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The Sensitivity of Adult, Embryonic, and Larval Carp *Cyprinus carpio* to Copper

Florence L. Harrison and David W. Rice, Jr.

Prepared for
U.S. Nuclear Regulatory Commission

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ABSTRACT

The copper sensitivity of adult, embryonic, and larval stages of carp Cyprinus carpio was determined using flow-through bioassay methods. Carp adults, embryos, and larvae were exposed continuously to copper concentrations that ranged from those producing an immediate effect to those producing none. Carp embryos were obtained after we induced adults to spawn. Exposure of embryos began at 4 to 6, 8 to 10, and 20 to 24 h after fertilization and continued until hatching. Exposure of larvae began 6 to 8 h after hatching and continued until yolk sac absorption. From the family of curves of cumulative mortality versus duration of exposure, median lethal times were determined and used to construct comparative toxicity curves.

The 24-h LC50s show the order of acute copper sensitivity of carp life-history stages, measured in micrograms per liter, as: larvae (180 µg/L) > embryos (240 µg/L) > adults (540 µg/L). Estimated incipient lethal concentrations give the order of subacute copper sensitivity of carp life-history stages as: larvae (110 µg/L) > adult (120 µg/L) > embryo (230 µg/L). The sensitivity of carp embryos to copper changed as embryogenesis progressed; for example, embryos were approximately twice as sensitive before as after blastopore closure.

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FOREWORD

This study is part of a larger research project that has three purposes: (1) to study the behavior of potentially toxic substances introduced into surface waters from nuclear power stations, (2) to determine the magnitude of the impact of these substances on representative economically important aquatic species, and (3) to develop models to predict the behavior and impact of these discharged substances. The initial thrust of the research was directed toward investigating the impact of cooling systems' corrosion products, in particular copper. Copper is of special interest because of its documented toxicity to aquatic organisms.

This investigation was funded by the Office of Nuclear Regulatory Research Division of Safeguards, Fuel Cycle and Environmental Research, under FY 1977 Nuclear Regulatory Research Order No. 60-78-04.

EXECUTIVE SUMMARY

Experiments were performed to determine the acute toxicity of copper to adults, newly hatched larvae, and embryos of the common carp Cyprinus carpio. Experimental animals were continuously exposed to copper at concentrations that ranged from those producing an immediate effect to those producing none. Concentrations of total and Chelex-100-labile copper were measured in the bioassay water. In addition, we determined the amount of copper in muscle and liver tissue of some experimental adult carp.

Sensitivity of adult carp was assessed in three different experiments. The toxicity curve generated from the combined mortality data indicated that the median lethal concentration after 48 h ($LC50_{48}$) was 170±20 µg Cu/L (micrograms of copper per liter) and the incipient lethal concentration (ILC50) was 120±3 µg Cu/L. Analyses of the bioassay water showed that more than 65% of the copper was in Chelex-100-labile forms. Copper concentrations in muscle and liver tissues taken from carp exposed to increased copper concentrations in the bioassay water did not significantly differ from concentrations in the same tissues in control animals.

Toxic response to copper was determined for groups of embryos that were 4 to 6, 8 to 10, and 20 to 24 h old. The sensitivity to copper appeared to decrease with increased age. The $LC50_{48}$ and ILC50 for 6- to 8-h carp embryos was 230 µg Cu/L. The percent of larvae that hatched decreased with increasing copper concentrations in the water and increased with increasing age of the embryo at the time the experiment began. More than 65% of the copper in the bioassay water was in Chelex-100-labile forms.

Effects of copper on newly hatched larvae were determined; the $LC50_{48}$ and ICL50 were 120 and 110 µg Cu/L, respectively. No change in sensitivity with age was established. Copper in the bioassay water was primarily in Chelex-100-labile forms.

1. Effects of Sublethal Exposure on Reproductive Capacity.

If carp live in the discharge zones of nuclear power stations whose cooling systems contain copper alloys, the fish are exposed throughout their entire life cycle to small increases in copper concentrations in the water. Information is needed about the effects of such exposures on reproductive capacity. Carp early life-history stages from pristine and polluted ecosystems must be examined to determine whether there are differences in mortality rates, number of abnormal individuals, and percent hatching.

2. Effects of Pollutant Interactions.

Carp living in the discharge area of nuclear power stations may be exposed to increased levels of chlorine, dispersants, and detergents as well as to copper and increased temperatures. Some information is available on the response of the fish at various life stages to these factors separately, but little or no data are available on the interactions of these environmental stresses. The synergistic effects of copper, chlorine, other chemicals, and temperatures on the carp during early life stages must be defined.

3. Detoxification of Copper in Carp.

Copper concentrations in muscle and liver tissues of carp were highly variable and did not appear to be related to exposure concentrations. The effect of increased concentrations of copper in tissues may not be related to the total copper present but to the concentration of an active form. Information is available indicating that heavy metals can be detoxified by complexing with metallothioneins; this reduces inhibition of metalloenzymes. Data are needed on the concentrations of copper in the metallothionein and metalloenzyme subcellular pools of adult carp exposed to increased concentrations of copper and on the relationship of the organo-metal concentrations to deleterious effects and total tissue copper concentrations.

INTRODUCTION

Effluents discharged from nuclear power stations that have copper alloys in the heat exchangers of their cooling systems may contain copper in concentrations that are deleterious to some aquatic organisms (Roosenberg, 1969; Martin and Knauer, 1973). Generally the differences in copper concentrations between influent and effluent waters are small (Young et al., 1977; Harrison et al., 1980). The exceptions are during start-up of water circulating through the condenser tubing after an enforced shutdown and when the mode of operation is being changed from open to closed cycle (Harrison 1979; Harrison et al., 1980).

Discharge zones of nuclear power stations located on freshwater ecosystems generally contain a rich flora and fauna. Many populations flourish in, and others are attracted to, the warmed waters. Among the fishes inhabiting the discharge zone of power stations, carp are a common species. It is not uncommon for this fish to form a stable population and breed in and near the effluent waters (Eder and Carlson, 1977).

The carp Cyprinus carpio is a native of Asia, where it has been cultured for thousands of years. It is highly esteemed for food and as a result was introduced to many countries. Although carp are generally not considered desirable sports fish in the United States, they are cultured in this country and when raised in ponds and fed well the flavor compares favorably with other species (Clemens, 1968).

Considerable information is available on the life history, growth, habits, and cultivation of carp (Clemens, 1968; Jester, 1974; Eder and Carlson, 1977). In an effort to increase productivity, attention has been directed to the artificially induced spawning of adults and growth of the early life stages (Clemens and Sneed, 1962; Makeyeva, 1975; Chaudhuri, 1976; Bhowmick et al., 1977). The literature contains some data on the effects of copper on carp. However, this information is confined to juvenile and adult stages and is restricted primarily to sublethal effects (Ozaki et al., 1972; Leonte, 1973; Labat et al., 1974, 1976a, 1976b).

The chemical form of copper to which fishes are exposed may play an important role in the toxic response (Lee, 1973; Neff and Anderson, 1976; Chapman and McCrady, 1977). Copper can exist in many forms in water.

Labile forms include ions, ion pairs, and weakly associated organic and inorganic complexes (Batley and Florence, 1976; Harrison et al., 1980). Although current copper emission standards are defined in terms of the total copper concentration in the water (California State Water Resources Control Board, 1972 and 1976; Klapow and Schueller, 1978), it is known that copper is less toxic to marine organisms when it is complexed (Lewis et al., 1973; Davey et al., 1973; Sunda and Guillard, 1976; Harrison et al., 1981a). Ionic copper was suggested as the form most toxic to freshwater fishes (Pagenkopf et al., 1974).

Because productivity can be affected both in the environment and in culture by potentially toxic pollutants, it is important to know the detrimental effects of these substances on populations at different life stages. The principal object of this study was to determine the toxicity of copper to developing embryos, newly hatched larvae, and adults of common carp. We will compare the sensitivity to copper at the different stages to identify the critical link in the life cycle and determine the amounts and the major chemical forms of copper in the bioassay waters.

