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A Comparison of the Toxicity of Nickel to the Developing Eggs and Larvae of Carp (*Cyprinus carpio*)[1,2]

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Natural concentrations of Ni in soil and water are not considered a biological hazard; however, higher concentrations can be toxic to man and biota (U. S. NATIONAL ACADEMY OF SCIENCES 1975, SCHROEDER et al. 1961, SCHROEDER and DARROW 1972, and U. S. ENVIRONMENTAL PROTECTION AGENCY 1975). Nickel is used primarily in the metal-plating industry and for the production of ferroalloys and stainless steel; however emissions from the combustion of fossil fuels are the major contributors to environmental releases in the United States (U.S. ENVIRONMENTAL PROTECTION AGENCY 1975). The combustion of coal releases Ni directly to the atmosphere and indirectly to aquatic environments by drainage from fly ash storage ponds (ELWOOD 1977, DAVISON et al. 1974). The electroplating industry is another potential source of Ni contamination. Nickel sulfate, the most important compound of Ni in commerce, is released to aquatic environments in significant amounts from the nickel-plating industry (U. S. ENVIRONMENTAL PROTECTION AGENCY 1975). Such releases resulting from man's activities increase the concentration of Ni in aquatic environments and should be viewed with concern.

According to a review by MCKEE and WOLF (1963), the toxicity of Ni to aquatic life depends upon the species, pH, water hardness, and other environmental factors. The 96-h TL_m (Median Tolerance Limits) for $NiCl_2$ in both hard and soft water for bluegills, fathead minnows, goldfish, and guppies ranged from 44.5 mg/L for fathead minnows in hardwater (360 mg/L) to 4.58 mg/L for guppies in soft water (20 mg/L) (PICKERING and HENDERSON 1964). PICKERING (1974) reported that a continuous exposure of fathead minnows to a concentration of 0.73 mg/L of $NiCl_2$ had no effect on their growth and survival but significantly reduced the number of eggs per spawning and the hatchability of the eggs.

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SAUTER et al. (1976) reported that fish eggs appeared to be more resistant than fry to Cd, Cu, Cr, and Pb. However their comparison can be questioned because the exposure times varied for the fry and the eggs. The objectives of this study were (1) to determine the toxicity of Ni to developing carp eggs and newly hatched carp larvae and (2) to compare the resistance of these two life stages to Ni toxicity.

MATERIALS AND METHODS

Spawning carp were collected from a local impoundment for artificially spawning in the laboratory. The method used was adapted from a technique developed by BLAYLOCK and GRIFFITH (1971). Eggs and milt were stripped from a single pair of fish into polypropylene bottles containing selected concentrations (3-10 ppm) of $NiSO_4$ in a 0.6% solution of NaCl and spring water. After fertilization, the eggs were transferred immediately to hatching boxes containing the same concentration of $NiSO_4$ in spring water as was used in the spawning bottle. Three replicates were run for controls and for each of the eight concentrations of $NiSO_4$.

Water in the hatching boxes was maintained at 25° ± 1. The solutions of $NiSO_4$ were changed once daily. After 72 h, the number of eggs that hatched, the number of unhatched eggs containing partially developed embryos, and the number of abnormal larvae were scored.

Carp larvae that hatched from the controls were used in the experiments to determine the toxicity of Ni to the larval stage. One-day-old carp larvae were exposed to concentrations of nickel ranging from 1 to 10 ppm. Two replicates of 25 larvae each were placed in polypropylene boxes containing $NiSO_4$ in 500 mL of spring water, and two replicates were maintained in spring water for controls. The temperature of the water ranged from 23° to 25°C. The mortality of the larvae was recorded at 24, 48, 72, and 96 h and continued on a daily basis at 6, 7, 8, 9, and 10 days.

Stock solutions were prepared by dissolving reagent grade $NiSO_4$ salt in spring water. The pH of the spring water was 7.4, and the total hardness was 128 ppm.

