253	15	0	0
	258	14	0	0
	271	10	2	20

Copper - Copper

120 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	239	15	3	20
	245	15	3	20
	253	15	4	26.7
	258	14	6	42.9
	271	15	10	66.7

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
16	239	15	1	6.7
	245	13	3	23.1
	253	14	3	21.4
	258	16	6	37.5
	271	12	7	58.3

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
30	239	15	1	6.7
	245	16	2	12.5
	253	16	3	18.8
	258	14	3	21.4
	271	15	8	53.3

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
76	239	15	0	0
	245	15	1	6.7
	253	15	0	0
	258	14	0	0
	271	10	2	20

Copper - Copper

144 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	239	15	3	20
	245	15	3	20
	253	15	4	26.7
	258	14	7	50
	271	15	11	73.3

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
16	239	15	1	6.7
	245	13	3	23.1
	253	14	4	28.6
	258	16	6	37.5
	271	12	8	66.7

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
30	239	15	1	6.7
	245	16	3	18.8
	253	16	3	18.8
	258	14	3	21.4
	271	15	8	53.3

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
76	239	15	0	0
	245	15	1	6.7
	253	15	1	6.7
	258	14	1	7.1
	271	10	2	20

Silver - Silver

48 Hours

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	2.7 (0.0)	16	0	0*
	3.3 (0.1)	16	0	0
	4.8 (0.1)	16	0	0
	7.2 (0.3)	15	2	13.3
	10.0 (0.5)	17	15	88.2

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.5 (0.0)	2.7*	16	0	0
	3.3	16	0	0
	4.8	15	0	0
	7.2	13	1	7.7
	10.0	15	10	66.7

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.3 (0.0)	2.7*	16	0	0
	3.3	14	0	0
	4.8	17	0	0
	7.2	13	3	23.1
	10.0	15	3	20

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
2.2 (0.0)	2.7*	16	0	0
	3.3	15	0	0
	4.8	14	0	0
	7.2	15	6	40
	10.0	16	14	87.5

Silver - Silver

60 Hours

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	2.7*	16	0	0
	3.3	16	0	0
	4.8	16	0	0
	7.2	15	6	40
	10.0	17	17	100

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.5	2.7	16	0	0
	3.3	16	0	0
	4.8	15	1	6.7
	7.2	13	3	23.1
	10.0	15	10	66.7

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.3	2.7*	16	0	0
	3.3	14	0	0
	4.8	17	0	0
	7.2	13	4	30.8
	10.0	15	3	20

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
2.2	2.7	16	0	0
	3.3	15	0	0
	4.8	14	4	28.6
	7.2	15	8	53.3
	10.0	16	16	100

Silver - Silver

72 Hours

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	2.7*	16	0	0
	3.3	16	0	0
	4.8	16	0	0
	7.2	15	9	60
	10.0	17	17	100

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.5	2.7	16	0	0
	3.3	16	0	0
	4.8	15	2	13.3
	7.2	13	4	30.8
	10.0	15	11	73.3

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.3	2.7	16	0	0
	3.3	14	0	0
	4.8	17	1	5.9
	7.2	13	7	53.8
	10.0	15	4	26.7

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
2.2	2.7	16	0	0
	3.3	15	0	0
	4.8	14	6	42.9
	7.2	15	10	66.7
	10.0	16	16	100

Silver - Silver

84 Hours

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	2.7	16	0	0
	3.3	16	0	0
	4.8	16	1	6.3
	7.2	15	10	66.7
	10.0	17	17	100

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.5	2.7	16	0	0
	3.3	16	0	0
	4.8	15	2	13.3
	7.2	13	6	46.2
	10.0	15	12	80

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.3	2.7	16	0	0
	3.3	14	0	0
	4.8	17	1	5.9
	7.2	13	7	53.8
	10.0	15	5	33.3

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
2.2	2.7	16	0	0
	3.3	15	0	0
	4.8	14	8	57.1
	7.2	15	13	86.7
	10.0	16	16	100