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

Adult Carp

The adult fish used in the experiments were trapped in wire cages placed in water about 1-m deep in the delta region of the San Joaquin River near Patterson, California. The traps were emptied at 1- to 2-d intervals and the fish transported to Lawrence Livermore National Laboratory (LLNL) where they were placed in 760-L bioassay tanks with recirculating water. The fish were held in the tanks for 3 d before the test began. They were not fed during the acclimation or testing periods. Before start-up of the experiment, the tanks were cleaned of debris and the water almost entirely exchanged. The mean wet weight of the fish used was 329.9±160.1[a] g and the mean standard length was 22.9±4.3 cm.

[a]For this and subsequent values, the mean ± one standard deviation is given.

Embryonic and Larval Carp

Two groups of embryos were used in the experiments. One was collected from the shallow waters of Clear Lake, California, in July 1978. The embryos were removed from plant material and transported to LLNL in plastic bags partially filled with Clear Lake water that was held at about 22°C in insulated containers.

The second group of embryos was obtained by artificially spawning fish in the laboratory. The procedure used to induce spawning was modified from those reported in the literature (Clemens and Sneed, 1962; Chaudhuri, 1976). Fresh pituitary glands were dissected from adult male and female carp after stunning the fish with a sharp blow to the head. A known number of glands were ground in 1 to 2 ml of physiological saline in a tissue homogenizer and then the supernatant was removed for injection into ripe males and females.

The fish were given a priming injection of approximately one-third of a prescribed dose. This was followed 24-h later by the final injection of the remaining two-thirds. For every 2 kg of fish, females received, in total, the extract from two to three glands, whereas males received the extract from one to two glands. Generally, two males and three females were injected. These were maintained after injection at 24°C in a 760-L tank. Spawning usually occurred after 48 h, and then embryos could be removed from the walls of the tank.

EXPOSURE CONDITIONS

Adult Carp

Bioassays on adult carp were conducted in 760-L fiberglass tanks through which water flowed at a rate of 1.5±0.2 L/min; 90% replacement required 24 h (Fig. 1a). Five to ten fish were present in each tank at the start of exposure. To provide good mixing and aeration, each tank was equipped with several air stones and a recirculating pump fitted with a plastic impeller and all-plastic tubing. The desired exposure solution in each tank was obtained by pumping water and a solution of copper chloride to a mixing area of the tank. The rates at which the water and copper solution were pumped were 1500±200 and 0.24±0.02 ml/min, respectively. The exposure solution was delivered to the bottom and side of the tank.

Embryonic and Larval Carp

Carp embryos and larvae were exposed to copper in 500-ml, clear-glass flow-through exposure chambers (Fig. 1b) that, in turn, were immersed in a constant-temperature water bath. From 20 to 25 embryos or larvae were present in each exposure chamber. Water containing a known concentration of copper was pumped into each chamber from a 19-L plastic jug at a rate of 5 ml/min. Approximately 5 h were required to replace 95% of the water in the exposure chamber. The mixture of seawater and copper in the 19-L plastic jugs was prepared daily.

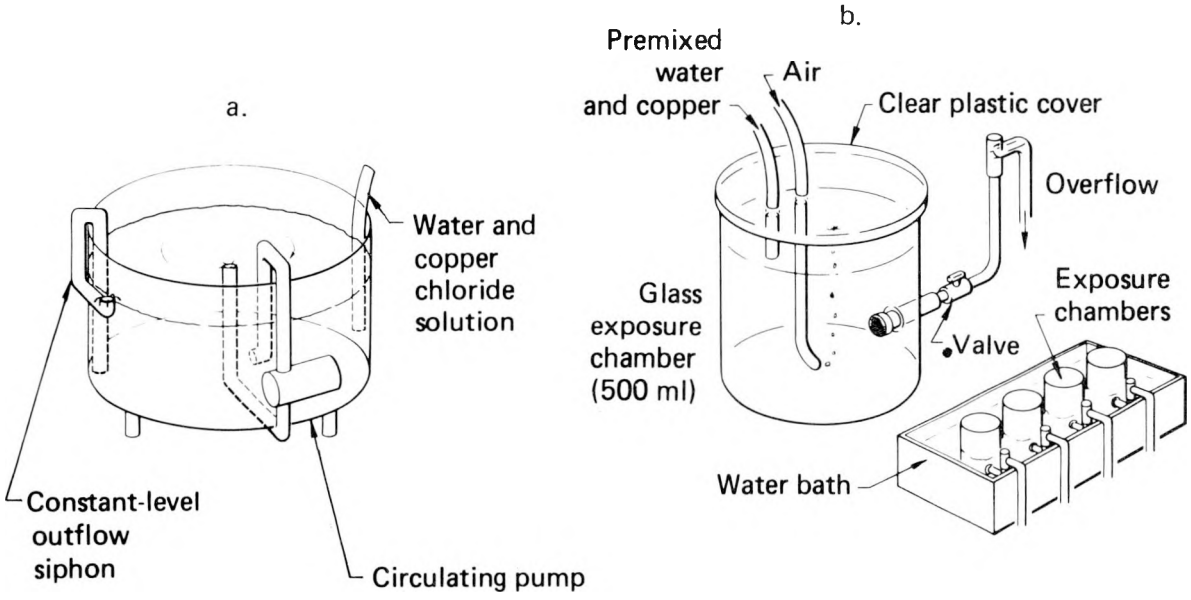

FIG. 1. a. Diagram of the apparatus used to study the effect of copper concentrations on adult carp. The water in the 760-L bioassay tank is maintained at a constant level with an outflow siphon. The water and copper chloride solutions that enter at prescribed rates are circulated throughout by a pump at the bottom of the tank. All parts of the apparatus are plastic or plastic coated. b. Diagram of the apparatus used to study the effects of copper on embryonic and larval carp. The water level in the 500-ml exposure chamber is maintained with an outflow siphon covered with nylon netting and fitted with a valve that could be closed when the chamber is removed from the water bath in which the chamber is kept. Embryos were aerated with a gentle stream of air, but larvae were not. All parts are plastic or plastic coated.

The height of water in the exposure chambers was maintained by a constant-level outflow siphon. The mouth of the siphon, located at the base of the exposure chamber, was covered with nylon netting (265-μm pore size) to prevent the loss of organisms. The bottom outlet from each exposure chamber was fitted with a valve that could be closed to allow the chamber to be removed from the water bath. By removing the exposure chambers and illuminating them from the side, both live and dead organisms could be observed more accurately. Observations were made every 2 to 4 h during the copper exposure. When embryos were exposed, a gentle stream of bubbles was delivered to the bottom of the exposure chamber; when larvae were exposed, no aeration was used. Overhead illumination was provided by the fluorescent lighting in the laboratory and followed the outdoor photoperiod.

Exposures continued (1) until all animals died; (2) in the case of the embryos, until hatching was complete; or (3) in the case of the larvae, until the yolk sac was absorbed. Larvae were considered dead when they failed to respond to a prod with a polished glass rod. Cumulative mortality with time, percent hatching, and the stage of development at mortality were taken to be indices of the toxic effect of copper.

WATER ANALYSES

Water Characteristics

Water delivered to the bioassay system was filtered through activated charcoal to remove chlorine. The primary source of the water was melting snow in the Sierra-Nevada Mountains (Hetch-Hetchy reservoir water); it is low in total dissolved solids (Table 1) and fully saturated with oxygen.

Temperature

Water temperature in the bioassay system was recorded on a regular basis. In the adult tests, the average temperature was 24.1±1.5°C; in the embryo and larvae tests, it was 19.5±0.7°C.

TABLE 1. Characteristics of water delivered to the bioassay system.