In the egg hatchability experiment, samples of the stock solutions and of the culture water were taken at 0, 22, 46.5, and 70 h for analysis. In the larval toxicity experiment, samples of the culture water were taken for analysis on day 1 and day 10. The water samples were acidified and kept at room temperature until they were analyzed by atomic absorption spectrophotometry. At 46.5 hr after fertilization, samples of ten eggs from the controls, 3, 6, and 10 ppm, test concentrations of Ni were collected, rinsed with distilled water, and analyzed to determine the accumulation of Ni within the eggs.

RESULTS

The effects of Ni on the hatchability of carp eggs and the production of abnormal larvae are shown in Table 1. Since a

TABLE 1

Effects of nickel sulfate on the hatchability of carp eggs and the production of abnormal embryos

Concentrations ppm	Total no.[a] eggs	Percent eggs hatched	Percent abnormal larvae	Percent unhatched eggs	Percent of unhatched eggs with embryos
0	1086	92.3	8.6	7.7	5.9
3	837	93.4	23.3	6.6	32.7
4	623	91.6	50.3	8.4	42.3
5	537	9.3	60.0	92.7	0.0
6	621	51.7	81.3	48.3	76.0
7	919	22.1	100.0	87.9	94.3
8	597	6.9	100.0	93.1	91.5
9	550	2.4	100.0	97.6	90.7
10	414	0.7	100.0	99.3	91.5

[a]Pooled total from three replicates.

combined chi-square value for the three replicates at each concentration indicated no significant difference ($P > 0.05$) in hatchability, the replicates were pooled to obtain the values in Table 1. Concentrations of up to 4 ppm of Ni had no effect on the hatchability of the eggs. At 5 ppm only a small percentage of the eggs hatched. This low percentage hatch was undoubtedly the result of unfertilized eggs and is attributed to technique error in stripping the male fish and fertilizing the eggs. This assumption is supported by the fact that in 507 unhatched eggs embryonic development did not take place, while at higher concentrations a large percentage of the unhatched eggs contained embryos (Table 1). At a concentration of 6 ppm, the percentage of eggs that hatched was approximately 50% and the percentage decreased as the concentration of Ni increased up to 10 ppm.

A chi-square analysis indicated a significant increase ($P < 0.05$) in abnormal larvae at a concentration of 3 ppm when compared with controls. The frequency of abnormal larvae increased as the concentration of Ni increased up to and including 7 ppm. At concentrations of 7 ppm and above, all larvae that hatched were abnormal.

Excluding the concentration of 5 ppm, the percentage of unhatched eggs that contained embryos increased with increasing

concentrations of Ni up to and including 7 ppm. At concentrations above 7 ppm, embryonic development continued through the eyed embryo stage but the number of eggs hatching decreased until at 10 ppm only three larvae which were abnormal hatched from 414 eggs. In a preliminary experiment involving concentrations of 10 ppm and lower, embryonic development took place; but at a concentration of 30 ppm and above, embryonic development was not observed.

The effect of Ni on the survival of carp larvae is shown in Fig. 1. The percentage of survival was obtained by combining the two replicates of 25 larvae each, since a combined chi-square value of the two replicates at each concentration indicated no significant difference ($P > 0.05$). These data were analyzed using a probit analysis (FINNEY 1974) to estimate the concentration of Ni that would be lethal to 50% of the larvae (LC_{50}) at various times. Likewise, a probit analysis was used to determine the LC_{50} of the developing eggs at the time of hatching (72 h). From these analyses a comparison was made of the sensitivity of the larvae and the developing egg (Table 2).

The LC_{50} for carp larvae exposed to nickel for 72 h is 8.46 ppm. The LC_{50} for developing eggs at the time of hatching (72 h) is 6.10 ppm. The numbers are significantly different ($P < 0.05$); therefore, the developing eggs can be considered more sensitive to Ni than the larvae. If the exposure time for the larvae is extended to 96 h, the LC_{50} decreases to 6.16 ppm, which is not statistically different from the LC_{50} for developing eggs at hatching.