Silver - Silver

96 Hours

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	2.7	16	0	0
	3.3	16	0	0
	4.8	16	4	25
	7.2	15	12	80
	10.0	17	17	100

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.5	2.7	16	0	0
	3.3	16	0	0
	4.8	15	3	20
	7.2	13	7	54
	10.0	15	14	93

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.3	2.7	16	0	0
	3.3	14	0	0
	4.8	17	3	18
	7.2	13	10	77
	10.0	15	9	60

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
2.2	2.7	16	0	0
	3.3	15	0	0
	4.8	14	10	71
	7.2	15	13	87
	10.0	16	16	100

Silver - Silver

120 Hours

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	2.7	16	0	0
	3.3	16	2	12.5
	4.8	16	11	68.8
	7.2	15	12	80
	10.0	17	17	100

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.5	2.7	16	0	0
	3.3	16	0	0
	4.8	15	7	46.7
	7.2	13	10	76.9
	10.0	15	15	100

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.3	2.7	16	0	0
	3.3	14	1	7.1
	4.8	17	5	29.4
	7.2	13	12	92.3
	10.0	15	12	80

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
2.2	2.7	16	0	0
	3.3	15	1	6.7
	4.8	14	12	85.7
	7.2	15	14	93.3
	10.0	16	16	100

Silver - Silver

144 Hours

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	2.7	16	0	0
—	3.3	16	3	19
	4.8	16	11	69
	7.2	15	14	93
	10.0	17	17	100

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.5	2.7	16	0	0
	3.3	16	0	0
	4.8	15	10	67
	7.2	13	12	92
	10.0	15	15	100

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.3	2.7	16	0	0
	3.3	14	2	14
	4.8	17	8	47
	7.2	13	13	100
	10.0	15	15	100

Pre-exp. Conc. (ug Ag/L)	Lethal Conc. (ug Ag/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
2.2	2.7	16	0	0
	3.3	15	1	7
	4.8	14	12	86
	7.2	15	15	100
	10.0	16	16	100

Nickel - Nickel

48 Hours

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	24.4 (1.5)	21	0	0
	28.2 (1.7)	19	0	0
	41.4 (2.5)	21	1	4.8
	46.3 (3.2)	19	0	0
	51.4 (3.1)	19	8	42.1

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.5 (0.0)	24.4	22	2	9.1
	28.2	22	4	18.2
	41.4	19	3	15.8
	46.3	23	10	43.5
	51.4	21	5	23.8

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.0 (0.1)	24.4	19	2	10.5
	28.2	21	3	14.3
	41.4	15	4	26.7
	46.3	20	9	45
	51.4	18	9	50

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
5.0 (0.4)	24.4	20	0	0
	28.2	15	1	6.7
	41.4	21	7	33.3
	46.3	21	8	38.1
	51.4	20	16	80

Nickel - Nickel

60 Hours

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	24.4	21	0	0
	28.2	19	2	10.5
	41.4	21	4	19
	46.3	19	5	26.3
	51.4	19	12	63.2

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.5	24.4	22	3	13.6
	28.2	22	6	27.3
	41.4	19	6	31.6
	46.3	23	17	73.9
	51.4	21	17	81

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.0	24.4	19	3	15.8
	28.2	21	7	33.3
	41.4	15	8	53.3
	46.3	20	14	70
	51.4	18	16	88.9

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
5.0	24.4	20	2	10
	28.2	15	3	20
	41.4	21	17	81
	46.3	21	13	61.9
	51.4	20	18	90

Nickel - Nickel

72 Hours

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	24.4	21	2	9.5
	28.2	19	3	15.8
	41.4	21	8	38.1
	46.3	19	8	42.1
	51.4	19	12	63.2

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.5	24.4	22	4	18.2
	28.2	22	8	36.4
	41.4	19	9	47.4
	46.3	23	21	91.3
	51.4	21	20	95.2