Characteristics	Mean	One standard deviation
pH	8.9	0.2
Total hardness as $CaCO_3$ (mg/L)	17.0	1
Calcium hardness as $CaCO_3$ (mg/L)	14.0	2
Total alkalinity	13.0	3
Phenolphthalein alkalinity	4.0	5
Chloride as NaCl (mg/L)	20.0	6

Copper

Both total and labile copper concentrations were measured. Total copper in samples containing $\geq$ 200 µg Cu/L was determined by direct aspiration of water into the flame of a Model 303 Perkin Elmer atomic absorption spectrophotometer (AAS) with deuterium background corrector; total copper in samples containing < 200 and > 10 µg Cu/L was determined by directly injecting a sample aliquot into an HGA 2100 model graphite furnace after diluting the sample one-to-one with ultrapure 2 N HNO_3. Labile copper, defined as that fraction passing through a 0.45-µm filter and retained by Chelex-100 resin, was determined by the method of Riley and Taylor (1968). Eluants from the columns were analyzed directly in the flame or the graphite furnace of the AAS.

TOXICITY CURVES

The primary measure of toxicity for this study was the copper concentration resulting in 50% mortality over a given time (median lethal concentration, LC50). This toxicity measure was determined by performing weighted least-squares estimates and maximum likelihood estimates for the parameters α and β in the logit model

$$P_{(x)} = \frac{e^{\alpha + \beta x}}{1 + e^{\alpha + \beta x}} \quad . \tag{1}$$

The linear transform of the logistic function is

$$\text{logit } P = \ln \left(\frac{P_{(x)}}{1-P_{(x)}} \right) = \alpha + \beta x \ ; \tag{2}$$

thus if logit P is plotted against x, the points should fall on a straight line with α as the intercept and β as the slope (Berkson, 1953). The weighted least-squares estimates of α and β were found first and then used as the initial estimates for the maximum likelihood estimates (Koshiver and Moore, 1979).

In our calculation of LC50, $P_{(x)}$ is the proportion responding at dose x. Our method followed that outlined by the American Public Health Association (1976), except that the logit analysis was used in place of a probit analysis. For each observation time, an estimated LC50 value was determined. The series of LC50 values obtained were used to construct a toxicity curve to estimate the incipient lethal concentration (ILC).

TISSUE ANALYSIS FOR COPPER

Adult carp were weighed and the standard length measured. The fish were then placed in labeled plastic bags, frozen, and later dissected. Before copper analysis, partially thawed fish were dissected and samples of liver and muscle removed. The tissues were dried at 100°C, ashed at 450°C, and dissolved in a mixture of concentrated HCl and HNO_3 (3:1). This solution was analyzed for copper with the AAS. Copper standards were dissolved in the same acid mixture. Both standard and unknown measurements were corrected for the reagent blank.

ADULT CARP

Mortality Tests

Three different experiments were performed to assess the sensitivity of adult carp to copper. In the first test (Exp. I), groups of fish were exposed to concentrations of either 1130, 850, 385, 235, or <1 µg Cu/L. The family of curves generated is shown in Fig. 2a; time for 50% mortality was related to the exposure concentrations.

In the second experiment (Exp. II), the highest exposure concentration was 850 µg Cu/L (Fig. 2b). We elected to include an exposure concentration of 120 µg Cu/L in this experiment in the hope that the ILC could be obtained. The ILC50 is defined as that level of toxicant lethal for 50% of individuals exposed for periods sufficiently long so that acute lethal action has ceased (Sprague, 1970). It is sometimes referred to as the lethal threshold concentration and is often equivalent to the 4-day LC50 (Sprague, 1970).

Exposure concentrations used in the third test (Exp. III) were 235, 120, 60, and <1 µg Cu/L (Fig. 2c). This experiment was performed to better define mortality at the lower exposure concentrations. Data from all three experiments were combined to generate composite mortality curves (Fig. 3). The $LC50_{48}$ was 170±20[a] µg Cu/L and the ILC50 was 120±3 µg Cu/L. The ILC50 was reached prior to 96 h and the ILC50 used as an estimate of the $LC50_{96}$.

Copper Concentrations in Bioassay Water

The concentrations of total and labile copper and the number of fish exposed in the three experiments from which the data were pooled are presented in Table 2. Exposure concentrations were relatively constant and most of the copper was in labile forms.

[a]The LC50 values for this and subsequent ones are given with 95% confidence limits.

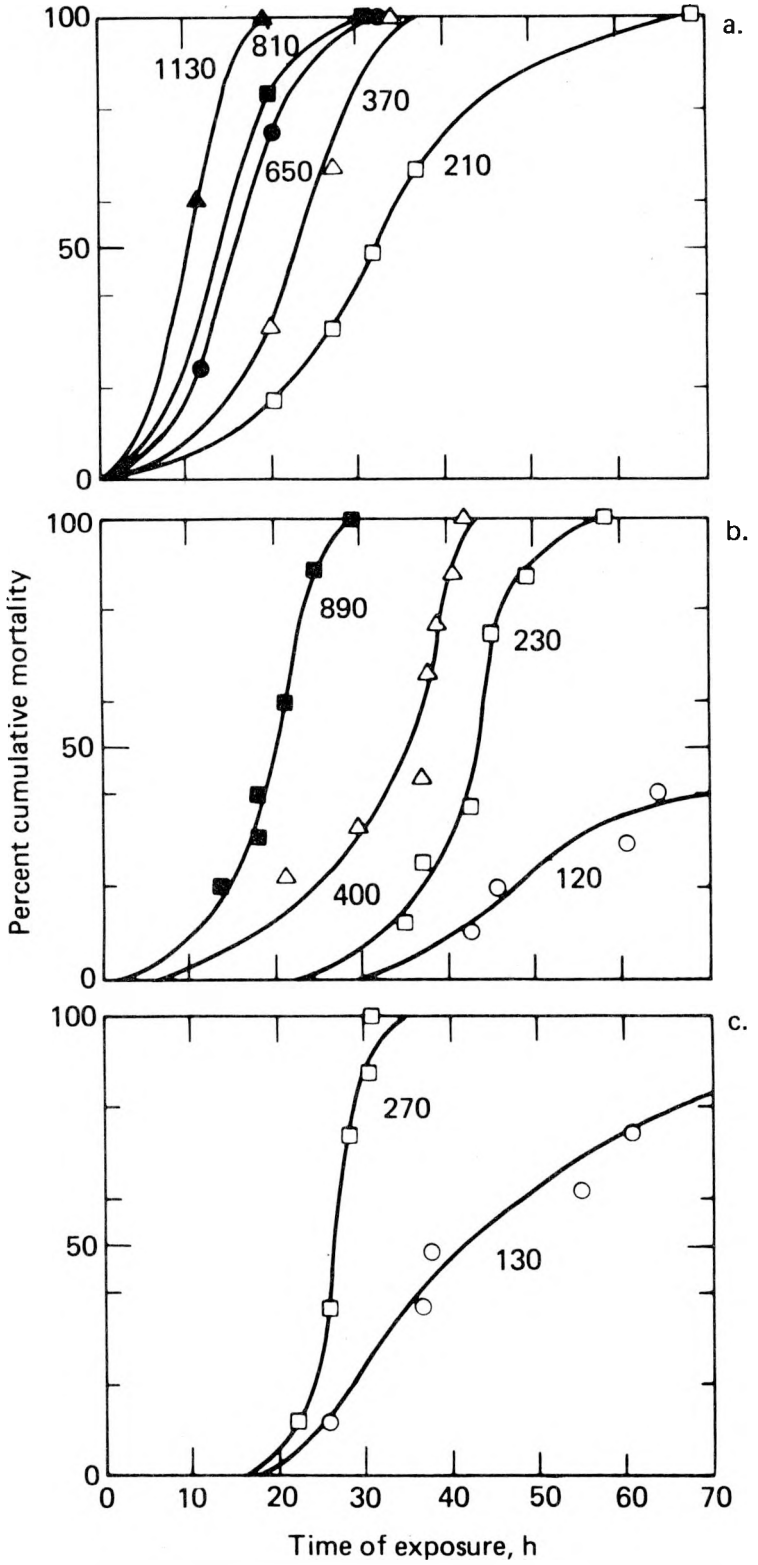

FIG. 2. Percent cumulative mortality of carp adults continuously exposed to copper. Numbers next to the curves give the exposure concentrations in µg Cu/L. a. Exp. I, no mortalities occurred in control water. b. Exp. II, no mortalities occurred in control and 55 µg Cu/L waters. c. Exp. III, no mortalities occurred in control and 70 µg Cu/L waters.

