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ACUTE AND CHRONIC EFFECTS OF WATER QUALITY CRITERIA BASED METAL MIXTURES ON THREE AQUATIC SPECIES

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16. ABSTRACT

Acute and chronic toxicity tests were conducted to determine the effects of metals combined as mixtures at proposed water quality criteria concentrations and at multiples of the LC50 and MATC obtained from tests on six metals with three aquatic species. These studies were the first part of a larger research effort to derive water quality criteria for combined pollutants by the U.S. EPA.

Arsenic, cadmium, chromium, copper, mercury, and lead combined at criterion maximum concentrations caused nearly 100 percent mortality to rainbow trout and daphnids (*C. dubia*) during acute exposure. Fathead minnows were not adversely affected at this or two times this concentration, although a mixture of 4 to 8 times the maximum value caused 15 to 60 percent mortality. Metals combined at the criterion average concentrations significantly reduced daphnid young production and fathead minnow growth after 7 and 32 days, respectively. Embryo hatchability and survival of rainbow trout were reduced at 4 times this criterion but not at the criterion average concentration.

Acute tests with metals mixed at multiples of the LC50 indicated that their joint action was more than additive to fathead minnows and nearly strictly additive to daphnids based on toxic units calculated from the individual components of the mixture. Chronic tests showed that the joint action was less than additive to fathead minnows but nearly strictly additive to daphnids indicating that long term metal interactions may be different in fish than in lower invertebrates. Adverse effects were observed at mixture concentrations of 1/2 to 1/3 of the MATC on fathead minnows and daphnids, respectively, suggesting that components of mixtures at or below no effect concentrations may contribute significantly to mixture toxicity on a chronic basis. These results point out the need for additional studies to determine the type and degree of interaction of toxicants because single chemical water quality criteria may not sufficiently protect some species when other toxicants are present concurrently.

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ABSTRACT

Acute and chronic toxicity tests were conducted to determine the effects of metals combined as mixtures at proposed water quality criteria concentrations and at multiples of the LC50 and MATC obtained from tests on six metals with three aquatic species. These studies were the first part of a larger research effort to derive water quality criteria for combined pollutants by the U.S. EPA.

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to determine the type and degree of interaction of toxicants because single chemical water quality criteria may not sufficiently protect some species when other toxicants are present concurrently.

Keywords: Mixtures, Metals, Fish, Invertebrates, Acute toxicity, Chronic toxicity.

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INTRODUCTION

Much of the information in the literature on the toxicity of chemicals to aquatic life deals with studies involving single toxicants. Numerous toxicity tests have been conducted to determine the acute and chronic effects of toxicants to provide data for the derivation of water quality criteria. To date, existing water quality criteria have been derived for single toxicants, yet it is rare to find natural waters in which only single toxicants are present. Aquatic organisms are usually exposed to a wide variety of toxicants from exposure to direct effluent discharges or from non-point source pollution due to chemical runoff. For this reason, the utility of water quality criteria on single toxicants is often questioned.

In an effort to establish more effective water quality criteria and hazard assessment programs, several mathematical models have been developed to predict the effect of mixtures of chemicals on aquatic organisms [1-6]. The application of these methods, however, has generally applied to acute lethality tests [7] and little work has been done to investigate the effects of mixtures on aquatic organisms on a chronic basis at sublethal concentrations [8-13]. Results from these tests appear to be somewhat contradictory and show no clear trend as to how chemicals interact as mixtures during acute and chronic exposure.

Due to the lack of adequate information, especially on the chronic effects of mixtures, little guidance has been given for setting water quality standards based on chemical mixtures. An early, tentative approach for evaluating joint toxicity has been to assume that there is an additive action between diverse toxicants [14]. A similar approach has more recently been taken by the European countries [7]. Based on their review of the literature on the effects of mixtures on freshwater fish and other aquatic organisms, it was proposed that for pollution control purposes, the concentration addition model is adequate to describe the acutely lethal joint effect of commonly occurring constituents of sewage and industrial wastes. This proposal is based on the rationale that the joint acute lethal toxicity of chemicals to fish can be predicted assuming simple addition of the proportional contribution from each toxicant, but that toxicity based on concentrations approaching no-effect are less than additive and probably do not contribute to the chronic toxicity of mixtures. It was concluded, however, that more empirical studies are needed on the long-term joint effect of mixtures of toxicants, especially, to determine the contribution of small fractions of the toxic units of the individual components.

The first objective of this research was to determine if the single chemical water quality criteria proposed by the U.S. Environmental Protection Agency (EPA) in 1984 [20][1] for selected inorganic chemicals were sufficient to protect selected aquatic species when they were present as mixtures. These criteria are not specific for individual species but are based on several species from a variety of aquatic families. The present studies would not show the type and degree of interaction of chemicals in these mixtures but would indicate their effect at proposed criteria concentrations.

The second objective was to measure the contribution of fractions of toxic units of mixtures by using acute (LC50) and chronic (MATC) values obtained from tests on individual chemicals in mixture tests at, above, and below these concentrations. In addition to the MATC, an estimate of

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[1] The numerical national water quality criteria proposed in 1984 [20], guidelines for deriving these values [26], and the corresponding terminology used in this text have been changed slightly since the completion of this research project due to the inclusion of new research data and public comments. For the most recent information on this subject see [48].

the level of 50 percent reduction in growth and reproduction was used as the toxic unit in chronic tests. Results from these tests would be species-specific and would indicate the possibility of concentration addition.

Inorganic chemicals, specifically arsenic, cadmium, chromium, copper, mercury and lead were selected for this study because of their importance to EPA in deriving individual chemical water quality criteria and because these chemicals are found together as mixtures in commonly occurring sewage and industrial wastes [15]. Tests were conducted with fathead minnows, rainbow trout, and daphnids. Fathead minnows and rainbow trout are important forage and game fish species, respectively, and are representatives of both warm and cold water aquatic organisms. Daphnids were chosen for this study because they are among the most sensitive aquatic organisms to most of the selected chemicals.

METHODS AND MATERIALS

Test Water

Tests were conducted in Lake Superior water that was filtered through sand and heated to 25 ± 3° C for tests with fathead minnows or cooled to 10 ± 3° C for tests with rainbow trout. Tests with daphnids were conducted at 25 ± 2° C with reconstituted hard water [16] and water from the Lester River located adjacent to the Environmental Research Laboratory in Duluth, MN. All organisms were cultured in the respective water before they were tested. Most of the tests conducted with daphnids were done in Lester River water because better survival and reproduction results were observed in this water than in either Lake Superior or reconstituted water. Lester River water was collected from just below the water surface and stored in polyethylene 5 gallon jugs at 6° C prior to testing. River water was filtered twice through 45 cm mesh screening and vigorously aerated before all daphnid tests. Routine chemical characteristics of all test waters were measured according to procedures described by the American Public Health Association et al. [17] and are shown in Table 1 for each species. Dissolved oxygen concentrations in the test chambers for all waters were at or above 70 percent saturation.

Exposure Systems and Toxicant Solutions

Flow-through tests with fathead minnows and rainbow trout were conducted using a dilution system [18] that delivered five concentrations at a 0.5 dilution factor and a control to up to 4 replicate chambers per treatment. Lake Superior water was fed from stainless steel headboxes to the diluter after it was vigorously aerated to remove excess dissolved gases. All toxicant solutions were delivered to the diluter mixing cell via FMI pumps (Fluid Metering Inc., Oyster Bay, N.Y. 11771) from 19 liter glass stock bottles containing either individual or mixtures of stock solutions.