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.0	24.4	19	5	26.3
	28.2	21	7	33.3
	41.4	15	10	66.7
	46.3	20	17	85
	51.4	18	17	94.4

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
5.0	24.4	20	5	25
	28.2	15	7	46.7
	41.4	21	19	90.5
	46.3	21	17	81
	51.4	20	19	95

Nickel - Nickel

84 Hours

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	24.4	21	4	19
	28.2	19	7	36.8
	41.4	21	9	42.9
	46.3	19	9	47.4
	51.4	19	18	94.7

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.5	24.4	22	6	27.3
	28.2	22	9	40.9
	41.4	19	11	57.9
	46.3	23	23	100
	51.4	21	20	95.2

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.0	24.4	19	6	31.6
	28.2	21	8	38.1
	41.4	15	11	73.3
	46.3	20	19	95
	51.4	18	18	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
5.0	24.4	20	7	35
	28.2	15	8	53.3
	41.4	21	20	95.2
	46.3	21	20	95.2
	51.4	20	20	100

Nickel - Nickel

96 Hours

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	24.4	21	10	47.6
	28.2	19	10	52.6
	41.4	21	15	71.4
	46.3	19	14	73.7
	51.4	19	18	94.7

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.5	24.4	22	7	31.8
	28.2	22	13	59.1
	41.4	19	15	78.9
	46.3	23	23	100
	51.4	21	21	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (SD) (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.0	24.4	19	8	42.1
	28.2	21	12	57.1
	41.4	15	13	86.7
	46.3	20	19	95
	51.4	18	18	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (SD) (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
5.0	24.4	20	13	65
	28.2	15	11	73.3
	41.4	21	21	100
	46.3	21	21	100
	51.4*	20	20	100

Nickel - Nickel

96 Hours

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	24.4	21	12	57.1
	28.2	19	14	73.7
	41.4	21	21	100
	46.3	19	17	89.5
	51.4	19	19	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.5	24.2	22	14	63.6
	28.2	22	17	77.3
	41.4	19	19	100
	46.3	23	23	100
	51.4*	21	21	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.0	24.4	19	15	78.9
	28.2	21	15	71.4
	41.4	15	15	100
	46.3	20	20	100
	51.4	18	18	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
5.0	24.4	20	18	90
	28.2	15	13	86.7
	41.4	21	21	100
	46.3	21	21	100
	51.4	20	20	100

Nickel - Nickel

144 Hours

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	24.4	21	18	85.7
	28.2	19	16	84.2
	41.4	21	21	100
	46.3	19	18	94.7
	51.4	19	19	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.5	24.4	22	20	90.9
	28.2	22	20	90.9
	41.4	19	19	100
	46.3	23	23	100
	51.4	21	21	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.0	24.4	19	18	94.7
	28.2	21	18	85.7
	41.4	15	15	100
	46.3	20	20	100
	51.4	18	18	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
5.0	24.4	20	20	100
	28.2	15	15	100
	41.4	21	21	100
	46.3	21	21	100
	51.4	20	20	100

Copper - Cadmium

48 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	1.2 (0.1)	15	0	0
	2.2 (0.2)	14	0	0
	4.1 (0.4)	15	1	6.7
	6.0 (0.3)	15	9	60
	8.5 (0.5)	15	11	3.30

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
20 (1)	1.2	15	0	0
	2.2	15	0	0
	4.1	16	1	6.3
	6.0	15	5	33.3
	8.5	19	17	89.5

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
30 (3)	1.2	15	0	0
	2.2	16	0	0
	4.1	14	1	7.1
	6.0	16	5	31.1
	8.5	15	12	80

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
52 (4)	1.2	15	0	0
	2.2	15	0	0
	4.1	20	1	5
	6.0	16	3	18.8
	8.5	15	7	46.7

Copper - Cadmium

60 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	1.2	15	0	0
	2.2	14	1	7.1
	4.1	15	2	13.3
	6.0	15	14	93.3
	8.5	15	13	86.7