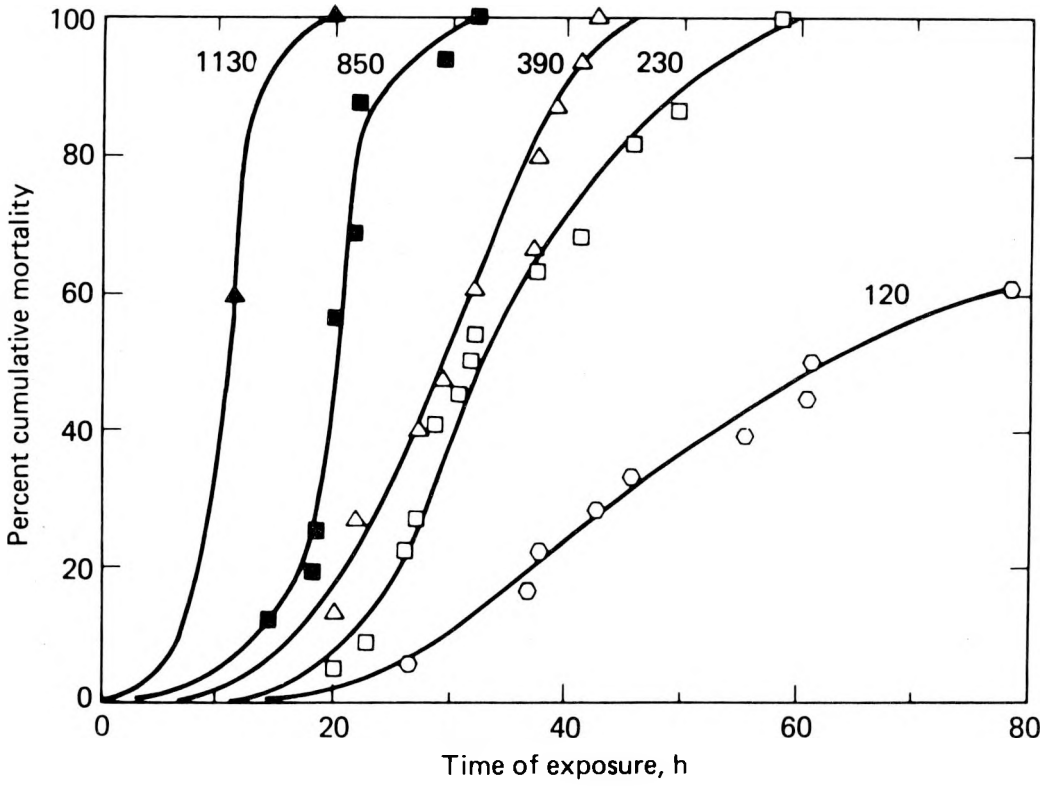

FIG. 3. Percent cumulative mortality of carp adults continuously exposed to copper. Data from Exp. I, II, and III pooled. Numbers next to the curves give the exposure concentrations in µg Cu/L. No mortalities occurred in control and 60 µg Cu/L waters.

TABLE 2. Copper concentrations in the adult carp bioassay water, and number of fish exposed at each concentration.

Measured copper,[a] µg/L	Number of analyses	Labile copper, %	Number of fish exposed	Experiments pooled
130	1	--	5	I
848±80	4	65	10	I, II
385±19	4	75	15	I, II
234±36	8	89	23	I, II, III
124±17	10	73	18	II, III
62±19	11	73	14	II, III
0.7 (control)	2	71	17	I, II, III

[a] ± one standard deviation.

Copper Concentrations in Tissue

Copper concentrations in muscle and liver tissue from carp exposed to increased copper concentrations in the bioassay water and from control carp did not differ significantly (Table 3). Except for fish in the control and 60 µg Cu/L bioassay water, the duration of the exposure was less than 3 d and may have been too short for copper to build up in the muscle tissue.

The highest average concentration in liver tissue was detected in fish exposed to 60 µg Cu/L. However, copper concentrations were so variable from animal to animal within a group that the differences among average values were not significant.

TABLE 3. Copper concentrations in tissues of adult carp exposed to different copper concentrations in the bioassay water.

Copper in bioassay water, µg/L	Average exposure, d	Number of fish	Tissue copper, µg/g wet weight[a]	
			Muscle	Liver
850	0.9	8	0.52±0.19	1.08±0.54
390	1.1	5	0.35±0.11	1.68±1.74
230	1.5	5	0.38±0.20	1.37±0.86
120	2.6	7	0.25±0.09	1.57±1.11
60	14.0	5	0.48±0.10	3.37±2.34
Control	14.0	5	0.37±0.15	0.96±0.49

[a] ± one standard deviation.

EMBRYONIC AND LARVAL CARP

Mortality of Embryos

Toxic response to copper was determined for three groups of embryos; each group was at a different age when first exposed to copper. The youngest embryos exposed were 4- to 6-h old (Exp. IV). Mortality was dose related and was rapid in animals held in water at the three highest concentrations, that is, 960, 720, and 470 µg Cu/L (Fig. 4a). We detected more deaths during the hatching period for embryos in water containing 230 µg Cu/L than for those in water containing <1 or 100 µg Cu/L. The $LC50_{48}$ and ILC50 for carp embryos tested in Exp. IV were both 230 µg Cu/L. The embryo ILC50 was reached in approximately 24 h, and ILC50 may be used as an estimate of the $LC50_{96}$ or the LC50 hatching.

Another test was performed with embryos that were 8-h old (Exp. V). The mortality curves obtained for animals held in water containing 720 or 470 µg Cu/L were atypical; the death rate was low between 5 and 35 and then high between 35 and 45 h of exposure (Fig 4b). On examining the

embryos, we found that high mortalities occurred at the developmental stage when pigmentation was occurring and the heart beat starting. Embryos appear to be sensitive at this stage. Again, we detected a greater number of embryo mortalities during the hatching period in the water containing 230 µg Cu/L than in the water containing <1 or 100 µg Cu/L.

Approximately 24-h-old embryos were exposed also (Exp. VI). Only three concentrations of copper in the water were tested; 100% mortality occurred in water containing 3050 or 960 µg Cu/L before the hatching period was reached (Fig. 4c).

The sensitivity of carp embryos to copper appears related to their age at the time of exposure (Fig. 5). Embryos that were exposed to copper 4 to 6, 8 to 10, or 20 to 24 h after fertilization reached 50% mortality in water containing 960 µg Cu/L at 3, 5, and 16 h, respectively.

The effects of copper on the hatchability of carp embryos are shown in Table 4. The percent of larvae that hatched decreased with increasing copper concentrations in the water and changed with increasing age of the embryo at the time the experiment began.

TABLE 4. Percent of embryos that hatched into larvae in experiments in which embryos of different ages were exposed to copper concentrations ranging from 960 to <1 µg/L.

