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Acute Toxicity and Toxic Interaction of Chromium and Nickel to Common Guppy *Poecilia reticulata* (Peters)

B. S. Khangarot and P. K. Ray

Preventive Toxicology Division, Industrial Toxicology Research Centre, Post Box No. 80, Mahatma Gandhi Marg, Lucknow-226 001, India

In general, polluted surface waters contain hundreds to thousands of toxic chemicals. Therefore, a knowledge of the joint effects of mixtures is important in determining water quality criteria, since interactions may increase the toxicity depending upon the nature of the individual chemicals. Several authors, for example Plackett and Hewlett (1952), Marking (1977), Muska and Weber (1977), Konemann (1981) have suggested methods of modeling and data analysis to describe the type of combined effects that occur. The acute toxicity of heavy metals in combination to the common guppy has been reported (Khangarot et al. 1981; Khangarot and Ray 1987). Information on the acute toxicity on combined effects of chromium and nickel to fish is rather scarce. The European Inland Fishery Advisory Commission in a recent report (EIFAC 1980) reviewed the literature on the joint effects of mixtures of toxicants to aquatic organisms. Toxicity of nickel and chromium to fish is generally low. These two elements are usually less toxic than silver, cadmium, copper and thallium; depending on test conditions, these may also be less hazardous than zinc, lead and arsenic. The acute toxicities of Ni and Cr to the common guppy were examined by Pickering and Henderson (1966) and Khangarot (1981). The present study was undertaken to investigate the acute toxicity of Ni and Cr singly and the toxic interaction of these two metal ions on survival of the common guppy, *Poecilia reticulata* (Peters). This species was selected for static bioassays because it can be easily cultured and raised under laboratory conditions through a complete life cycle, and it is one of the most common fish used for laboratory toxicity studies.

MATERIALS AND METHODS

The average total length of fish was 15 mm (range 12-17 mm) and average wet weight was 184 mg (range, 160-200 mg). Fish were acclimatized for 10 days in glass aquaria at room temperature before use. Test animals were fed fish food (Shalimar Fish Food Co., Bombay) *ad libitum* once a day but were not fed for 48 h before the start of the bioassay and during the test period. Test aquaria were wide-mouth glass jars of 10 liters. A minimum of eight concentrations, three replicates per concentration plus three controls, were used for each bioassay. Stock solutions in distilled water were prepared by dissolving reagent grade nickel chloride ($NiCl_2.6H_2O$] and potassium dichromate

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Send reprint request to B.S. Khangarot at above address.

($K_2Cr_2O_7$). Test concentrations are given as mg/L of metal ions. The acute renewal static bioassay as described by APHA et al (1975) in the Standard Methods were followed. Ten fish were used for each concentration and three replicates were conducted for each bioassay. Test solutions were renewed after every 24 h. The number of fish that died in each concentration was recorded after 30 minutes and 1,2,4,8,12 and 24 h thereafter. Dead specimens were removed immediately. Tests were conducted for 240 hours.

The test water used was dechlorinated tap water. The physico-chemical properties of the test water are given in Table 1. Dissolved oxygen and alkalinity in chromium toxicity tests were not determined, but values were determined in control jars. The results of replicates using the same toxicant were pooled to increase sample size, and the median period of survival (LT_{50} values) and median lethal concen-

Table 1: Physico-chemical characteristics of dilution water used in acute toxicity tests with common guppy, Poecilia reticulata

Characteristics	Unit	No. of observations	Mean	Range
pH		20	7.6	7.4-7.8
Temperature (air)	°C	20	32	30-34
Water temperature	°C	20	28.5	27-30
Acidity	mg/L as $CaCO_3$	20	Nil	Nil
Free CO_2	mg/L	20	2.5	1.5-3.5
Alkalinity	mg/L as $CaCO_3$	10	135	125-150
Total hardness	mg/L as $CaCO_3$	10	178	165-200

trations (LC_{50} values) were determined on the pooled data. Survival time distributed approximately lognormal and LT_{50}'s and other 95% confidence limits were calculated by the method of Litchfield (1949). Similarly, the LC_{50} values and their 95% confidence limits and slope functions were determined by the nomographic method of Litchfield and Wilcoxon (1949).