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
20	1.2	15	0	0
	2.2	15	0	0
	4.1	16	3	18.8
	6.0	15	7	46.7
	8.5	19	19	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
30	1.2	15	0	0
	2.2	16	0	0
	4.1	14	1	7.1
	6.0	16	8	50
	8.5	15	13	86.7

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
52	1.2	15	0	0
	2.2	15	0	0
	4.1	20	1	5
	6.0	16	8	50
	8.5	15	8	53.3

Copper - Cadmium

72 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	1.2	15	0	0
	2.2	14	1	7.1
	4.1	15	4	26.7
	6.0	15	14	93.3
	8.5	15	15	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
20	1.2	15	0	0
	2.2	15	0	0
	4.1	16	5	31.3
	6.0	15	10	66.7
	8.5	19	19	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
30	1.2	15	0	0
	2.2	16	0	0
	4.1	14	1	7.1
	6.0	16	8	50
	8.5	15	15	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
52	1.2	15	0	0
	2.2	15	0	0
	4.1	20	4	20
	6.0	16	9	56.3
	8.5	15	10	66.7

Copper - Cadmium

84 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	1.2	15	0	0
	2.2	14	1	7.1
	4.1	15	5	33.3
	6.0	15	14	93.3
	8.5	15	15	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
20	1.2	15	0	0
	2.2	15	1	6.7
	4.1	16	5	31.3
	6.0	15	12	80
	8.5	19	19	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
30	1.2	15	0	0
	2.2	16	1	6.3
	4.1	14	2	14.3
	6.0	16	9	56.3
	8.5	15	15	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
52	1.2	15	0	0
	2.2	15	1	6.7
	4.1	20	6	30
	6.0	16	13	81.3
	8.5	15	11	73.3

Copper - Cadmium

96 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	1.2	15	0	0
	2.2	14	1	7.1
	4.1	15	8	53.3
	6.0	15	14	93.3
	8.5	15	15	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
20	1.2	15	0	0
	2.2	15	1	6.7
	4.1	16	6	37.5
	6.0	15	12	80
	8.5	19	19	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
30	1.2	15	0	0
	2.2	16	1	6.7
	4.1	14	2	14.3
	6.0	16	10	62.3
	8.5	15	15	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
52	1.2	15	0	0
	2.2	15	2	13.3
	4.1	20	6	30
	6.0	16	13	81.3
	8.5	15	11	73.3

Copper - Cadmium

120 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	1.2	15	0	0
	2.2	14	2	14.3
	4.1	15	8	53.3
	6.0	15	14	93.3
	8.5	15	15	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
20	1.2	15	0	0
	2.2	15	1	6.7
	4.1	16	8	50
	6.0	15	12	80
	8.5	19	19	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
30	1.2	15	0	0
	2.2	16	1	6.3
	4.1	14	3	21.4
	6.0	16	11	68.8
	8.5	15	15	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
52	1.2	15	0	0
	2.2	15	2	13.3
	4.1	20	6	30
	6.0	16	13	81.3
	8.5	15	12	80

Copper - Cadmium

144 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	1.2	15	0	0
	2.2	14	2	14.3
	4.1	15	8	53.3
	6.0	15	14	93.3
	8.5	15	15	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
20	1.2	15	0	0
	2.2	15	1	6.7
	4.1	16	8	50
	6.0	15	12	80
	8.5	19	19	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
30	1.2	15	0	0
	2.2	16	1	6.3
	4.1	14	3	21.4
	6.0	16	11	68.8
	8.5	15	15	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Cd/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
52	1.2	15	0	0
	2.2	15	2	13.3
	4.1	20	6	30
	6.0	16	13	81.3
	8.5	15	12	80

Copper - Nickel

48 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	19.7 (1.2)	15	0	0
	29.8 (2.1)	12	0	0
	33.5 (2.4)	16	0	0
	42.3 (3.0)	16	2	12.5
	54.5 (5.1)	18	9	50