Concentration of copper in water, µg/L	Age of embryos, h		
	4 to 6	8 to 10	20 to 24
	Percent hatching		
960	0	0	5
470	0	0	78
230	12	8	--
100	72	59	100
<1	62	60	100

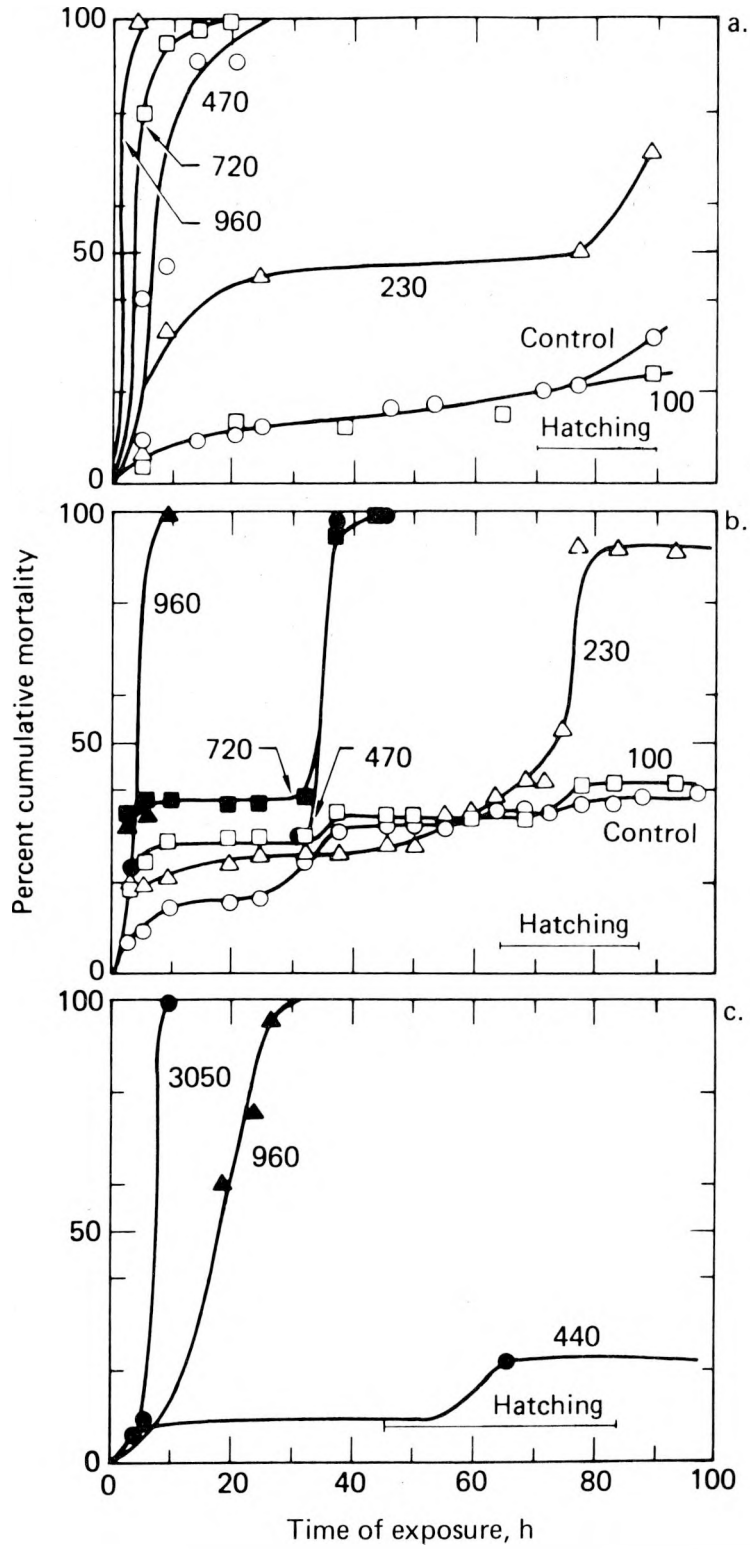

FIG. 4. Percent cumulative mortality of embryonic carp continuously exposed to copper. Numbers next to the curves gives the exposure concentrations in µg Cu/L. a. Exp. IV. b. Exp. V. c. Exp. VI.

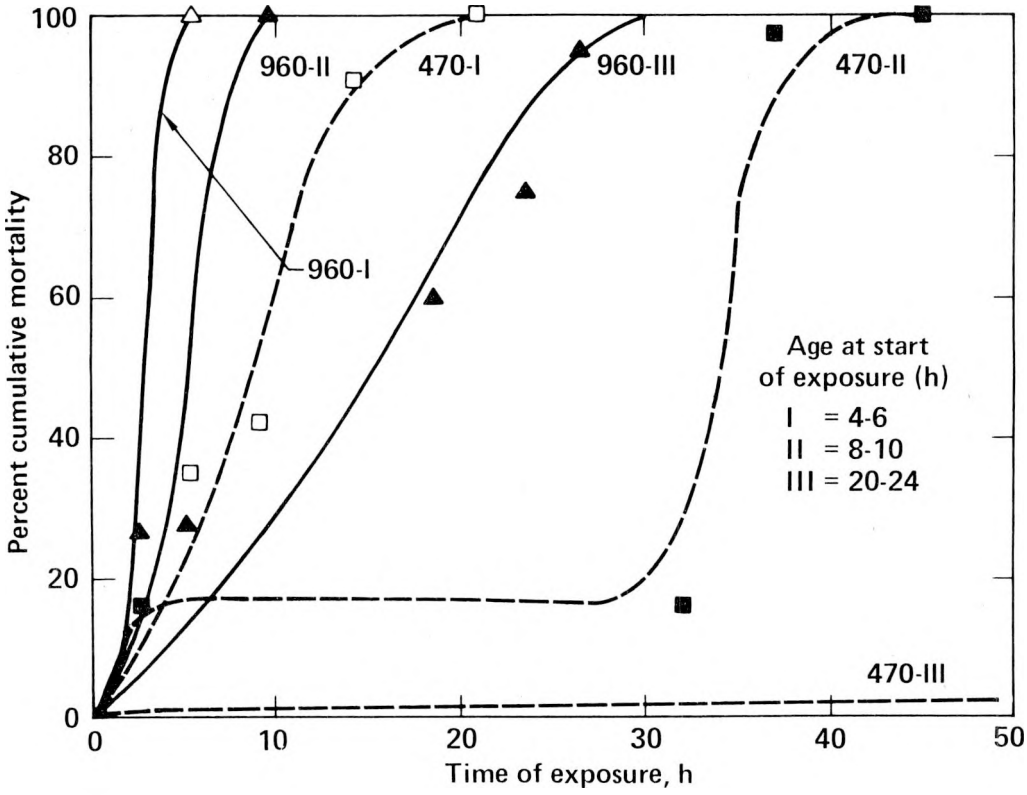

FIG. 5. Percent cumulative mortality of embryonic carp of different ages continuously exposed to copper. Numbers next to the curves give the exposure concentrations in µg Cu/L.

Mortality of Larvae

The effects of copper on larvae hatched from eggs maintained in control water (Exp. VII and VIII) and on eggs maintained in water containing different concentrations of copper were examined (Exp. IX). Mortality curves obtained in Exp. VII and VIII were similar even though the larvae used in Exp. VII were approximately 24-h old and those in Exp. VIII were 1- to 2-h old (Fig. 6). However, the effects of yolk sac absorption were seen about 24-h earlier in Exp. VII than in Exp. VIII. The $LC50_{48}$ and ILC50 for carp larvae tested in Exp. VIII were 120 and 110 µg Cu/L, respectively. The ILC50 was reached before 96 h and the ILC50 may be used as an estimate of the $LC50_{96}$.

One group of 24-h embryos was exposed to copper continuously until death or yolk sac absorption ensued (Fig. 7). In this group, hatching began earlier in the animals that were in water containing 500 µg Cu/L than those in water with 100 µg Cu/L. Insufficient data were available to determine whether there was a change in sensitivity of those larvae exposed to copper before hatching.