Analysis of the culture water for Ni in the hatchability test showed that the solutions at the beginning of the experiment and at 22, 46.5, and 70 h were within 0.5 ppm of the expected concentrations (Table 3). In the larval toxicity experiment, the concentrations did not vary more than 1 ppm during the 10-day test period. Analysis of developing eggs at 46.5 h after exposure to concentrations of 3, 6, and 10 ppm of Ni gave concentration factors of 11, 8, and 6, respectively.

DISCUSSION

As the exposure time increased, the concentration of nickel required to produce 50% mortality in carp larvae decreased (Fig. 1). Whereas concentrations of up to 7 ppm had little effect on the 24-h survival of larvae, 1 ppm produces approximately 50% mortality after 10 days of exposure (Table 2). The LC_{50} value at 96 h was significantly lower than at 72 h. PICKERING and HENDERSON (1964) obtained a similar decrease for TL_m values at 24, 48, and 96 h for fathead minnows, bluegill, goldfish, and guppies exposed to $NiCl_2$. In their study the TL_m value for goldfish in soft water (hardness, 20 mg/L)

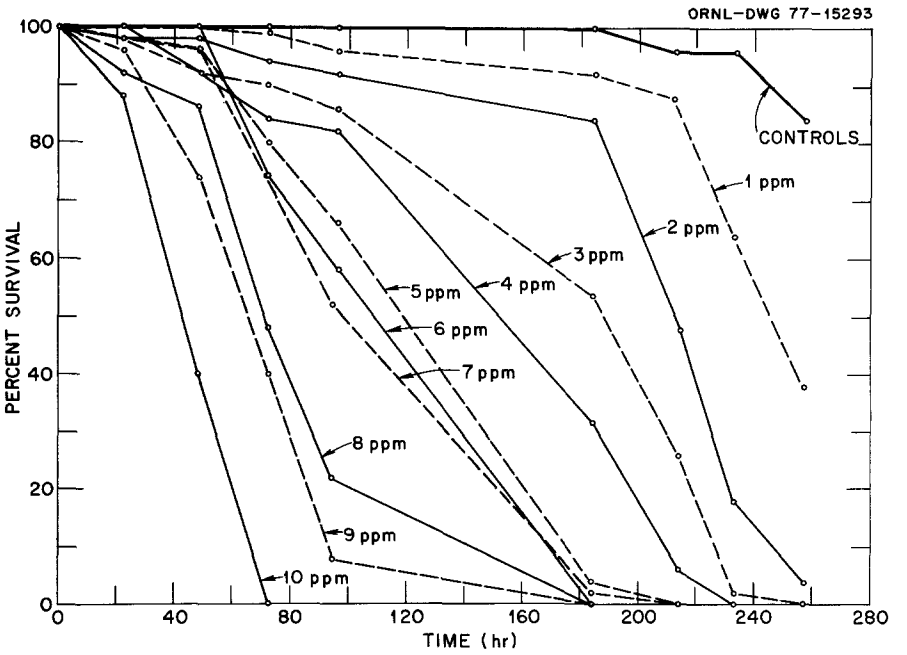

Fig. 1. The effect of nickel sulfate on the survival of carp larvae is shown for different exposure times. The percent survival of one-day-old carp larvae exposed to concentrations of nickel ranging from 1 to 10 ppm was plotted against time. Each data point represents the pooled values from two replicates of 25 larvae.

TABLE 2

The LC_{50} values estimated by probit analysis for carp eggs and larvae exposed to nickel sulfate

Life stage	Exposure time (hr)	LC_{50} Ni (ppm)	95% Confidence limits	
			Lower	Upper
Egg	72	6.10	6.00	6.19
Larvae	72	8.46	7.85	9.26
Larvae	96	6.16	5.79	6.54
Larvae	184	3.18	2.92	3.43
Larvae	212	2.14	1.89	2.38
Larvae	233	1.30	1.10	1.50
Larvae	257	0.75	0.54	0.94

TABLE 3

Concentration of nickel in 46.5-hr-old carp (Cyprinus carpio) eggs after exposure to nickel sulfate from the time of fertilization

Ni (ppm) culture water	Ni (ppm) eggs[a] (wet wt)	Concentration factor (c/f)
0.0014	0.35	250.0
3.20	36.26	11.3
6.30	52.63	8.4
9.70	61.04	6.3