The effects of a mixture of nickel and chromium on survival of common guppy were also studied. The acute toxicity of mixtures were determined by the method of Sprague (1973). In this procedure, the concentration of toxicant in a mixture is reported as a proportion of the LC_{50}'s determined singly. The toxicity ratio of Ni and Cr in the mixture study was 1:1 of their LC_{50} values. The sum of the ratio of the Ni and Cr toxicant mixture is then determined. The additive indices of the mixture of chemicals were determined by the following formula:

$$\frac{A_m}{A_i} + \frac{B_m}{B_i} = S \ \ldots\ldots (1)$$

Where A_m= LC_{50} Ni in mixture, A_i = LC_{50} for Ni individually, B_m = LC_{50} for Cr in mixture, B_i=LC_{50} for Cr individually and S is the sum of the biological activity. If the sum of the toxicity of chemical is simply additive, S=1.0. A total less than 1.0 is considered synergistic, while one greater than 1.0 is considered antagonistic. Marking (1977) presented a system in which the index represents simple additive, greater than additive, and less than additive effects by zero, positive and negative values, respectively. The values equaling zero indicate additive toxicity, positive values indicate synergistic toxicities and negative values suggest antagonistic mixtures. Marking's Additive Index (MAI) can be calculated as:

$$MAI = (1/S - 1.0 \text{ if } S \leq 1.0 \text{ and} \quad \text{.... (2)}$$
$$MAI = 1.0 - S \text{ if } S \geq 1.0 \quad \text{.... (3)}$$

The significance of the Marketing Additive Index (MAI) from zero can be calculated by substituting values from the 95% confidence limits for the LC_{50} in the formula (1) to determine the range of MAI. If the range includes zero, the deviation is not considered significant. The values that yield the greatest deviation from MAI are used to establish the range whenever an MAI overlaps zero and the additive toxicity is assumed.

RESULTS AND DISCUSSIONS

The median survival time (LT_{50} values) for the metals tested singly are given in Table 2. Nickel and chromium have approximately the same acute toxicity. Survival time in the Ni-Cr mixture was much reduced (Table 4), suggesting that metals potentiate each other's toxicity and caused the rapid death of fish. At lethal concentrations, behavioral changes were surfacing, rapid mouth and opercular movements and convulsions. All the test fish loss their equilibrium before death. The LT_{50} values for mixtures seem to be simply additive. In stronger mixtures (e.g., 2 and 5 toxic units), fish died faster than would be expected from their resistance to individual metals. The 96 h mixture toxicity test results were used for calculating the LT_{50} values, and the 95% confidence limits and slope functions of Ni and Cr are given in Table 2. Expressed in mg/L of metal ions, the 96 h LC_{50} values were 29.28 (confidence limits = 24.81-34.55) for Cr (Table 3). Mixtures of Ni and Cr (ratio 1:1 of LC_{50}'s) were tested for 48 h and 96 h and their combined toxicity to common guppy was found to be simply additive. MAI was determined to be + 0.312 (range 0.04 to + 0.80) for the 48 h test and + 0.086 (range -0.244 to +1.466) for 96 h test. These values indicate that acute toxicity of Ni-Cr mixtures were merely additive because the range overlapped zero (see Table 4).

Pickering and Henderson (1966) observed a 96 h LC_{50} of 4.45 mg/Ni/L (C.L. = 2.83-5.39 mg/L) with common guppy in soft water (hardness 20 mg/L as $CaCO_3$) at 25°C. Other workers have studied the acute and chronic toxicity of Ni to various other freshwater fishes. In carp, the 72 h LC_{50}'s for freshly fertilized eggs and one-day old larvae were 6.1 and 6.2 mg/L, respectively (Blaylock and Frank 1979). Pickering (1974) reported a 96 h LC_{50} for fathead minnows ranging from 27 to 32 mg/L of Ni, based on nominal concentrations.

The 96 h LC_{50} values for chromium to the common guppy was 29.28mg/L and 95% confidence limits calculated were 24.81-34.55 mg/L (hardness 178 mg/L as $CaCO_3$), while Pickering and Henderson (1966) observed a 24 h LC_{50} of 113 mg/L of chromium for guppy in soft water (hardness 20 mg/L as $CaCO_3$). The 96 h LC_{50} values for fathead minnows and bluegills were 17.6 and 118 mg/L Ni, respectively, when tested under soft-water conditions (Pickering and Henderson, 1966). Differences

Table 2: The LT_{50} values (median survival time) with 95% confidence limits and slope functions for the common guppy against nickel and chromium toxicants

