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Kinetic Analysis of the Swimming Behavior of the Goldfish, *Carassius auratus*, Exposed to Nickel: Hypoactivity Induced by Sublethal Concentrations

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The discharge of nickel into aquatic environments from numerous industries poses a threat to fish populations because of its toxicity. Although little is known, however, about the precise mechanism of its toxicity in freshwater fish (Chaudry and Nath 1985), it produces some of the symptoms associated with heavy-metal poisoning in general; it accumulates in fish tissues (Kulikova et al. 1985) and results in alterations in gill structure, including hypertrophy of respiratory and mucous cells, separation of the epithelial layer from the pillar cell system, cauterization and sloughing, and necrosis of the epithelium (Nath and Kumar 1989). The destruction of the gill lamellae decreases the ventilation rate and if severe, as after acute exposure, may cause blood hypoxia and death (Heath 1987). The effects of short-term exposure of fish to sublethal concentrations of nickel are not as well defined. The kinetic method of Ellgaard et al. (1975, 1977), which uses locomotor activity to assess the general health of fish, is ideally suited to examine whether sublethal concentrations of nickel adversely affect fish. In previous studies, the measured changes in locomotor activity observed when fish are exposed to pollutants correlate with more specific changes, e.g., physiological, biochemical, histological or neurosensory changes, which occur under the same conditions. Thus, the kinetic method also meets the criteria for pollution early warning systems as discussed by Cairns and van der Schale (1980). This method has previously been used to demonstrate that short-term exposure to sublethal concentrations of the heavy metals cadmium, chromium, and zinc (Ellgaard et al. 1978) and copper (Ellgaard and Guillot 1988) are detrimental to the health of bluegills. The present study examines the effects of short-term exposures of sublethal concentrations of nickel on the locomotor activity of the goldfish, *Carassius auratus*.

MATERIALS AND METHODS

Goldfish, *Carassius auratus*, approximately 3-5 cm in length, were obtained

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from a commercial pet store. In the laboratory, they were held in tanks of dechlorinated tap water at 27 ± 1° C [hardness = 135 mg/l (as $CaCO_3$), alkalinity = 80 mg/l (as $CaCO_3$), pH = 8.7]. Aquaria were filtered with external filters equipped with activated charcoal and glass wool. Water in the aquaria was air-saturated with air stones. The fish were fed tropical fish food; fish were not fed during periods of experimentation or 60 min preceding an experimental run.

In order to study the effects of sublethal concentrations of nickel (nickelous chloride hexahydrate) on the locomotor activity of the goldfish, the concentration of nickel which caused 50% mortality at 96 hr (96-hr LC50) was determined. A single mortality test, using 8 fish per aquarium, was conducted at each concentration.

The locomotor activity experiments were conducted on 9 groups of fish (3 control groups, 2 groups at 25 ppm nickel, 1 group at 50 ppm nickel and 3 groups at 75 ppm nickel) in each of nine 38-l aquaria. The actual number of kinetic runs for each group is detailed below and in Fig. 1. Each aquarium was divided into two identical compartments, A and B, by a clear Plexiglas partition with a diamond-shaped opening in its center, approximately 80 cm^2 in area. Note that the size, shape and number of openings affect the rate at which the kinetic reaction proceeds and therefore the parameters can be varied until one establishes a reaction that can be monitored, ideally over a short period of time. Thus the present configuration of the opening differs, for example, from the 5 circular openings each of 25 mm radius used when testing bluegill (Ellgaard et al. 1975). Prior to an experimental run, filters and air sources were removed. The Plexiglas partition was temporarily removed, and the 30 goldfish were gently herded to one half of the aquarium (compartment A) by means of a porous screen; the Plexiglas partition was then reinserted, and its opening was blocked by the porous screen. After the fish voluntarily distributed themselves in a random fashion in compartment A, usually within 2-3 minutes (a period of 10 minutes was routinely allotted), the porous screen was withdrawn from the aquarium, leaving only the Plexiglas partition, and the fish were allowed to disperse throughout the aquarium. The number of fish in compartment A was recorded after each minute for a total period of 5 minutes (during this period the initial reaction kinetics is described); equilibrium is reached several minutes later.