[a]One analysis of ten eggs.

decreased from 26.9 ppm at 24 h to 9.8 ppm at 96 h. RAO et al. (1975) found that the TL_m value for 4- to 5-cm carp (Cyprinus carpio) exposed to $NiCl_2$ in water with a total hardness of 112 mg/L decreased from 49.0 ppm at 24 h to 35 ppm at 96 h. REHWOLDT et al. (1972) investigated the effects of increasing temperature on the acute toxicity of heavy metal ions and concluded that temperature had no effect on the toxicity of Ni to several species of fish. In the same study a 96 h TL_m of 10.4 ppm was estimated for juvenile carp in 28°C water with a hardness of 55 mg/L. Compared to these literature values, newly hatched larvae of carp in the present experiment were more sensitive to Ni toxicity than carp which were several months old.

SHAW and BROWN (1971) found that 1 ppm of Ni had no effect on the fertilization of rainbow trout eggs and the subsequent hatchability of their eggs. Fathead minnows chronically exposed to concentrations of 0.73 ppm of Ni from the time they were approximately 6 weeks old until they spawned produced significantly fewer eggs per female. In addition, the hatchability of the eggs from these chronically exposed adults was significantly reduced (PICKERING 1974). In the present study the gametes of carp were exposed to Ni during fertilization and throughout embryonic development, a period of 72 h. Although 6 ppm reduced the hatchability of the eggs by approximately 50%, the fertilization process was apparently unaffected. This is apparently true for concentrations of Ni up to 10 ppm, because a high percentage of the unhatched eggs contained developed embryos (Table 1).

In the present study for equivalent exposure times, the developing eggs of carp were more sensitive to Ni toxicity than newly hatched larvae. Nickel toxicity to the different life stages has not been compared previously and these results differ from the ones obtained with some other metals. In seven species of fish, not including carp, the eggs were more resistant to the

toxicity of Cd, Cr, Cu, and Pb than were the fry (SAUTER et al. 1976). Because of the variability in the exposure time and the embryonic stage at which exposures were initiated, these comparisons can be questioned; but in another experiment (PICKERING and VIGOR 1965) in which the exposure times to $ZnSO_4$ was the same for eggs and fry of fathead minnows, the TL_m value was significantly less for fry than for the hatchability of the eggs. SAUTER et al. (1976) and PICKERING and VIGOR (1965) did not include analyses of metal concentration in the eggs and fry; therefore it is not known whether a differential concentration in the two life stages could account for the difference in sensitivity.

A concentration factor of 100 has been reported for Ni in freshwater fish (THOMPSON et al. 1972); however, the concentration factor for fish eggs was not available. Our analysis of carp eggs gave concentration factors ranging from 250 for eggs developing in spring water to 6 for eggs developing in water containing 10 ppm of Ni (Table 3). Although uptake data for Ni by fish eggs are not available, a rapid uptake of radioactive Ni was reported for the sea urchin eggs (Lytechnius pictus) (TIMOURIAN and WATCHMAKER 1972). The uptake rate was the highest immediately after fertilization but continued throughout development. Since carp eggs imbibe water immediately after fertilization, the uptake rate for Ni in carp eggs is probably similar to the uptake rate in sea urchin eggs. If this is the case, our analyses for Ni which were made at the eyed embryo stage, 46.5 h after fertilization, would be after the rapid rate of uptake and should be representative of the Ni concentration in the developing egg.

The stage of the life cycle most sensitive to an environmental pollutant is generally considered the crucial stage in regards to the success of a population exposed to an environmental pollutant. Of the two life stages of the carp which were tested in the present experiment, the developing egg was the most sensitive stage when the exposure times were equal. Since the exposure time for a developing egg is limited by hatching, the larval stage becomes more sensitive when the exposure time is extended. Thus, for an acute short-term release of Ni to an aquatic environment, the egg would be the more crucial stage, but for a long-term chronic release the toxicity of Ni to the larval stage would be more crucial to the survival of a population.

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