Glass test chambers measured 7 cm wide x 18 cm long x 9 cm high with a water depth of 6.4 - 7.0 cm. The flow rate to each chamber was ~15 ± 1 ml/min. Wide spectrum fluorescent bulbs provided a light intensity of 30-45 and 110-320 lux for tests with rainbow trout and fathead minnows, respectively, at the water surface during a 16 hr photoperiod. This included a 30 min. gradual brightening and dimming period with incandescent lights to simulate dawn and dusk [19].

Static renewal tests with daphnids were conducted in 30 ml plastic disposable beakers (Plastics, Inc., Minneapolis, MN) containing 15 ml of solution. Replicate test beakers (10 per concentration) were used in all tests for each of 6 toxicant concentrations and a control, utilizing a 0.5 dilution factor scheme. Test beakers were located in a water bath to provide the necessary temperature control. Light intensity at the water surface was 25-100 lux for the 16 hr automatically controlled photoperiod.

Stock solutions for all tests were prepared by dissolving reagent grade sodium arsenite ($Na_2AsO_4.7H_2O$), cadmium nitrate ($Cd(NO_3)_2.H_2O$), sodium dichromate ($Na_2Cr_2O_7.H_2O$), cupric nitrate ($Cu(NO_3)_2.3H_2O$), mercuric nitrate ($Hg(NO_3)_2.H_2O$) and lead nitrate ($Pb(NO_3)_2$) in distilled water. For mixture tests, stock solutions of arsenic and chromium were kept separate from each other and from the other chemicals because of the formation of insoluble compounds at high concentrations. Although several valence states of arsenic and chromium exist in natural waters, trivalent arsenic and hexavalent chromium were chosen for our studies because numerous toxicological studies with aquatic organisms on these forms were available in the literature and because their chemical properties were suitable for mixture testing. Both forms, however, are easily converted to different valence states depending upon water quality (i.e., reversible reactions occur between trivalent and pentavalent arsenic and hexavalent and trivalent chromium [20]). Therefore, arsenic and chromium species were analyzed by methods described by Ficklin [21] and Martin and Riley 1982 [22], respectively, to determine the species present under our test conditions. Trivalent arsenic was found to stay in this form when arsenic was tested individually, however, pentavalent arsenic was measured periodically in the presence of the other chemicals. The ratio of trivalent to pentavalent arsenic ranged from 0 to 100 percent in chambers containing mixtures. Hexavalent chromium, essentially stayed in this form (> 95 percent) in all tests. Nitrates of copper, cadmium, mercury and lead were used in this study because of their solubility properties and stability in these mixtures.

Quality Control

Samples of all test solutions were taken from the test chambers or mixing flasks and analyzed according to a monitoring program that characterized the test pattern. Procedures for metal water analyses were those described by the U.S. EPA [23]. Measurements of all of the metals except for mercury are expressed as total acid exchangeable metal. Mercury measurements are reported as total recoverable metal. Detection limits for these procedures are included in Tables 2 and 3. To verify the accuracy of the method of analyses, known amounts of metal were added to control water to obtain percentage recoveries each time water samples were taken. Mean, standard deviation and number of analyses in parentheses of percentage recoveries for arsenic, cadmium, chromium, copper, mercury, and lead in all tests were 100 ± 6 (61), 98 ± 6 (58), 100 ± 5 (61), 99 ± 7 (65), 97 ± 6 (59), 92 ± 9 (60), respectively. Quality control samples from the Environmental Monitoring and Support Laboratory, Cincinnati, OH were also obtained for use as checks on our quality control. All values were similar to those stated above for spiked recoveries. In addition, samples, except for mercury, were periodically filtered through a 0.45 μm Millipore filter to measure the portion of dissolved metal. Mean, standard deviation and number of analyses for dissolved measurements (in percent) of total acid exchangeable arsenic, cadmium, chromium, copper and lead were 101 ± 8 (11), 93 ± 9 (11), 102 ± 3 (11), 92 ± 6 (11), and 75 ± 14 (16), respectively. Because mercury loss is high during the filtration procedure, samples were differentiated instead as to their inorganic and organic components to characterize the metal in solution [23]. Mercury samples were, generally, found to be > 90 percent inorganic mercury in our test water. All concentrations are expressed as the metal, not as the compound tested.

Biological Procedures

The test animals used in this study were fathead minnows (*Pimephales promelas*), rainbow trout (*Salmo gairdneri*) and ceriodaphnids (*Ceriodaphnia dubia*). The name ceriodaphnids will hereafter be used in the text as daphnids for simplicity. Rainbow trout embryos (< 24 hr old after fertilization) were obtained from the Minnesota Department of Natural Resources cold water fish hatchery located at the mouth of the French River, near Duluth, Minnesota and were incubated and reared at our laboratory at 10° C prior to testing. Both fathead minnows and daphnids were obtained from existing cultures at our laboratory. Procedures for conducting acute lethality tests to determine LC50 values closely followed those described by the American Society for Testing and Materials (ASTM) [16]. Acute tests with fish were continuous flow through tests and were initiated by randomly distributing 10 rainbow trout (~ 90 day old, ~ 1.5 g) and fathead minnows (~ 30 day old, ~ 0.15 g) to each duplicate test chamber per treatment. Tests with fish were for 96 hr. Acute (48 hr) static tests with daphnids were initiated by randomly distributing five (< 24 hr) daphnids to each duplicate exposure chamber. Procedures for conducting flow-through chronic tests with fish were similar to those described by ASTM [24] and renewal tests with daphnids were done according to Mount and Norberg [25]. Survival, growth and/or young production were used as the response variables in these chronic tests.

Testing Design

The first part of this study began in May of 1983 by exposing each species to a mixture of the six metals at water quality criteria concentrations proposed by the U.S. EPA [20] (Table 4). Water quality criteria concentrations for aquatic life are expressed as two numbers, a maximum and average concentration. The definition and guidelines for deriving these concentrations are given by Stephan et al. [26]. Measured water concentrations in our tests correlating to these criteria are shown in Tables 2 and 3. Tests included concentrations at, above, below and in proportion to the criteria for each metal. Acute lethality tests were used to determine effects at criterion maximum concentrations and chronic tests were utilized in tests at criterion average concentrations [26]. Criteria concentrations of cadmium, copper and lead were adjusted for water hardness according to guidelines for deriving national water quality criteria [26].

The second part of this study was initiated by exposing fathead minnows and daphnids to each metal to determine respective LC50 and chronic (MATC) values. Subsequently, each species was exposed to mixtures at, above, and below these concentrations to determine possible additive interactions of the six selected metals. Rainbow trout were not used in these studies because tests with this species were too long (up to 90 days) for the number of tests required.

Statistical Procedures

Forty-eight and 96 hr LC50 values were determined with a computerized, modified trimmed Spearman-Karber method described by Hamilton et al. [27]. Daily mortality data from replicate exposure tanks were combined before LC50 values were calculated.

For early life-stage tests with rainbow trout and fathead minnows, survival, embryo hatchability, and larval deformity data were transformed to arcsin % [28] for variance stabilization. Individual weights of fish in replicate chambers were pooled before data were subjected to Dunnett's one-sided comparison of treatment means to control means ($P = 0.05$) [29]. Survival and young production data for life cycle daphnid tests were analyzed using the procedure of Hamilton [30] as modified by Rogers [31].

RESULTS AND DISCUSSION

Criteria Exposures

Survival of species exposed to metals mixed at their respective criterion maximum concentrations is shown in Table 5. Metal mixtures at this criterion concentration reduced the survival of rainbow trout by 95% and killed all of the daphnids in acute tests but caused no significant effect on fathead minnow survival after 96 hours. Concentrations between 4 and 8 times this criterion reduced survival of fathead minnows from 15 to 60 percent and killed all of the rainbow trout. A mixture of one half of the maximum concentration had no significant effect on any of the three species. The sensitivities of these species appear to be directly related to their sensitivities to each of the individual metals. Both rainbow trout and daphnids are in families that were consistently the most sensitive to each of these metals when compared to other aquatic species [20]. Conversely, fathead minnows were usually found to be more tolerant than trout to all of the metals except mercury, where their sensitivity ranked between that of daphnids and rainbow trout.