Under these experimental conditions, the movements of fish describe an opposed first order reaction (Ellgaard et al. 1975, 1977). This reaction can be expressed by a rate expression as follows: $Kt = \ln [((A)_0 - (A)_e) / ((A) - (A)_e)]$, where K = rate constant; (A) = number of fish in compartment A at time t ; $(A)_0$ = number of fish in compartment A at time t = 0; $(A)_e$ = number of fish in compartment A at equilibrium = 15 and t = time. Using this relation, when $\ln [((A)_0 - (A)_e) / ((A) - (A)_e)]$ is plotted against time, t,

a straight line results. All lines are best-fit lines determined by least squares simple regression analysis (Cricket-graph computer program). The rate constant, K, of the reaction is equal to the slope of the best-fit line. The rate constant thus describes the locomotor activity of the goldfish.

To determine the effects of nickel on motility, the locomotor rate constants of groups (n=9) of fish before (pretreatment period) and after (treatment period) exposure to nickel were compared. All groups of fish were kept in the same aquaria throughout the entire experiment and the kinetic runs were done in the same aquaria in which the fish were maintained between runs. The pretreatment periods lasted for 5 days, during which two kinetic experiments were conducted each day. After the pretreatment period, the same nine groups of fish were exposed to either 0, 25, 50 or 75 ppm of nickelous chloride hexahydrate for 5 days. The groups (n=3) of fish maintained at 0 ppm during the treatment period served as external controls. The temperature was 27 ± 1° C throughout all test periods.

The number of fish (n=30) used in the present studies is inadequate to define a reliable rate constant for an opposed first-order reaction, if only a simple run is used for analysis; kinetic analysis generally depends on large numbers of reactants (Avogadro's number in chemical kinetics). To overcome the limitations imposed by the small number of fish being used in each kinetic run, the experiments are repeated several times (10 times in the present study) over several days using the same group of fish. The data from all of the individual runs are then pooled to describe a single reaction rate plot and thus a single rate constant. In this way a single rate constant for all of the pretreatment experiments can be compared to a single rate constant for all of the treatment period experiments. Specifically, kinetic studies on each group of fish were conducted twice daily during the 5-d treatment period for a total of 10 kinetic runs. All data collected during the entire pretreatment period were pooled to derive a single semilogarithmic plot and, thus, a single rate constant for each group of fish for the entire pretreatment period. To do this, all values of (A) obtained for each time t, from all experiments on the same group of fish in the pretreatment period, were averaged. This mean value of (A) was then used to determine the log value, $\ln [((A)_0 - (A)_e) / ((A) - (A)_e)]$, at each time t. The same method was used to derive a single or overall rate constant for that same group of fish for the entire period of exposure to nickel (treatment period). For example, see Fig. 1, where one line, the pretreatment control, is defined by pooling the data from 10 independent runs carried out over a 5-d period, prior to the exposure to nickel, and the other is a line derived from the same group of fish by pooling the data from 10 independent runs over a subsequent 5-d period in which the fish were exposed to 75 ppm nickel.

The number of fish that died during the 5-d experimental periods were recorded after each day. Fish mortality was similar in all tanks; an