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
16 (1)	19.7	16	0	0
	29.8	17	0	0
	33.5	18	1	5.6
	42.3	18	1	5.6
	54.5	17	2	11.8

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
40 (3)	19.7	17	0	0
	29.8	18	0	0
	33.5	15	0	0
	42.3	18	0	0
	54.5	18	0	0

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
70 (4)	19.7	18	0	0
	29.8	18	0	0
	33.5	18	0	0
	42.3	18	0	0
	54.5	16	1	6.3

Copper - Nickel

60 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	19.7	15	0	0
	29.8	12	0	0
	33.5	16	2	12.5
	42.3	16	8	50
	54.5	18	12	66.7

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
16	19.7	16	0	0
	29.8	17	0	0
	33.5	18	2	11.1
	42.3	18	4	22.2
	54.5	17	10	58.9

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
40	19.7	17	0	0
	29.8	18	0	0
	33.5	15	1	6.7
	42.3	18	3	16.7
	54.5	18	4	22.2

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
70	19.7	18	0	0
	29.8	18	0	0
	33.5	18	0	0
	42.3	18	1	5.6
	54.5	16	4	25

Copper - Nickel

72 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	19.7	15	0	0
	29.8	12	2	16.7
	33.5	16	4	25
	42.3	16	9	56.3
	54.5	18	12	66.7

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
16	19.7	16	0	0
	29.8	17	0	0
	33.5	18	4	22.2
	42.3	18	9	50
	54.5	17	15	88.2

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
40	19.7	17	0	0
	29.8	18	1	5.6
	33.5	15	3	20
	42.3	18	6	33.3
	54.5	18	9	50

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
70	19.7*	18	0	0
	29.8	18	0	0
	33.5	18	0	0
	42.3	18	5	27.7
	54.5	16	6	37.5

Copper - Nickel

84 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	19.7	15	0	0
	29.8	12	4	33.3
	33.5	16	5	31.3
	42.3	16	11	68.8
	54.5	18	17	94.4

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
16	19.7	16	0	0
	29.8	17	2	11.8
	33.5	18	8	44.4
	42.3	18	14	77.8
	54.5	17	17	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
40	19.7	17	0	0
	29.8	18	2	11.1
	33.5	15	6	40
	42.3	18	11	61.1
	54,5	18	15	83.3

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
70	19.7	18	0	0
	29.8	18	1	5.6
	33.5	18	4	22.2
	42.3	18	12	66.7
	54.5	16	10	62.5

Copper - Nickel

96 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	19.7	15	0	0
	29.8	12	6	50
	33.5	16	7	43.8
	42.3	16	12	75
	54.5	18	17	94.4

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
16	19.7	16	0	0
	29.8	17	2	11.8
	33.5	18	8	44.4
	42.3	18	17	94.4
	54.5	17	17	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
40	19.7	17	1	5.9
	29.8	18	6	33.3
	33.5	15	9	60
	42.3	18	14	77.8
	54.5	18	17	94.4

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
70	19.7	18	0	0
	29.8	18	6	33.3
	33.5	18	6	33.3
	42.3	18	15	83.3
	54.5	16	14	87.5

Copper - Nickel

120 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	19.7	15	0	0
	29.8	12	8	66.7
	33.5	16	12	75
	42.3	16	16	100
	54.5	18	18	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
16	19.7	16	4	25
	29.8	17	8	47.1
	33.5	18	12	66.7
	42.3	18	18	100
	54.5	17	17	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
40	19.7	17	4	23.5
	29.8	18	12	66.7
	33.5	15	13	86.7
	42.3	18	18	100
	54.5	18	18	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
70	19.7	18	0	0
	29.8	18	14	77.8
	33.5	18	12	66.7
	42.3	18	18	100
	54.5	16	16	100