Toxicant	Concentrations (in mg/L)	LT_{50} and 95% confidence limits (in hours)	Slope function and 95% confidence limits
Nickel	150	10.00 (8.13-12.30)	1.81 (1.56-2.10)
	100	20.00 (16.40-24.40)	1.73 (1.50-1.99)
	75	46.00 (37.09-57.04)	1.86 (1.60-2.16)
	49	84.00 (62.69-112.56)	2.30 (1.85-3.47)
	28	130 (92.20-183.30)	2.53 (1.85-3.57)
Chromium	150	7.00 (5.56-8.82)	1.26 (1.16-1.37)
	100	11.00 (9.56-12.65)	1.47 (1.34-1.62)
	75	29.00 (23.59-35.96)	1.85 (1.59-2.15)
	49	60.00 (41.96-85.80)	2.78 (2.12-3.64)
	28	125.00 (100.00-156.20)	1.85 (1.57-2.18)

in acute toxicity in Ni and Cr to the common guppy between the present study and those found by other investigators are mainly due to differences in total hardness, temperature and slight variations in pH of the exposure procedures. At 48 and 96 h, the value of the Cr+Ni in combination (of MAI) overlapped zero; therefore, the acute toxicity of the Ni-Cr mixture to the common guppy was additive. Little information is available concerning the acute toxicity of mixtures of these two metals on aquatic animals. In a recent report of the European Inland Fisheries Advisory Commission (EIFAC 1980), the information on the combined effects of mixtures of toxicants on aquatic organisms

Table 3: Calculated LC_{50} values with 95% confidence limits for common guppy against nickel and chromium toxicants

Toxicant	Time (in hours)	LC_{50} and 95% CL as mg/L of metal	Slope functions and and 95% CL
Nickel	24	95.00 (84.70-104.30)	1.34 (1.22-1.47)
	48	63.00 (54.78-73.08)	1.64 (1.41-1.90)
	72	50.12 (40.10-62.65)	1.71 (1.40-2.09)
	96	36.00 (29.76-43.57)	1.91 (1.55-2.35)
	168	33.11 (28.54-38.41)	1.65 (1.42-1.91)
	240	31.15 (26.13-36.13)	1.52 (1.32-1.75)
Chromium	24	49.00 (38.28-67.20)	1.94 (1.43-2.61)
	48	39.80 (31.85-49.76)	1.75 (1.90-2.18)
	72	32.00 (27.58-37.12)	1.50 (1.26-1.79)
	96	29.28 (24.81-34.55)	1.72 (1.34-2.22)
	168	19.05 (14.11-25.71)	1.77 (1.26-2.48)
	240	19.25 (17.57-20.67)	1.17 (1.09-1.36)

CL: Confidence limits

has been reviewed. The ratio of 3:1 of the 96 h LC_{50} values for Ni and Cr in hard water showed the ratio of 1:1; the additive toxicity was apparently 13 and 21 times more than additive for rainbow trout (EIFAC 1980). Khangarot et al. (1981) noted synergistic effects while studying the acute toxicity of Zn-Ni-Cu mixtures to the common guppy. Anderson and Weber (1975) observed that the acute toxicities of Cu-Zn mixtures were supra-additive (synergistic) to the male common guppy, <u>Poecilia reticulata</u>, in water of intermediate hardness (hardness 125 mg/L $CaCO_3$). In general, environmental toxicants occur in mixtures

Table 4: Toxicity and Marking Additive Index (MAI) for nickel and chromium applied individually and combination to the common guppy in 48 and 96 h tests

Metals	48 h LC_{50} and 95% CL (in mg/L) Individually	in combination	(S*) and range	MAI and range
Nickel and	63.00 (54.78-73.08 **)	24.74 (21.89-27.96)	0.762 (0.555-1.045)	+ 0.312 (-0.04 to +0.80)
Chromium	39.80 (31.85-49.76)	14.68 (12.70-17.03)		
(1:1 of 48 h LC_{50}'s)				
	96 h LC_{50} and 95% CL (in mg/L)			
Nickel and	36.00 (29.76-43.57)	16.46 (14.70-18.44)	0.921 (0.628-1.244)	+0.086 (-0.244 to +1.466)
Chromium	29.28 (24.81-34.55)	13.58 (11.91-15.48)		
(1:1 of 96 h LC_{50}'s)				

*) sum of biological activity
**) as percent confidence limits and range represented in parenthesis

in natural waters, therefore, the interaction of toxicity is an important factor which must be taken into account when assessing the hazards of environmental pollutants to aquatic life and for setting valid water quality standards for diverse uses. Additional studies on the effects of toxicants, singly and in mixtures, on biochemical and physiological processes are needed to gain more knowledge of interactions and their toxic effects.

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