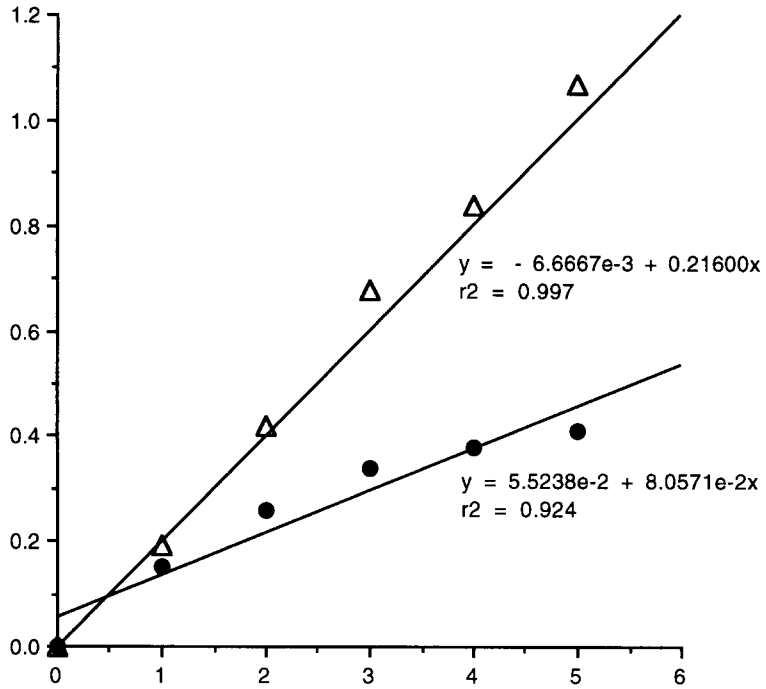

Figure 1. Reaction kinetics for goldfish locomotor activity before (open triangles) and after (closed circles) exposure to nickel at 75 ppm. The value of ln $[((A)_0 - (A)_e) / ((A) - (A)_e)]$ is plotted against time for the first five minutes of the reaction. Ten experiments were routinely run during both a 5-d pretreatment period and a 5-d treatment period to give 10 values of (A) for each minute during the 5-min kinetic run. The mean of the values of (A) for each minute was then used to determine the log value for each minute. The slope of the best fit line is equal to the rate constant for each 5-d period.

average of 1.6 fish died per tank per day in all aquaria throughout the experiment. Dead fish were replaced with goldfish that came from a separate holding tank that contained only dechlorinated tap water.

Consequently, the total number of fish in each aquarium remained at 30; however, the length of their exposure to nickel varied. This procedure reduces any effect that the nickel might have on the whole "nickel-exposed" population because some of its replacement members have indeed had limited exposure to nickel. This in turn minimizes any differences between the locomotor activities of the control and nickel-treated groups and results in a conservative measure of the nickel effects.

RESULTS AND DISCUSSION

In the present study, the concentration of nickel that caused 50% mortality of goldfish in 96 hr (96-hr LC50) was found to be between 75 and 100 ppm (Table 1). Pickering and Henderson (1966) found that nickel was more toxic in soft water than in hard water. For example, the 96-hr LC50 was approximately 5 ppm for both bluegills *(Lepomis macrochirus)* and fathead minnows *(Pimephales promelas)* in soft water (alkalinity of 18 ppm and hardness of 20 ppm, both as $CaCO_3$), whereas it was approximately 40 ppm for both species in hard water (alkalinity of 300 ppm and hardness of 80 ppm). They also observed that goldfish *(Carassius auratus)* were more resistant to nickel, with 96-hr LC50 values in soft water approximating 10 ppm. Thus, if one assumes that goldfish are twice as resistant to nickel as bluegills and fathead minnows, one might expect, by extrapolation, that the 96-hr LC50 for goldfish in hard water might be in the neighborhood of 80 ppm.

Table 1. Mortality of goldfish, *Carassius auratus*, exposed to nickelous chloride for a 96-hr period. Each of the 8 tanks initially contained 8 fish.

Concentration of nickel (ppm)	Percent of fish living at 96 h
0	87.5
1	87.5
10	100.0
25	87.5
50	75.0
75	75.0
100	12.5
125	12.5

Based on the LC50 results, kinetic experiments were conducted at the sublethal concentrations of 25, 50 and 75 ppm. A typical log-rate plot described by the locomotor activity of the fish before and after treatment at 75 ppm is shown in Fig. 1. The rate constants derived from the slopes of the best fit lines at all concentrations are presented in Table 2. Nickel elicited a hypoactive response from the goldfish at all concentrations tested and the ratio of treatment to pretreatment rate constants was concentration dependent. For example, fish exposed to 25 ppm of nickelous chloride were only 69% as active as they were prior to exposure, and the activity of those exposed to 50 and 75 ppm was reduced to 59% and 41% of their pretreatment activity, respectively. This of course results in a reduction in the dispersion of these fish.

Table 2. Effects of nickel on rate constants for locomotor activity of goldfish, *Carassius auratus*.