Survival, growth and young production of species exposed to metals mixed at their respective criterion average concentrations are presented in Tables 6 and 7. Concentrations of 8 and 16 times this value reduced the survival of developing rainbow trout embryos and fathead minnow larvae by nearly 100 percent (Table 6). A lower concentration of 4 times this criterion also significantly reduced survival of trout sac larvae after approximately 45 days of exposure and that of early juvenile fathead minnows after a 32-day test. A further reduction in the survival of juvenile trout was observed at this concentration by the end of the 90-day test, however, due to fungal disease in two of the four replicate control chambers during the last three weeks of the test, significant differences were not observed. Growth determinations of trout exposed for a 70-day period were also hampered by control temperatures that were ~ 1° C lower than the other treatments for approximately 21 days during the 90-day exposure. This resulted in slightly smaller control fish by the end of the test. Although fish appeared smaller in chambers at 4 times the criterion average compared to those in lower concentrations, statistical decreases in growth were not observed at this concentration after 70 days. Growth determinations on fathead minnows, however, indicated dramatic decreases in weight at lower mixture concentrations including a significant 30 percent reduction at the criterion average concentration (Table 6). Visual decreases in growth were noted at this concentration as early as 3 weeks after the test started. Significant adverse effects of mixtures at the criterion average concentration were also observed in tests with daphnids which caused approximately 80 percent reduction in young production after a 7-day test (Table 7). Although survival of daphnids was decreased by 40 percent by this concentration, the differences were not statistically significant.

The tolerance of rainbow trout to the criterion average mixtures was surprising due to their normally sensitive nature to these individual metals. However, this response may have been due to the lack of statistical differences in the data as a result of the decreased growth of fish in the controls, as was previously stated. Although trout appeared healthy in a mixture at the criterion average concentration, it is likely that this concentration could have been an effect concentration if control growth was normal. This hypothesis is supported by the fact that this concentration caused adverse effects on both daphnids and fathead minnows; species which, generally, have been shown to be as sensitive or less sensitive, respectively, than trout to individual metals [20]. In contrast to the observed response for rainbow trout, the high sensitivity of fathead minnow growth was unexpected. The reason for their sensitivity to the present mixtures is not known, although copper could have been the major factor contributing to the decrease in growth. Copper was shown to decrease growth of this species at very low levels in individual metal tests conducted as part of this study (see the following text) and in recent studies conducted by Benoit [32] in Lake Superior water. Copper concentrations causing effects were nearly the same as the criteria concentrations in our mixtures.

The above results suggest that all three test species may not be sufficiently protected if the selected metals were present in water as mixtures at proposed water quality criteria concentrations. These data are particularily meaningful because rainbow trout are an economically important species and were adversely affected at least at the criterion maximum concentrations. Although presently proposed criteria do not attempt to protect all species, the chronic adverse effects of metal mixtures observed in this study on daphnids and fathead minnows also indicate that important forage organisms may not be protected which could ultimately cause a decrease in more desirable fish populations.

Individual Metal and Mixture Exposures

Acute LC50 values for fathead minnows and daphnids exposed to individual metals are shown in Tables 8 and 9. Comparison of the results indicate that daphnids were, generally, more sensitive to these metals than were fathead minnows even though daphnids were tested in Lester River water which was higher in hardness, alkalinity and organic content than that of Lake Superior water. These water quality characteristics usually decrease the toxicity of metals by decreasing their bioavailability by complexation in water [33-35]. Both species were less sensitive to arsenic, chromium, and lead than to mercury, cadmium and copper but their sensitivity to these metals was not the same after the respective exposure periods. Fathead minnows were most sensitive to cadmium then copper and mercury, whereas daphnids were most sensitive to mercury then cadmium and copper. Sensitivity differences in these tests were also probably related to water quality differences since some water quality parameters may effect some metals differently. For example, water hardness has been found to effect the toxicity of cadmium, copper and lead more than that of arsenic, mercury and chromium [20]. However, the acute values obtained for both species in these tests were similar to those observed by other investigators [20].

The LC50 values calculated for these metals in mixture tests for each species are included in Tables 8 and 9 along with the fractions of toxic units (LC50s) calculated for each metal. The toxic unit approach for calculating the combined effects of mixtures of toxicants was reviewed by Sprague [2]. In this method, fractions of toxic units of the individual toxicant in the mixture adding up to a total of 1.0 indicate a strictly additive joint action, < 1.0 more than additive and > 1.0 less than additive. The sum of the fractions of toxic units calculated for fathead minnows from Table 8 was 0.53 indicating that these metals were more than additive in their acute joint action. Conversely, the value calculated for daphnids was 1.47 (Table 9) suggesting a nearly strictly additive joint action. Differences in the joint action correlate to the sensitivity differences of these species to the individual metals as was noted above. Differences in the behavior of metals in mixtures have also been reported in a review of the literature [7] on effects of mixtures on aquatic organisms. This review showed that the same metals may be additive, more than additive or less than additive depending upon the species, combination of metals, or water quality present. Anderson and Weber [3] suggested that discrepancies found between additive action and more than additive actions of metals in organisms may be due to water quality characteristics such as water hardness, which may alter metallic forms. They discussed that more than additive responses were shown in tests with copper and zinc using soft water and additive responses were observed from exposures with hard water. More recent studies by Anderson and others [36, 37] indicate that the reduction in toxicity of a metal with increasing hardness, however, may not be great when other metals are present concurrently. Although additional work is needed to make correlations between water quality characteristics and the reaction of chemicals in mixtures, water quality effects on toxicity may also be a plausible explanation for the slight differences observed in this study since water quality parameters in these tests were different.

The chronic effects of individual metals on fathead minnows are shown in Table 10. Growth in these tests was the most or among the most sensitive measure of response to all metals. A significant value obtained in the arsenic test at 1,340 ug/l was not used in the calculation of the MATC because significant adverse effects were not observed in the next higher concentration. The MATC's or chronic values (calculated as the geometric mean of the highest no-effect and lowest effect concentrations from Table 10) for this species and each metal are included in Table 11. MATC's calculated for this species were similar to chronic values observed for the respective metals by other investigators [20]. Comparison of the above MATCs for fathead minnows showed that species sensitivity to the metals differed slightly in chronic tests compared to that from acute tests. Cadmium was the most toxic metal in acute tests followed by copper, mercury, lead, arsenic and chromium; whereas mercury was much more toxic on a chronic basis followed by copper, cadmium, lead, chromium, and arsenic. Acute to chronic ratios for each metal are also included in Table 11. Tests with mercury showed the highest ratio (193) for fathead minnows indicating that it is much more toxic on a chronic basis than on an acute basis to this species than the other metals.

The effects of these metals on the survival and young production of daphnids are presented in Table 12. Both mortality and young production were used to determine the overall toxicity comparison (scaled T-statistic) [30, 31] in these tests. Young production was the most sensitive parameter measured with all the metals except chromium for which survival was slightly more sensitive. The high sensitivity of daphnid young production to metals and other chemicals including several industrial effluents has also been observed by Mount [38]. The MATC's for each of the metals and daphnids are shown in Table 11. As with the acute tests, chronic values obtained for daphnids were generally lower than those calculated for fathead minnows indicating their high chronic sensitivity to these metals. Comparisons of the above values for daphnids to these metals showed that their sensitivity differed slightly on an acute versus chronic basis as it did with fathead minnows. Mercury was the most toxic metal to daphnids in acute tests but cadmium was more toxic on a chronic basis. This was the opposite of that observed for fathead minnows. The ranking of acute to chronic ratios for these two metals and daphnids was also opposite that observed for fathead minnows with the highest ratio being for cadmium (12.4) and lowest for mercury (0.73) (Table 11). Generally, acute to chronic ratios were lower for daphnids than fathead minnows, especially for mercury. This difference may be attributed to the greater influence of food on metal toxicity in chronic tests with daphnids which could have decreased biological availability and/or toxicity of these chemicals. Biesinger and Christensen [39] and Lima et al. [40] showed that the presence of food decreases the toxicity of metals to these types of organisms.