Copper - Nickel

144 Hours

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	19.7	15	2	13.3
	29.8	12	10	83.3
	33.5	16	15	93.8
	42.3	16	16	100
	54.5	18	18	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
16	19.7	16	9	56.3
	29.8	17	15	88.2
	33.5	18	17	94.4
	42.3	18	18	100
	54.5	17	17	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
40	19.7	17	6	35.3
	29.8	18	15	83.3
	33.5	15	14	93.3
	42.3	18	18	100
	54.5	18	18	100

Pre-exp. Conc. (ug Cu/L)	Lethal Conc. (mg Ni/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
70	19.7	18	5	27.8
	29.8	18	17	94.4
	33.5	18	18	100
	42.3	18	18	100
	54.5*	16	16	100

Nickel - Copper

48 Hours

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	254 (15)	18	0	0
	268 (19)	18	1	5.6
	276 (19)	18	4	22.2
	281 (28)	18	2	11.1
	293 (23)	18	7	38.9

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.6 (0.0)	254	19	0	0
	268	18	1	5.6
	276	18	5	27.8
	281	18	9	50
	293	18	12	66.7

Pre-exp. Conc. (mg Ni/L)	Lethal Conc.. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.4 (0.1)	254	18	1	5.6
	268	18	4	22.2
	276	18	16	88.9
	[illegible]	18	15	83.3
	293	18	17	94.4

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
6.0 (0.4)	254	19	9	47.4
	268	18	16	88.9
	276	18	17	94.4
	281	18	18	100
	293	18	18	100

Nickel - Copper

60 Hours

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	254	18	1	5.6
	268	18	5	27.8
	276	18	9	50
	281	18	8	44.4
	293	18	10	55.6

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.6	254	19	1	5.3
	268	18	7	38.9
	276	18	9	50
	281	18	14	77.8
	293	18	17	94.4

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.4	254	18	2	11.1
	268	18	10	55.6
	276	18	16	88.9
	281	18	17	94.4
	293	18	18	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
6.0	254	19	12	63.2
	268	18	18	100
	276	18	18	100
	281	18	18	100
	293	18	18	100

Nickel - Copper

72 Hours

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	254	18	1	5.6
	268	18	8	44.4
	276	18	12	66.7
	281	18	12	66.7
	293	18	13	72.2

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.6	254	19	3	15.8
	268	18	12	66.7
	276	18	12	66.7
	281	18	16	88.9
	293	18	17	94.4

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.4	254	18	6	33.3
	268	18	13	72.2
	276	18	18	100
	281	18	18	100
	293*	18	18	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
6.0	254	19	16	84.2
	268	18	18	100
	276	18	18	100
	281	18	18	100
	293	18	18	100

Nickel - Copper

84 Hours

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	254	18	1	5.6
	268	18	10	55.6
	276	18	14	77.8
	281	18	14	77.8
	293	18	14	77.8

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.6	254	19	4	21.1
	268	18	14	77.8
	276	18	16	88.9
	281	18	16	88.9
	293	18	18	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.4	254	18	8	44.4
	268	18	16	88.9
	276	18	18	100
	281	18	18	100
	293*	18	18	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
6.0	254	19	17	89.5
	268	18	18	100
	276	18	18	100
	281	18	18	100
	293	18	18	100

Nickel - Copper

96 Hours

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
Control	254	18	6	33.3
	268	18	13	72.2
	276	18	14	77.8
	281	18	15	83.3
	293	18	17	94.4

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
0.6	254	19	10	52.6
	268	18	15	83.3
	276	18	16	88.9
	281	18	17	94.4
	293	18	18	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
1.4	254	18	12	66.7
	268	18	17	94.4
	276	18	18	100
	281	18	18	100
	293*	18	18	100

Pre-exp. Conc. (mg Ni/L)	Lethal Conc. (ug Cu/L)	# of Fish Per Cage	# of Deaths	Percent Mortality
6.0	254	19	17	89.5
	268	18	18	100
	276	18	18	100
	281	18	18	100
	293	18	18	100