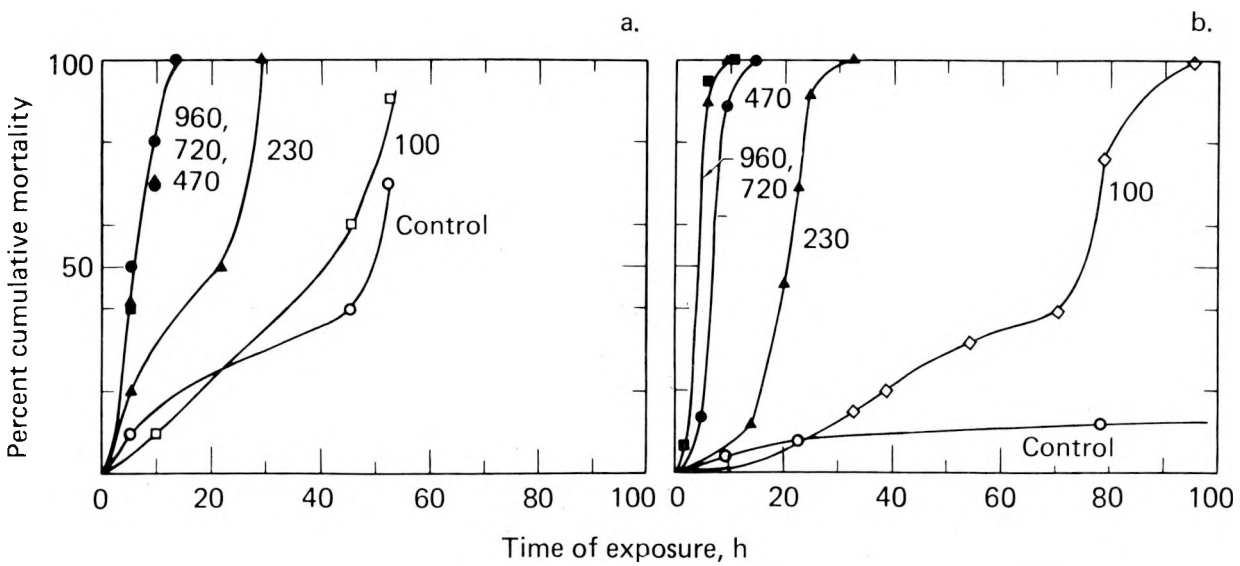

FIG. 6. Percent cumulative mortality of larval carp continuously exposed to copper. Numbers next to the curves give the exposure concentrations in µg Cu/L. a. Exp. VII, larvae were approximately 24-h old. b. Exp. VIII, larvae were approximately 1- to 2-h old.

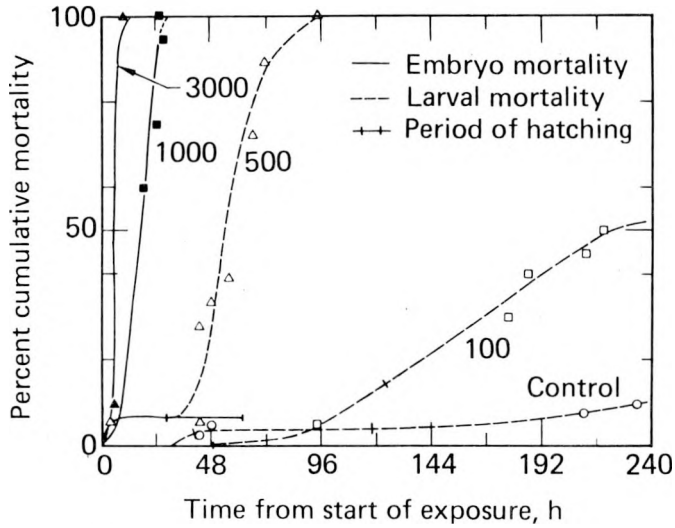

FIG. 7. Percent cumulative mortality of embryonic and larval carp continuously exposed to copper. Embryos were 24-h old when exposure was initiated. Numbers next to the curves give the exposure concentration in µg Cu/L.

Copper Concentrations in Bioassay Water

Copper concentrations in the bioassay water during the exposure of the embryos and larvae varied less than that of the adult exposure; the standard deviation was always less than 10% of mean (Table 5). Like that in the adult bioassay water, most of the copper was labile.

TABLE 5. Copper concentrations in the embryonic and larval bioassay water, and number of organisms exposed at each concentration.

Measured copper,[a] µg/L	Number of analyses	Labile copper, %	Number of organisms	
			Embryos	Larvae
960±80	6	65	62	55
720±60	6	78	60	101
470±50	6	89	59	100
230±30	7	87	60	26
100±10	8	99	60	25
0.7 (control)	2	71	50	25

[a] ± one standard deviation.

TOXICITY CURVES

Mortality data from the experiments were used to generate toxicity curves. Examples of the cumulative mortality data used in the calculations and of the results obtained for adults, larvae, and embryos are given in Tables 6, 7, and 8, respectively. From a log-log plot of the time to 50% mortality versus concentration, a straight line that terminates in the ILC can be generated (Figs. 8a, 8b, 8c). The slope of the line and the ILC differed with life stage.

TABLE 6. Sample data used to generate LC50's for adult carp. The LC50s were then used to construct a toxicity curve.

Exposure time, h	Measured exposure concentration, µg Cu/L						LC50,[a] µg Cu/L	Chi square value[b]	Degrees of freedom
	60	120	230	390	850	1130			
	Proportion dead[c]								
14.0	--	--	--	0.00	0.13	0.60	1100[d]		
20.0	--	--	0.00	0.13	0.63	1.00	738±162	0.65	2
25.5	--	0.00	0.09	0.27	0.88	1.00	558±106	0.85	3
29.5	0.00	0.06	0.41	0.47	0.94	1.00	386±78	4.42	4
37.0	0.00	0.17	0.64	0.67	1.00	--	252±60	3.98	3
39.0	0.00	0.22	0.64	0.87	1.00	--	215±51	1.64	3
42.5	0.00	0.28	0.68	1.00	--	--	186±44	1.60	2
49.5	0.00	0.33	0.86	1.00	--	--	157±36	1.37	2
58.5	0.00	0.39	1.00	--	--	--	122[d]	--	--
64.0	0.00	0.56	1.00	--	--	--	119[d]	--	--
78.0	0.00	0.61	1.00	--	--	--	118[d]	--	--

[a] ±95% confidence limit.

[b] All values not significant $p \geq 0.05$.

[c] Corrected for control mortality.

[d] Determined according to method of Litchfield and Wilcoxon (1949).

TABLE 7. Sample data used to generate LC50s for larval carp. The LC50s were then used to construct a toxicity curve.

Exposure time, h	Measured exposure concentration, µg Cu/L						LC50,[a] µg Cu/L	Chi square value	Degrees of freedom
	Control	100	230	470	720	960			
	Proportion dead[b]								
2.5	--	--	0.00	0.15	0.28	0.29	1207±258	3.71	2
5.0	--	--	0.00	0.21	0.74	0.71	656±87	7.32[c]	2
7.0	--	--	0.00	0.49	0.96	0.89	479±72	3.95	2
9.3	--	--	0.00	0.88	1.00	1.00	384±95	1.01	2
11.7	--	--	0.00	0.95	1.00	--	370[d]	--	-
13.8	--	0.00	0.08	1.00	--	--	240[d]	--	-
19.8	--	0.00	0.44	1.00	--	--	220[d]	--	-
23.0	--	0.00	0.67	1.00	--	--	190[d]	--	-
29.0	--	0.00	0.92	1.00	--	--	180[d]	--	-
37.8	0.00	0.09	1.00	--	--	--	110[d]	--	-
54.3	0.00	0.26	1.00	--	--	--	110[d]	--	-
70.8	0.00	0.35	1.00	--	--	--	110[d]	--	-

[a] ± 95% confidence limit.

[b] Corrected for control mortality.

[c] $P \leq 0.05$.

[d] Determined according to method of Litchfield and Wilcoxon (1949).

TABLE 8. Sample data used to generate LC50s for embryonic carp. The LC50s were then used to construct a toxicity curve.