Measured water concentrations and results of chronic mixture tests for fathead minnows and daphnids are presented in Tables 13-17. Two 32-day flow-through tests with fathead minnows were conducted to determine the effects of mixture concentrations at 0.5 dilution ratios of the MATC's. Results from these tests (Table 16) show that metals mixed at MATC, and 4/3 MATC concentrations caused nearly 100 percent mortality and decreased growth of juvenile fish after 32 days of exposure. All larvae that hatched at these concentrations were deformed and all but a few fish died only one week after hatching. Metals combined at 1/2 and 2/3 of the MATC values also caused 40 to 50 percent mortality, respectively, although statistical differences at P = 0.05 were just on the border of not being significant. The lack of statistical significant difference here appears to be attributed to the fact that mortalities in duplicate chambers at these concentrations were more dissimilar than usual. The authors, however, feel that these are real differences because deformities and abnormal behavior of newly hatched larvae were observed at these concentrations early in each experiment at hatch. In addition, growth of juvenile fish exposed to mixtures of 2/3 of the MATC were reduced by as much as 25 percent by the end of the test.

The effects of mixture concentrations on survival and young production of daphnids are shown in Table 17. Metals combined at 4/3 MATC, MATC, and 2/3 MATC concentrations all caused 100 percent mortality. The next lower concentration of metals mixed at 1/3 of the MATC did not adversely effect survival but significantly reduced young production by approximately 60 percent. The lower effect level observed for daphnids compared to that for fathead minnows agrees with the findings from individual metal tests and suggests that the mechanism of toxic action of individual metals may be similar to those in mixtures. The adverse effects of mixtures at fractions of the MATC also indicate that chronic joint action may occur in these species at levels presumed acceptable based on tests with individual metals. Other studies on the effects of metal mixtures on fish [8, 41] and more recent ones on invertebrates [42] have also indicated the adverse effects of mixtures occur at concentrations having no significant effect on an individual basis. The literature on the sublethal effects of combinations of metals on aquatic organisms indicate that joint action may occur at sublethal levels, but that there is no clear trend as to the degree of response [7]. As in acute tests, chronic responses vary in the literature [7] from less than additive to more than additive depending upon the chemical or species tested.

Because the MATC is not a point estimate level, the degree of sublethal joint action based on this endpoint in the present chronic tests cannot be calculated precisely. In order to determine the type and degree of joint action, an estimate of the value causing a 50 percent reduction in weight and the number of young produced per female for fathead minnows and daphnids, respectively, exposed to individual metals and metal mixtures, was obtained from response curves relating these endpoints to exposure concentration. Figures 1 and 2 show that these response relationships were curvilinear for the metals tested on an individual as well as mixture basis, respectively. This pattern was similar for both species and all metals in both types of tests and was characterized by a gradual decrease in the curve followed by a rapid decline as exposure concentration increased. Slopes for the steep part of the curves were, generally, greater for metals in the mixture than for metals tested individually indicating that the observed joint action caused greater toxicity than that caused by each metal during the same exposure period. The similarity in the slopes of the response curves for individual as well as combined metals suggest that modes of action of metals may also be similar under these conditions and not changed but merely enhanced by metal interactions in some fashion. This has been postulated previously for lethal tests with metals and binary metal mixtures by Hewitt [43].

All values below the 50 percent response level in Figures 1 and 2 were significantly less than the control (P = 0.05) and are shown by the open symbols. However, some values above this level were also statistically significant for both species exposed to the individual metals (Figure 1) suggesting that the 50 percent response is probably too high a level to estimate chronic no effect concentrations. In general, no effect concentrations (i.e, MATC) are estimated from chronic endpoints that are significantly different from the control. These endpoints are not estimates of the degree of adverse effect and may or may not be biologically significant. Additional chronic studies are needed to define meaningful point estimates which would better correlate to biological effect concentrations.

Chronic concentrations and fractions of this toxic unit based on the response curves for each species exposed to individual metals and to the mixture are shown in Table 18. Sums of the fractions of toxic units of 3.31 and 1.08 indicate that the chronic joint action of these metals was less than additive with fathead minnows and nearly strictly additive with daphnids, respectively. Comparisons of these results with the acute joint action determined above for these species show that the interaction of metals may be different on an acute and chronic basis and between different classes of organisms. The joint action of the selected metals was more than additive to fathead minnows at acutely lethal levels but was less than strictly additive at sublethal concentrations as determined by effects on growth. The joint action displayed on daphnids was nearly strictly additive on both an acute and chronic basis. These differences agree with tentative conclusions made on the joint action of metal mixtures on fish and invertebrates by the European countries [7]. Generally, the few sublethal mixture tests reviewed using growth as the endpoint showed that chemical interactions were less than additive than the corresponding effect on survival. The data available for aquatic invertebrates showed a generally additive joint action. However, the review reported that one study [44] indicated that sublethal metal mixtures may be more than additive on fish reproduction and suggested that this work be extended to include other commonly occurring toxicants. The reason for differences in the joint action of metals on the present test species is not clear but it may be linked to the species sensitivity differences noted above and thus attributed to differences in metabolic defense mechanisms of these different types of organisms. These mechanisms are primitive in daphnids, and may be the same in this species during both acute and chronic exposure. On the other hand, defense mechanisms in fish are more specialized than daphnids which may allow fish to become more tolerant to metal toxicity during long-term exposures as they develop resulting in a lesser joint action of the toxicant. Such mechanisms of detoxification may be related to the binding of metals to special proteins (i.e., metallothioneins) as suggested in the literature [45]. However, further study of the mechanisms of toxic action of chemicals are needed and may be the key to understanding long-term chemical interactions.

The mode of action of metals in fish is obviously different on an acute basis than on a chronic basis. It is generally considered that metals cause acute toxicity by destruction of the gill membranes. Studies by Hewitt [43] indicate that the gills of zebra fish (*Brachydanio rerio*) were the primary site of significant metal accumulation during acute exposure and that the relative bioconcentration of metals was significantly greater in gill tissue of fish exposed to metal mixtures than to individual metal solutions. These results may explain the more than additive response obtained in our acute tests with fathead minnows. Knowledge of metal concentrations in fish exposed to mixtures on a long-term basis would help to explain the difference in metal interactions observed between acute and chronic tests.

Although it appears from the present studies that mixtures of metals may be less than additive to fish in long-term exposures, the adverse effect observed on survival of this species at concentrations of 2/3 to 1/2 of the MATC are low enough to cause concern that these organisms may not be protected by criteria that are based on MATC concentrations derived for individual metals. Chronic adverse effects on daphnid reproduction caused by metals mixed as low as 1/3 of the MATC can be correlated to the nearly strictly additive effect shown above, and suggests that these organisms may be more susceptible to metal interactions than fish. Recent studies by Biesinger et al. [42] have also indicated that metal interactions may be nearly additive on daphnid reproduction based on complete life-cycle studies. Similarly, Hermans et al. [12] suggested that although the joint toxicity at sublethal levels is lower than at lethal levels to daphnids, the toxicity of mixtures containing primarily organic chemicals remains much higher than that of individual chemicals and may be near concentration addition. Still other studies on the effects of mixtures on fish [8, 43] and algae [46] indicate that some metals as well as other chemicals such as pesticides [37] may be even more than additive on a chronic basis.