Nickel concentration (ppm) during treatment period	Rate constant during: Pretreatment period	Rate constant during: Treatment period	Ratio of treatment to pretreatment rate constants
0 (control)	.114	.103	.90
0 (control)	.146	.143	.98
0 (control)	.204	.184	.90
0 (control; average)			.93
25	.101	.070	.69
25	.204	.141	.69
25 (average)			.69
50	.158	.093	.59
75	.200	.079	.40
75	.100	.053	.53
75	.151	.040	.29
75 (average)			.41

The effects of stressors on any organism can be examined at several levels of complexity or integration, increasing in order as follows: 1) gene structure and function and enzyme activity, 2) cell integrity and metabolism, 3) histological lesions, 4) organ behavior, 5) homeostasis, 6) growth and reproduction, 7) ecology and behavior (Heath 1987). In this hierarchy, changes at higher levels represent a more generalized response within the organism. One generally finds that effects observed at the higher levels of complexity reflect effects at lower levels. In the case of nickel, the generalized response, locomotor hypoactivity, may be a reflection of metal-induced changes in neurophysiology leading to changes in exploratory behavior or to tissue damage, such as nickel-induced gill histopathology (Nath and Kumar 1989), which may indirectly affect muscle physiology. Support for the latter is found in the fact that similar alterations in gill structure including hypertrophy, necrosis and epithelial lifting are caused by other heavy metals such as copper, cadmium, chromium and zinc (Heath 1987). This histopathology is associated with decreases in the ventilation rate and in oxygen diffusion across the gill lamellae. If such a histopathological effect were to begin immediately upon exposure to heavy metals one might expect a concomitant decrease in locomotor activity. This was observed when bluegill were exposed to copper (Ellgaard and Guillot 1988) and now appears to be the case when goldfish are exposed to nickel. However, contrary to this prediction cadmium, chromium and zinc induce

locomotor hyperactivity in bluegill (Ellgaard et al. 1978). This suggests that short-term exposure of fish to sublethal concentrations of the latter heavy metals may effect changes in addition to the gill histopathologies. Alternatively, the effects may vary with respect to the fish species and the heavy metal concerned. It is also possible that the hypoactivity induced by nickel or copper may reflect an approach to death, which may be observed following chronic exposure to concentrations which are considered sublethal by 96-hr bioassay tests. This interpretation is consistent with lethality studies, comparing acute and long-term exposure of molluscs to heavy metals, which suggested that the lethal concentrations determined by 96-hr bioassays are artificial and leads to the use of concentrations defined as "sublethal" (by 96-hr LC50) which are actually lethal to organisms exposed for longer than 96 hr (Nelson et al. 1988). For example, surf clams had a 96-hr LC50 value of 0.051 mg/L copper, while 0.010 mg/L was 100% lethal in 45 days and 50% lethal in 28 days.

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REFERENCES

Cairns J Jr, van der Schalie WH (1980): Biological monitoring. I. Early warning systems. Water Res 14:1179.

Chaudry HS, Nath K (1985): Effects of nickel intoxication on liver glycogen of a freshwater teleost, *Colisa fasciatus.* ACTA Hydrochim Hydrobiol 13:245-248.

Ellgaard EG, Bloom KS, Malizia AA Jr, Gunning GE, Jensen RE (1975): The locomotor activity of fish: an analogy to the kinetics of an opposed first-order chemical reaction. Trans Am Fish Soc 104:752-754.

Ellgaard EG, Guillot JL (1988): Kinetic analysis of the swimming behavior of bluegill sunfish, *Lepomis macrochirus* Rafinesque, exposed to copper: hypoactivity induced by sublethal concentrations. J Fish Biol 33:601-608.

Ellgaard EG, Ochsner JC, Cox JK (1977): Locomotor hyperactivity of the bluegill sunfish, *Lepomis macrochirus,* by sublethal concentrations of DDT. Can J Zool 55:1077-1081.

Ellgaard EG, Tusa JE, Malizia AA Jr (1978): Locomotor activity of the bluegill, *Lepomis macrochirus.* Hyperactivity induced by sublethal concentrations of cadmium, chromium, and zinc. J Fish Biol 12:19- 23.

Heath A (1987): Water Pollution and Fish Physiology. Boca Raton, FL: CRC Press.

Kulikova I, Seisuma Z, Legzdina M (1985): Heavy metals in water

organisms. Symp Biol Hung 29:141-154.
Nath K, Kumar N (1989): Nickel-induced histopathological alterations in the gill architecture of a tropical freshwater perch, *Colisa fasciatus*. Sci Total Environment 80:293-296.
Nelson DA, Miller JE, Calabrese A (1988): Effect of heavy metals on bay scallops, surf clams, and blue mussels in acute and long-term exposures. Arch Environ Contam Toxicol 17:595-600.
Pickering QH, Henderson C (1966): The acute toxicity of some heavy metals to different species of warm water fishes. Air Wat Pollut Int J 10:453-463.