Exposure time, h	Measured exposure concentration, µg Cu/L						LC50,[a] µg Cu/L	Chi square value	Degrees of freedom
	Control	100	230	470	720	960			
	Proportion dead[b]								
5.3	--	--	0.00	0.35	0.78	1.00	562±70	3.24	2
9.0	--	0.00	0.27	0.43	0.95	1.00	444±51	12.67[c]	3
14.5	--	0.00	0.27	0.91	0.98	1.00	320±39	3.30	3
21.0	--	0.20	0.27	0.91	1.00	--	311±38	2.84	2
25.5	0.00	0.02	0.36	1.00	--	--	250±53	0.17	2
39.8	0.00	0.02	0.36	1.00	--	--	250±53	0.17	2
78.3	0.00	0.02	0.36	1.00	--	--	250±53	0.17	2
91.3	0.00	0.02	0.58	1.00	--	--	220±48	0.05	2

[a] ± 95% confidence limit.

[b] Corrected for control mortality.

[c] $P \leq 0.01$.

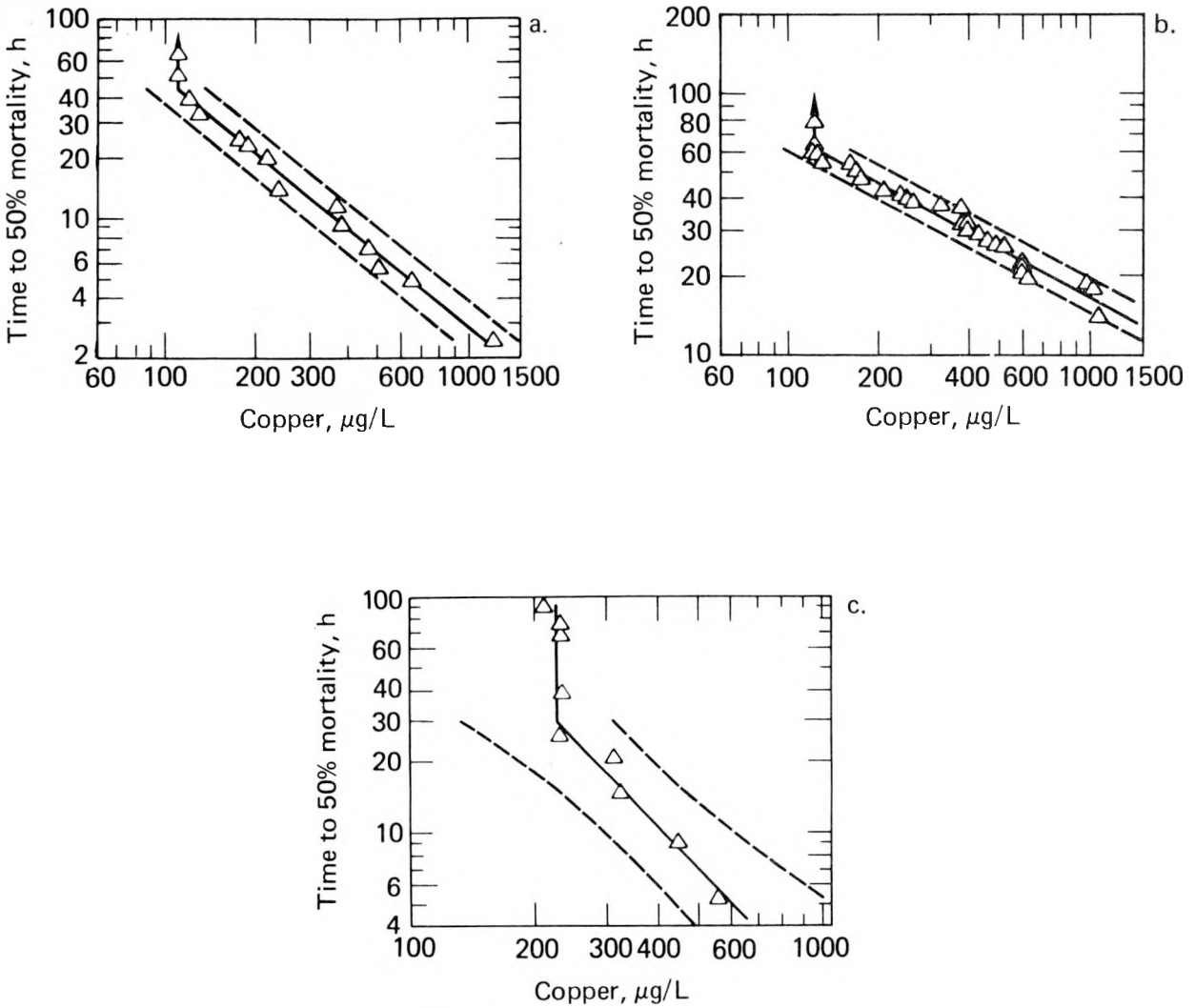

FIG. 8. a. Toxicity curve for adult carp continuously exposed to copper. Vertical arrow indicates the estimated ILC. The equation of the line is log y = 3.0912 - 0.6214 log x. The coefficient of determination (r) was -0.9824. Dotted lines indicate the 95% confidence intervals. b. Toxicity curve for larval carp continuously exposed to copper. Vertical arrow indicates the ILC. The equation of the line is log y = 4.1734 - 1.2369 log x. The coefficient of determination (r) was -0.9920. Dotted lines indicate the 95% confidence intervals. c. Toxicity curve for embryonic carp continuously exposed to copper. Vertical arrow indicates the ILC. The equation of the line is log y = 5.7214 - 1.8060 log x. The coefficient of determination (r) was -0.9747. Dotted lines indicate the 95% confidence intervals.

TABLE 9. Toxicity of copper to freshwater fishes.

Fish	Criteria[a]	Copper, µg/L	Exposure type[b]	Reference
Rainbow trout	48-h TLm	70	Field	Calamari and Marchetti (1975)
Coho salmon				Lorz and McPherson (1976)
Yearlings	96-h TLm	74	S	
Smolts	96-h TLm	60	S	
Stone loach	63-d TLm	250	FT	Solbe and Cooper (1976)
Rainbow trout	96-h TLm	250-680	FT	Lett et al. (1976)
Fathead minnow	96-h TLm	600-980	FT	Brungs et al. (1976)
Fathead minnow	MATC	66	FT	Brungs et al. (1976)
Bluegills	96-h TL50	1100	FT	Benoit (1975)
	MATC	21-40	FT	Benoit (1975)
Brown bullheads	96-h TLm	170-190	FT	Brungs et al. (1973)
Cutthroat trout	96-h LC50	16-367	FT	Chakoumakos et al.(1979)
Carp	48-h LC50	170	FT	This work

[a]TL50 = median tolerance limit; LC50 = median lethal concentration. TLm = TL50 = LC50; MATC = maximum acceptable toxicant concentration.
[b]S = static water; FT = flow-through water.

DISCUSSION

The effects of copper have been assessed for a number of species of freshwater fishes. Sensitivity to copper varied widely with the fish (Table 9). Our results indicate that the carp can tolerate higher copper concentrations in the water than can most fish.

Little information is available on the acute effects of copper on adult carp. However, some data are available on sublethal effects. Ozaki et al. (1972) determined that carp could survive more than 30 d at 80 µg Cu/L and that they grew slower at 245 µg Cu/L. Labat et al. (1974) exposed adult

carp to copper at concentrations from 500 to 1500 µg/L and found histological changes in the gill structure. Exposure resulted also in mucous-cell depletion followed by inhibition of mucosecretion. Pequignot and Moga (1975) exposed carp to 200 µg Cu/L and reported degenerative changes in gill tissue and in hematopoietic tissue. Reduced oxygen consumption (Leonte, 1973) and changes in electrocardiograms (Labat et al., 1976) were reported also.