The effects of metals mixed at fractions of both acute and chronic values on the selected species correspond with the effects noted in the first part of this study from mixtures based on proposed water quality criteria concentrations. Water quality criteria are based on data from tests with individual chemicals on several families of aquatic organisms and are not species-specific. However, endpoints such as the LC50 and MATC are species-specific and are used to determine the criteria for individual chemicals. Thus, the adverse effects caused by metal mixtures at proposed water quality criteria concentrations appear to be due to the joint action of the selected metals at concentrations having no significant effect on an individual basis, and suggest that components of mixtures at or below no observable effect concentrations may contribute significantly to the toxicity of these mixtures. The effects of mixtures at sublethal levels are of particular importance since these concentrations would be allowed to exist continuously throughout the year as criteria and may not be sufficient to protect some aquatic species. These effects may also be magnified in waters having a low buffering capacity and pH which may increase the bioavailability and/or toxicity of metals. Recently completed studies by Hutchinson and Sprague [47] show that reproductive failure of fish was complete when fish were exposed to trace metal mixtures in water of low pH (pH 5.8). This level of pH alone caused no effects on fish reproduction in very soft (6.0 mg/l as $CaCO_3$) water.

The results of the present tests suggest that further study is needed to determine the type and degree of interaction of toxicants on both an acute and chronic basis and to determine the possible effects of water quality characteristics on these interactions. Results obtained from these tests would help identify general characteristics of certain mixtures and may provide data for new methods and possibly the rationale for deriving water quality criteria for combined pollutants.

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Table 1. Measured water quality characteristics for metal tests conducted in Lake Superior, reconstituted and Lester River water.

Test Species	Hardness as $CaCO_3$ (mg/l)	Alkalinity as $CaCO_3$ (mg/l)	pH[a]	Total Organic Carbon (mg/l)	Conductivity (μmhos)
			Lake Superior Water		
Rainbow trout	45.3±0.8 (23)[b]	42.7±1.3 (24)	7.7 (7.2-7.9)	-	-
Fathead minnow	43.9±1.0 (90)	42.4±1.9 (90)	7.4 (6.0 8.1)	2.0±1.4 (2)	91±1.4 (2)
			Reconstituted Water[c]		
C. dubia	165±4.0 (2)	112±4.0 (2)	8.1 (7.8-8.3)	1.6 (1)	451±1.4 (2)
			Lester River Water		
C. dubia	100±7.9 (11)	97±9.3 (22)	8.2 (8.0-8.5)	7.1±0.7 (9)	194±20 (9)

[a] Mean (range)

[b] Mean ± standard deviation (number of samples)

[c] Reconstituted hard water [16]

Table 2. Measured water concentrations for tests with fathead minnows, rainbow trout, and daphnids exposed to metal mixtures at criterion maximum concentrations.

Metal	Treatment (μg/l)					
	Control	1/2 Max	Max[a]	2x Max	4x Max	8x Max
			Fathead Minnow			
As	<1.0[b]	69±0.4[c]	143±1.4	262±3.5	556±4.2	1,092±93
Cd	<0.25	0.7±0.1	1.5±0.2	2.9±0.1	6.0±0.0	13±0.0
Cr	<1.0	6.8±0.6	15±4.8	26±2.4	50±1.6	90±0.8
Cu	1.0[d]	4.4±0.1	7.9±0.7	15±0.2	30±1.0	58±1.6
Hg	<0.05	0.6±0.0	1.3±0.0	2.3±0.2	4.2±0.0	7.9±0.6
Pb	<0.5	9.9±1.4	23±4.7	40±4.0	79±0.9	161±13.4
			Rainbow Trout			
As	<1.0	81±3.4	148±9.2	288±18.4	536±42	1,075±122
Cd	<0.25	0.8±0.0	1.6±0.0	3.2±0.1	5.7±0.4	12±1.1
Cr	<1.0	7.3±0.2	14±1.3	26±1.8	47±0.2	90±6.8
Cu	1.0[d]	6.1±1.1	9.2±1.6	17±0.9	30±0.6	57±1.8
Hg	<0.05	0.5±0.1	0.9±0.1	1.9±0.2	3.6±0.4	7.2±0.0
Pb	<0.5	11±0.5	17±1.6	36±0.5	74±6.4	157±17.0
			Ceriodaphnia dubia[d]			
As	<1.0	72	157	308	645	-[e]
Cd	<0.25	3.4	7.0	11.1	34.4	-
Cr	<1.0	4.2	10.2	21.7	42.4	-
Cu	1.0[d]	8.5	19.0	39.1	81.5	-
Hg	<0.05	0.34	0.9	1.8	4.2	-
Pb	<0.5	64	143	284	611	-

[a] Criterion maximum concentrations

[b] Detection limit

[c] Mean ± standard deviation

[d] One measurement was made at the beginning of the test

[e] Daphnids were not tested at this level.

Table 3. Measured water concentrations for tests with fathead minnows, rainbow trout, and daphnids exposed to metal mixtures at criterion average concentrations.

Metal	Treatment (μg/l)					
	Control	Avg[a]	2x Avg	4x Avg	8x Avg	16x Avg
			Fathead Minnow			
As	<1.0[b]	66±9.3[c]	139±14.7	275±26	548±45	1,225±266
Cd	<0.25	1.4±0.2	3.2±0.6	6.9±0.6	14±1.6	31±5.6
Cr	<1.0	6.4±1.8	13±1.9	26±2.1	64±1.6	130±27
Cu	1.0	5.7±0.7	11±0.4	19±0.7	39±1.6	101±42
Hg	<0.05	0.2±0.1	0.3±0.1	0.6±0.1	1.3±0.2	2.3±0.0
Pb	<0.5	0.9±0.2	1.7±0.4	2.9±0.3	7.1±1.7	16±5.8
			Rainbow Trout			
As	<1.0	79±9.6	154±1.8	306±40	571±49	1,260±128
Cd	<0.25	1.7±0.3	3.8±0.4	7.5±0.7	14±1.1	31±2.0
Cr	<1.0	8.4±1.7	15±2.8	29±3.1	56±6.4	122±9.0
Cu	1.0	7.3±1.0	13±1.2	23±2.2	43±6.1	86±4.3
Hg	<0.05	0.2±0.1	0.4±0.1	0.7±0.2	1.5±0.2	2.9±0.5
Pb	<0.5	1.1±0.3	1.8±0.4	3.4±0.7	6.0±0.7	12±1.4
			Ceriodaphnia dubia			
As	<1.0	68±2.3	137±7.5	286±14	575±32	1,152±26
Cd	<0.25	6.7±0.3	13±1.3	28±2.5	5.6±6.2	117±15.6
Cr	<1.0	7.0±0.3	14±0.4	29±0.6	59±0.5	121±1.5
Cu	1.0	13±0.6	28±1.4	58±2.6	120±5.5	248±12.7
Hg	<0.05	0.13±0.01	0.27±0.01	0.57±0.04	1.3±0.02	3.4±0.2
Pb	<0.5	3.6±0.5	8.3±0.6	16±0.4	33±3.9	70±6.3

[a] Criterion average concentration

[b] Detection limit

[c] Mean ± standard deviation

Table 4. Proposed National Water Quality Criteria (1984) for selected metals and freshwater aquatic life.

	Criteria (µg/l)	
Metal	Max. Conc.	Avg. Conc.
Arsenic^{+3}	140	72
Cadmium^{+2}	1.8[a], 8.2[b]	1.8[a], 8.2[b]
Chromium^{+6}	11	7.2
Copper^{+2}	7.6[a], 25.1[b]	5.2[a], 17.3[b]
Mercury^{+2}	1.1	0.2
Lead^{+2}	22[a], 128[b]	0.9[a], 5.1[b]

[a] Adjusted for hardness of Lake Superior water (45 mg/l as $CaCO_3$) [26]

[b] Adjusted for hardness of reconstituted water (168 mg/l as $CaCO_3$) [26].

Table 5. Survival of fathead minnows, rainbow trout, and daphnids exposed to metal mixture at multiples of criterion maximum concentrations.

Treatment[a]	Fathead minnow[b]	Rainbow trout[b]	*C. dubia*[c]
Control	100±0.0[d]	100±0.0	90±32
1/2 Max	100±0.0	100±0.0	100±0.0
Max	100±0.0	5±10	0±0.0
2x Max	100±0.0	0±0.0	0±0.0
4x Max	85±7.1	0±0.0	0±0.0
8x Max	40±0.0	0±0.0	0±0.0

[a] Treatment number corresponds to measured concentrations in Table 2.

[b] Tests conducted in Lake Superior water for 96 hr.

[c] Test conducted in reconstituted water [16] for 48 hr.

[d] Mean ± standard deviation.

Table 6. Survival and growth of fathead minnows and rainbow trout exposed to metal mixtures at multiples of criterion average concentrations for 32 and 90 days, respectively in Lake Superior water.

Treatment[a]	Embryo Hatchability (%)	Normal Larvae at Hatch (%)	Survival (%)	Weight (mg)
		Fathead Minnow		
Control	100±0.0[b]	100±0.0	87±19	174±46
Avg	100±0.0	100±0.0	93±0.0	129±36*
2x Avg	100±0.0	100±0.0	94±9.2	44±29*
4x Avg	100±0.0	94±9.2	43±14*	6.5±2.5*
8x Avg	97±5.0	10±4.2*	4±5.0*	2.0±0.0*
16x Avg	97±5.0	0±0.0*	0±0.0*	0±0.0*
		Rainbow Trout		
Control	96±2.3	99±1.0	96±2.3[c]	121±42[d]
Avg	92±2.3	99±1.0	92±2.8	195±85
2x Avg	95±1.2	99±1.0	95±1.2	185±62
4x Avg	88±3.5*	98±2.9	89±3.5*	150±48
8x Avg	8±5.3*	0±0.0*	8±5.3*	-
16x Avg	0±0.0*	0±0.0*	0±0.0*	-

[a] Treatment corresponds to measured concentrations in Table 3

[b] Mean ± standard deviation

[c] Based on 45-day exposure period (see text)

[d] Based on 70-day exposure period (see text)

* Significant decrease from control (P = 0.05)

le 7. Survival and number of young per female of C. dubia exposed to metal mixtures at multiples of criterion average concentrations after 7 days in reconstituted water.

eatment[a]	Survival (%)	Young per Female	Scaled T-statistic of Overall Comparison
ntrol	90 ± 32[b]	15.6 ± 1.0	–[c]
/2x Avg	80 ± 42	12.5 ± 1.1	0.81
vg	60 ± 52	3.3 ± 1.0*	3.37*
x Avg	0 ± 0.0*	–[d]	2.08*
x Avg	0 ± 0.0*	–[d]	2.08*
x Avg	0 ± 0.0*	–[d]	2.08*
.6x Avg	0 ± 0.0*	–[d]	2.08*

Treatment corresponds to measured concentrations in Table 3 except for /2x Avg concentration (not shown in Table 3)

lean ± standard deviation

lo value because other values are compared to the control

lo value because all animals died

Significant decrease from control (P = 0.05)

Table 8. Ninety-six hour LC50 (µg/l) and fractions of this toxic unit for 30-d old fathead minnows exposed to individual metals and to a metal mixture in Lake Superior water.

As	Cd	Cr	Cu	Hg	Pb
		Individual Metal Tests			
12,600 (9,900-15,900)[a]	13.2 (10.9-15.9)	43,300 (36,600-51,300)	96 (83-111)	172 86-347)	2,100 (1,100-4,000)
		Metal Mixture Test			
1,200 (1,000-1,500)	1.2 (1.0-1.5)	4,550 (3,710-5,590)	7.8 (6.6-9.2)	13.9 (11.4-17.0)	125 (104-149)
		Fraction of Toxic Unit[b]			
0.10	0.09	0.12	0.08	0.08	0.06

[a] Ninety-five percent confidence limits

[b] Mixture LC50 divided by individual metal LC50 (sum of fractions = toxic unit of 0.53)

Table 9. Forty-eight hour LC50 (μg/l) and fractions of this toxic unit for < 24-hr old *C. dubia* exposed to individual metals and to a metal mixture in Lester River water.

As	Cd	Cr	Cu	Hg	Pb
		Individual Metal Tests			
1,448 (1,214-1,727)[a]	27.3 (21.9-34.1)	144 (110-189)	66 (55-81)	8.8 (6.6-11.8)	248 (212-290)
		Metal Mixture Test			
344 (303-390)	6.1 (5.3-7.0)	34.7 (30.6-39.4)	16.8 (14.7-19.1)	2.3 (2.0-2.6)	60.7 (53.0-69.4)
		Fraction of Toxic Unit[b]			
0.24	0.22	0.24	0.26	0.26	0.25

[a] Ninety-five percent confidence limits

[b] Mixture LC50 divided by individual metal LC50 (sum of fractions = toxic unit of 1.47)

Table 10. Survival and growth of fathead minnows exposed to individual metals for 32 days in Lake Superior water.

Measured Concentration (μg/l)	Embryo Hatchability (%)	Normal Larvae at Hatch (%)	Survival (%)	Weight (mg)
Arsenic				
<1.0[a] (control)	100+0.0	100+0.0	84+5.0	109+3.0
1,340+79[b]	100+0.0	100+0.0	100+0.0	91+2.0*
2,520+78	100+0.0	100+0.0	87+0.0	100+1.0
4,400+199	100+0.0	100+0.0	77+14	88+8.0*
8,340+428	100+0.0	100+0.0	84+5.0	48+5.0*
16,410+791	100+0.0	97+5.0	0*	-[d]
Cadmium				
<0.1[a] (control)	100+0.0	100+0.0	93+0.0	96+1.0
1.8+0.1[b]	100+0.0	100+0.0	97+5.0	94+2.0
3.8+0.3	100+0.0	100+0.0	97+5.0	93+3.0
7.6+0.6	100+0.0	97+4.9	77+14	107+13
15.6+1.4	100+0.0	0*	2.0+4.0*	44[c]*
29.5+2.3	97+4.9	0*	0*	-[d]
Chromium				
<20[a] (control)	100+0.0	97+5.0	94+9.0	123+6.0
220+18[b]	100+0.0	100+0.0	97+5.0	113+4.0
435+20	100+0.0	97+5.0	97+5.0	113+8.0
863+48	100+0.0	94+9.0	94+9.0	115+6.0
1,630+72	100+0.0	100+0.0	100+0.0	105+1.0
3,170+130	100+0.0	100+0.0	90+4.0	86+11*
Copper				
<2.0[a] (control)	100+0.0	100+0.0	90+4.0	128+1.0
4.8+0.3[b]	100+0.0	100+0.0	100+0.0	112+16
8.0+1.3	100+0.0	100+0.0	63+14*	74+5.0*
16.0+1.9	100+0.0	100+0.0	47+19*	27+10*
31.0+2.8	94+9.0	0*	2.0+4.0*	9[c]*
65.0+5.0	100+0.0	0*	0*	-[d]
Mercury				
<0.05[a] (control)	100+0.0	100+0.0	100+0.0	124+1.0
0.23+0.03[b]	100+0.0	100+0.0	94+9.0	121+13
0.36+0.05	100+0.0	100+0.0	93+0.0	123+1.0
0.65+0.07	94+9.0	100+0.0	80+0.0	120+5.0
1.21+0.06	100+0.0	100+0.0	94+9.0	80+4.0*
2.24+0.08	100+0.0	70+0.04*	79+11	28+3.0*
Lead				
<1.0[a] (control)	100+0.0	100+0.0	86+9	122+13
69+5[b]	100+0.0	100+0.0	90+14	110+10
123+10	100+0.0	100+0.0	94+9	104+14
232+20	100+0.0	100+0.0	64+23	123+26
466+65	100+0.0	0*	0*	-[d]
946+145	100+0.0	0*	0*	-[d]

[a] Detection limit

[b] Mean ± standard deviation

[c] Weight of one surviving fish

[d] No value because all animals died

* Significant decrease from the control (P = 0.05)

Table 11. MATCs and acute to chronic ratios for fathead minnows and daphnids exposed to individual metals.