The cause of death in the adult animals in our bioassay tests is not known. Nearly all animals had respiratory and locomotory difficulties before death, and mortality may have resulted from any of the sublethal responses described above acting singly or in concert.

Copper concentrations in muscle tissue from adult carp exposed to increased copper concentrations in the water did not differ significantly from those in control fish. However, the data on copper concentrations in the liver of animals held in 60-µg Cu/L bioassay water suggests that copper may have built up in some animals. Data on concentrations in muscle and liver tissues from the stone loach Noemacheilus barbatulus (L.) (Solbe and Cooper, 1976) and from the striped bass Morone saxatilis (Heit, 1979) are available.

Copper concentrations in muscle tissue of striped bass collected from the field were similar to those we determined in control animals, but those in liver tissue were higher; concentrations in both tissues of the stone loach were higher. In groups of stone loach exposed to increased copper concentrations in water, concentrations in the tissues were not significantly different from those in control animals; the range of copper values was large for both control and exposed animals.

The sensitivity of carp to copper differed with the life-history stage. The 24-h LC50s show the order of acute copper toxicity of carp life stages as: larvae (180 µg/L) > embryos (240 µg/L) > adults (540 µg/L). Estimated incipient LC50s give the order of sublethal copper sensitivity of carp life-history stages as: larvae (110 µg/L) > adult (120 µg/L) > embryo (230 µg/L).

The toxicity of copper at different life stages of freshwater fishes has been investigated for a number of species (Grande, 1967; Hughes, 1968; Mount, 1968; Mount and Stephan, 1969; Hazel and Meith, 1970; McKim and Benoit, 1971, 1974; O'Rear, 1972; Benoit, 1975; McKim et al., 1978). Larvae

and early juveniles of all species tested were more sensitive to copper than were the embryos (McKim et al., 1978). Our results indicate that the carp larva also is more sensitive than the embryo. However, we detected large differences in sensitivity associated with the stage in embryonic development; the earlier exposure began, the greater the sensitivity. In our experiments the youngest embryos exposed were 4- to 6-h old, so it is not known whether eggs fertilized in the test solutions would have shown greater sensitivity to copper than these embryos. If they had, the difference in response between embryos and larvae would have been even smaller and probably not significant.

A number of environmental factors affect the toxicity of copper to aquatic biota. Most of these factors are related to the formation in the water of organic and inorganic complexes of copper. A number of investigations indicate that the presence of organic chelators in the water reduces the toxic response (Grande, 1967; Sprague, 1968; Wilson, 1972; Zitco et al., 1973; Biesinger et al., 1974; Shaw and Brown, 1974; Lewis et al., 1973; Sunda and Guillard, 1976; Harrison et al., 1981a). This reduction appears to be related to the stability constant of the copper-ligand complex (Nishikawa and Tabata, 1969; Harrison et al., 1981a).

Formation of inorganic complexes of copper is related to the concentration of inorganic ions in the water. It has been well documented that sensitivity to copper is inversely related to hardness and alkalinity (Lloyd and Herbert, 1962; Stiff, 1971; Chapman and McCrady, 1977; Chakoumakos et al., 1979). According to Stiff (1971) and Chapman and McCrady (1977), the phenomenon occurs because more copper carbonate complexes form at the higher alkalinities that accompany the higher hardness values. Because total hardness and alkalinity were low in the water used in our bioassay system, near maximum sensitivity of the carp would be expected.

Water quality criteria for copper that were proposed and published for review by the Environmental Protection Agency take water hardness into consideration. For total recoverable copper the criterion to protect freshwater aquatic life should not exceed the numerical value given by $e(0.94[\ln(\text{hardness})]-1.23)$ at any time. For example, at hardness of 50, 100, and 200 mg/L $CaCO_3$ the concentration of total recoverable copper should not exceed 12, 22, and 43 µg/L at any time (EPA, 1980).

Analytical problems are encountered in quantifying the different chemical species of copper in the water. In the past, models were used to predict the concentrations of copper species present. Computer models currently available for determining chemical speciation in marine and freshwaters were reviewed recently (Nordstrom et al., 1979). The concentrations of species predicted by these models differ considerably.

It is generally accepted that Cu^{2+} is toxic to aquatic organisms. However, it is not clear that it is the only toxic form of copper. Shaw and Brown (1974) concluded that Cu^{2+} and $CuCO_3$ are the forms toxic to rainbow trout (Salmo gairdnerii). Pagenhopf et al. (1974) indicate that Cu^{2+} and $CuOH^+$ are toxic. Andrew et al. (1977) state that copper toxicity is directly related to activities of Cu^{2+}, $CuOH^+$, and $Cu_2(OH)_2^{2+}$. Engel and Sunda (1979) attributed the toxicity of copper to the activity of Cu^{2+}. Chakoumakos et al. (1979) considers Cu^{2+}, $CuOH^+$, and $Cu(OH)_2^0$ to be important copper species that cause toxicity within the pH range he tested. However, the importance of the $Cu(OH)_2^0$ depends on the stability constant used in the model to calculate species information.

Information on the toxicity of different chemical forms of copper is limited and it is not known whether all aquatic organisms are sensitive to the same chemical forms. For some organisms the sites on the membranes critical in accumulating copper may only bind Cu^{2+}. However, the loss of Cu^{2+} from the water (as for example, when it is taken up by an aquatic organism) will result in the formation of more Cu^{2+} from dissociation of the other labile forms of copper with which it is in equilibrium. Under these circumstances it is expected that the entire labile pool of copper could be available to the organisms.

In our investigation we measured not only total copper but the Chelex-100-labile fraction. At the flow rates of water through the columns that we used, thermodynamically stable metal complexes such as Cu-EDTA and Cu-humus are not retained completely by the resin. Figura and McDuffie (1979) attribute this to slow dissociation kinetics of soluble metal-ligand complexes. In their scheme of classifying trace metals they consider the Chelex-100-labile forms of copper to include Cu^{2+}, very labile metal ligands, moderately labile ligands, and (possibly) slowly exchangeable ligands bound in colloidal matter.

Acute mortality data are seldom used directly for estimating safe levels of chemicals in natural waters because concentrations used in acute studies are much greater than those found in the environment. Most organisms in polluted ecosystems are exposed to chronic low-levels of copper. However, because of the absense of relevant data, acute mortality data were often used in setting standards. In these cases, the 96-h LC50 values are multiplied by a fractional application factor. The factor recommended by the National Academy of Sciences and National Academy of Engineering (1973), in the report on Water Quality Criteria was 0.1 for copper.

The use of application factors or other means of extrapolating from acute to environmental levels of copper entails an element of risk (Chapman and McCrady, 1977). To estimate safe levels of copper in the environment, more information is needed on the sublethal responses of chronic exposure to copper.

CONCLUSIONS

The results of our study on the acute effects of copper on carp indicate that the carp Cyprinus carpio can tolerate higher copper concentrations in the water than most freshwater fishes. The sensitivity of carp to copper differed with the life-history stage. Whereas the 24-h LC 50s show the order of sensitivity to be > embryos > adults, the ILC shows it to be larvae > adults > embryos. For embryos we detected that the sensitivity decreased with increased age of the embryos.

Copper in the bioassay water was primarily in Chelex-100-labile forms. Concentrations of Chelex-100-labile copper were determined in the effluent from three nuclear power stations located on freshwater ecosystems (Harrison et al., 1981b). Because Chelex-100-labile copper should be available to carp, we would expect that life stages of these fish would be affected adversely by power station effluents that contain Chelex-100-labile copper in excess of those of the ILC50 of the respective stages.

The ILC50s determined in this study are higher than labile copper concentration in effluents when nuclear power stations are operating normally, that is, when large volumes of water are pumped continuously through the condensers. Few, if any, mortalities due to copper would be expected in the life stages we examined. However, large copper pulses of considerable duration were documented during start-up (Warrick et al., 1975). If copper pulses as large as these occur at nuclear power stations that have carp living in the discharge zone, the effects on the carp may be detectable.

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