Test Species	As	Cd	Cr	Cu	Hg	Pb
			MATCs (ug/l)			
Fathead minnow[a]	3,330	10.9	2,270	6.2	0.89	329
C. dubia[b]	1,140	2.2	63	45	12	52
			Acute/Chronic Ratios[c]			
Fathead minnow	3.8	1.2	19	16	193	6.4
C. dubia	1.3	12.4	2.3	1.5	0.73	4.8

[a] Tests were conducted for 32-d in Lake Superior water.

[b] Tests were conducted for 7-d in Lester River water.

[c] Acute LC50 (individual values, tables 8 and 9) divided by the MATCs.

Table 12. Survival and number of young per female of *C. dubia* exposed to individual metals after 7 days in Lester River water.

Measured Concentration (ug/l)	Survival %	Young per Female	Scaled T-Statistic of Overall Comparison
Arsenic			
< 1.0[a] (control)	80+42	19.8+1.5	-[b]
102+6[c]	100+0.0	19.8+1.4	0.44
188+6	90+32	14.8+1.5	0.97
404+15	100+0.0	14.6+1.8	1.00
793+52	100+0.0	18.3+1.2	0.54
1636+58	50+53	6.2+0.5*	3.36*
3250+60	0+0.0*	-[d]	2.08*
Cadmium			
< 0.25[a] (control)	100+0.0	25.4+1.6	-[b]
0.6+0.1[c]	100+0.0	22.6+1.5	0.50
1.5+0.1	90+32	24.2+1.4	0.50
3.2+0.2	100+0.0	18.8+1.7*	1.15*
6.6+0.3	100+0.0	11.3+1.0*	2.90*
13.4+1.0	90+32	5.3+1.2*	4.04*
25.6+2.1	0+0.0*	7.5+0.9*	4.14*
Chromium			
< 1.0[a] (control)	100+0.0	18.3+1.1	-[b]
10.3+1.1[c]	90+32	17.2+0.9	0.53
21.0+1.0	100+0.0	17.3+2.2	0.16
41.4+2.4	90+32	18.9+1.2	0.15
94.8+5.7	20+42*	14.2+0.8	1.20*
171+11	0+0.0*	5.5+1.9*	-[e]
345+2	0+0.0*	-[d]	2.47*
Copper			
3.4+0.3[a] (control)	90+32	29.0+0.8	-[b]
9.9+0.3[c]	90+32	26.2+1.4	0.70
16.7+0.3	100+0.0	28.2+0.8	0.53
31.6+2.6	100+0.0	27.5+1.9	0.53
63.9+2.5	80+42	10.0+3.0*	2.28*
122+7	60+52	1.5+0.5*	11.86*
237+1	0+0.0*	-[d]	2.08*
Mercury			
< 0.05[a] (control)	100+0.0	29.6+2.0	-[b]
0.55+0.02[c]	100+0.0	25.9+1.0	0.66
1.05+0.01	100+0.0	27.8+1.0	0.32
2.07+0.03	80+42	29.9+1.6	0.45
4.36+0.10	100+0.0	28.5+1.5	0.18
8.69+0.24	100+0.0	27.7+1.7	0.29
16.9+0.15	20+42*	6.7+3.9*	-[e]
Lead			
< 1.0[a] (control)	90+32	19.3+0.6	-[b]
36+1[c]	100+0.0	21.3+0.7	0.98
74+3	100+0.0	16.6+0.8*	1.16*
165+9	30+48*	16.0+1.3*	1.55*
321+7	30+48*	8.0+2.4*	-[e]
608+22	0+0.0*	-[d]	2.10*
1284+3	0+0.0*	-[d]	2.10*

[a] Detection limit

[b] No value because other values are compared to the control

[c] Mean ± standard deviation

[d] No value because all animals died

[e] Too few degrees of freedom to calculate value

* Significant decrease from the control (P = 0.05)

Table 13. Measured water concentrations for a test with fathead minnows exposed to metal mixtures at multiples of the MATC in Lake Superior water (Test 1).

	Treatment (μg/l)					
Metal	Control	1/16 MATC	1/8 MATC	1/4 MATC	1/2 MATC	MATC
As	< 1.0[a]	199±6[b]	403±21	805±54	1,545±64	3,203±158
Cd	< 0.1	0.6±0.1	1.3±0.1	2.6±0.3	5.3±0.5	10.8±0.9
Cr	< 1.0	135±9	272±18	557±30	1,137±30	2,237±61
Cu	1.0	1.3±0.4	1.7±0.3	2.4±0.3	3.8±0.4	6.7±0.7
Hg	< 0.05	0.05±0.01	0.1±0.02	0.2±0.03	0.4±0.03	0.7±0.1
Pb	< 1.0	16.3±0.9	34±1.4	70±2.3	143±6	237±91

[a] Detection limit.

[b] Mean ± standard deviation.

Table 14. Measured water concentrations for a test with fathead minnows exposed to metal mixtures at multiples of the MATC in Lake Superior water (Test 2).

	Treatment (μg/l)					
Metal	Control	1/12 MATC	1/6 MATC	1/3 MATC	2/3 MATC	4/3 MATC
As	< 1.0[a]	315±23[b]	575±28	1,153±65	2,156±128	3,974±380
Cd	< 0.1	0.8±0.1	1.6±0.1	3.3±0.2	6.7±0.3	12.2±1.3
Cr	< 1.0	212±19	400±29	792±31	1,600±111	2,894±130
Cu	1.0	1.6±0.3	2.0±0.3	3.0±0.4	4.7±0.6	8.0±1.0
Hg	< 0.05	0.08±0.01	0.14±0.01	0.3±0.04	0.6±0.06	1.0±0.16
Pb	< 1.0	24±19	46±4	93±11	185±17	339±32

[a] Detection limit.

[b] Mean ± standard deviation.

Table 15. Measured water concentrations for a test with *C. dubia* exposed to metal mixtures at multiples of the MATC in Lester River water.

	Treatment (μg/l)						
Metal	Control	1/12 MATC	1/6 MATC	1/3 MATC	2/3 MATC	MATC	4/3 MATC
As	< 1.0[a]	84.3±1.6[b]	171±2	351±10	707±13	1,027±18	1,426±42
Cd	< 0.1	0.17±0.03	0.39±0.05	0.69±0.08	1.30±0.1	1.9±0.1	2.5±0.2
Cr	< 1.0	5.5±0.4	10.2±0.3	20.7±0.4	41.2±0.9	62.9±1.1	82.1±1.6
Cu	4.9	8.4±0.8	11.8±0.9	20.0±0.8	35.6±0.7	48.6±1.5	66.3±3.4
Hg	< 0.05	0.9±0.1	1.8±0.1	3.8±0.03	7.6±0.1	10.0±0.1	15.2±0.5
Pb	< 1.0	4.0±0.2	7.1±0.1	15.1±1.3	30.0±1.3	44±1.8	59.2±2.8

[a] Detection limit.

[b] Mean ± standard deviation.

Table 16. Survival and growth of fathead minnows exposed to metal mixtures at multiples of the MATC for 32-days in Lake Superior water.

Treatment[a]	Embryo Hatchability (%)	Normal Larvae at Hatch (%)	Survival (%)	Weight (mg)
		Test 1		
Control	100±0.0[b]	100±0.0	87±9	109±5
1/16 MATC	100±0.0	100±0.0	86±10	106±13
1/8 MATC	100±0.0	100±0.0	74±9	114±3
1/4 MATC	100±0.0	100±0.0	83±14	102±5
1/2 MATC	100±0.0	100±0.0	63±14	104±23
MATC	100±0.0	0±0.0*	10±4*	43±24*
		Test 2		
Control	100±0.0	100±0.0	84±5	114±13
1/12 MATC	100±0.0	100±0.0	90±14	107±13
1/6 MATC	100±0.0	100±0.0	87±0.0	118±6
1/3 MATC	100±0.0	100±0.0	87±0.0	106±2
2/3 MATC	100±0.0	100±0.0	50±24	85±14
4/3 MATC	97±4.9	0±0.0*	3.5±4.9*	9±0*

[a] Treatment corresponds to measured water concentrations in Tables 13 and 14, respectively.

[b] Mean ± standard deviation.

* Significant decrease from the control (P = 0.05)

Table 17. Survival and number of young per female of *C. dubia* exposed to metal mixtures at multiples of the MATC for 7 days in Lester River water.

Treatment[a]	Survival (%)	Young per Female	Scaled T-statistic of Overall Comparison
Control	100±0.0[b]	17.8±1.5	–[c]
1/12 MATC	90±32	18.0±2.1	0.03
1/6 MATC	90±32	19.6±1.8	0.53
1/3 MATC	90±32	6.9±1.5*	2.16*
2/3 MATC	0±0.0	–[d]	2.47*
MATC	0±0.0	–[d]	2.47*
4/3 MATC	0±0.0	–[d]	2.47*

[a] Treatment corresponds to measured water concentration in Table 15.

[b] Mean ± standard deviation.

[c] No value because other values are compared to the control.

[d] No value because all animals died.

* Significant decrease from the control (P = 0.05).

Table 18. Chronic concentrations[a] (µg/l) and fractions of this toxic unit based on a 50 percent reduction in weight and the number of young per female for fathead minnows and *C. dubia*, respectively, exposed to individual metals and a metal mixture.

Species	As	Cd	Cr	Cu	Hg	Pb
			Individual Metal Tests			
Fathead minnow	7,079	14.5	3,467	11	1.4	331
C. dubia	1,259	6.0	132	56	12.6	269
			Metal Mixture Test			
Fathead minnow	2,630	8.9	1,998	5.9	0.7	234
C. dubia	275	0.6	17	18	3.2	15
			Fraction of Toxic Unit[b]			
Fathead minnow	0.37	0.61	0.58	0.54	0.50	0.71
C. dubia	0.22	0.10	0.13	0.32	0.25	0.06

[a] Concentrations are recalculated from log units at the 50 percent value (Figures 1 and 2).

[b] Mixture value divided by individual metal value (sum of fractions = toxic units of 3.31 and 1.08 for fathead minnows and daphnids, respectively).

Figure 1.

Percent reduction (50%) in weight and number of young per female of fathead minnows and *C. dubia*, respectively, exposed to individual metals for 32 and 7 days. Open symbols = significant decrease from control (P = 0.05), X = total fathead mortality, S = steep slope.

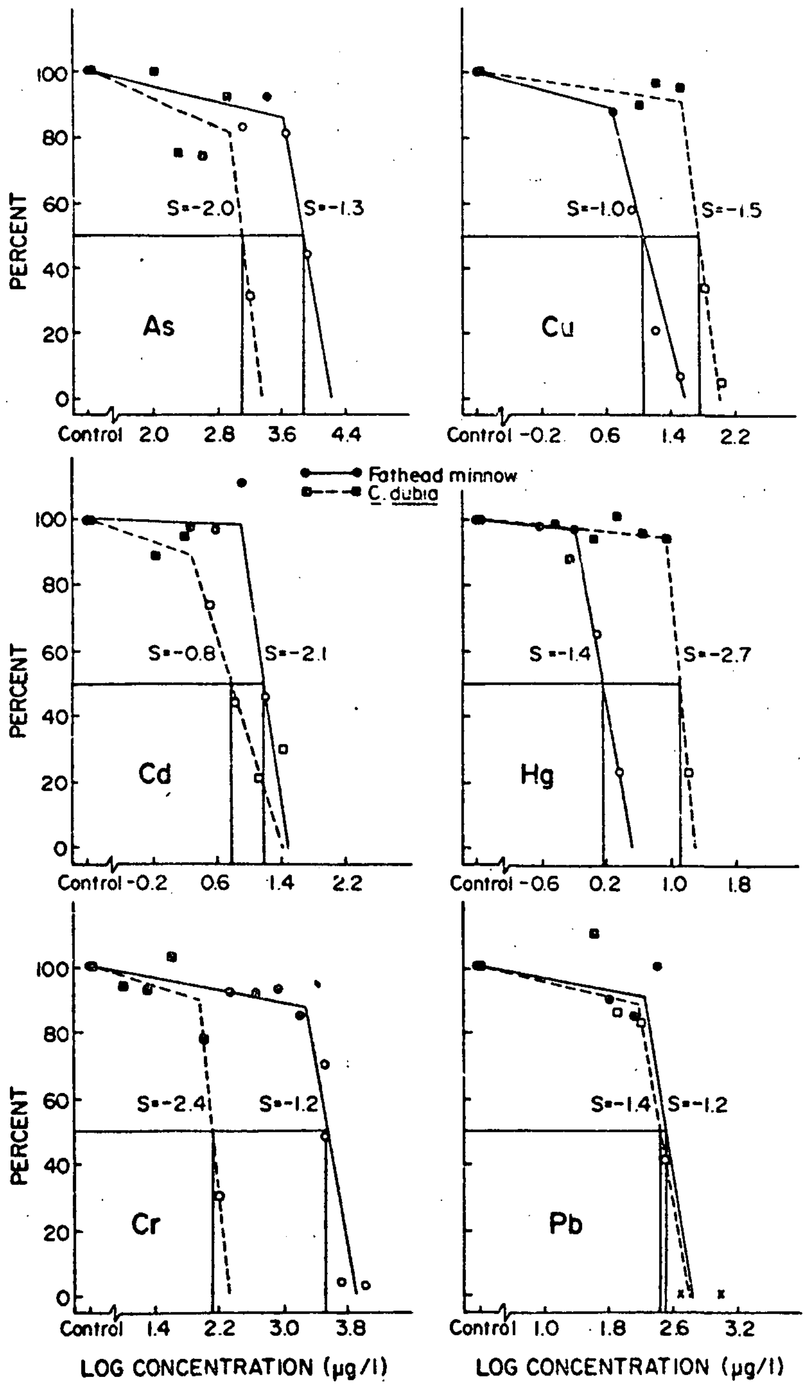

Figure 2.

Percent reduction (50%) in weight and number of young per female of fathead minnows and C. dubia, respectively, exposed to a mixture of metals for 32 and 7 days. Open symbols = significant decrease from controls (P = 0.05), S = steep slope.