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Factors Influencing Thiocyanate Toxicity in Rainbow Trout *Salmo gairdneri*

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The toxicity of thiocyanate (SCN^-) to fish is influenced by the level of fish activity (Heming et al. 1985). This is evidenced most dramatically when fish are forced to perform short bouts of strenuous swimming, such as occurs during capture avoidance. Strenuous exercise of SCN^--exposed fish results in "sudden death syndrome", characterized by the immediate onset of convulsions, loss of equilibrium and buoyancy, flaring of the operculum, darkening of the skin epithelium and, within minutes, cessation of ventilation and extreme rigor. Similar signs of SCN^- toxicity have been observed in mammals, including tonic convulsions, hyper-reactivity, vertigo, and extensor rigidity (Garvin 1939, Barnett et al. 1951, Smith 1973). In fish, the magnitude of the exercise effect decreases as the frequency of the exercise bouts increases (Heming et al. 1985).

The present study was undertaken to examine the accumulation and toxicity of SCN^- in rainbow trout (Salmo gairdneri), in relation to exercise stress and ambient water quality. The effect of a single bout of exercise on blood SCN^- concentration was measured. In addition, effects of water hardness and Cl^- concentration on the accumulation of SCN^- in blood were determined.

MATERIALS AND METHODS

Rainbow trout were obtained from Sam Livingston Hatchery, Calgary, Alberta. The fish were held in dechlorinated tapwater (Table 1, full strength), under a 12-h light/12-h darkness photoperiod. The animals were fed a commercial trout diet (Martin Feed Mills Limited, Elmira, Ontario), but were not fed for 48 h prior to, or during, the experiments. The tests were conducted under static-flow conditions in 40-L polyethylene-lined tanks (46 cm diameter, 76 cm height), with 25% water replacement daily. The tanks were continuously aerated. Fish for a given test were distributed randomly among the tanks and allowed 48 h

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Table 1. Chemical characteristics (mg/L) of test waters: charcoal-filtered tapwater full strength or diluted to half-strength with reverse osmosis water.

Characteristic	Full strength tapwater	Half-strength tapwater
alkalinity, as $CaCO_3$	93	53
calcium	41	24
chloride	5	3
chlorine	<0.010	<0.010
hardness, as $CaCO_3$	152	86
magnesium	12	7
potassium	1.3	1.0
sodium	6	4

to acclimate to the test environment without toxicant. Reagent grade KSCN was used as the toxicant. Full strength tapwater (Table 1) was used as the diluent, unless otherwise indicated.

Three separate tests were conducted. First, an acute lethality bioassay was conducted which included a control and four toxicant concentrations. Dead fish were counted and removed from each tank at least once daily. To assess the effects of exercise, the fish were chased with a hand-held dipnet and forced to swim vigorously for 30 s at 96 h into the test. Mortality counts were repeated 1 h later (97 h into the bioassay).

The second test examined the effect of a single exercise bout on blood SCN^- concentrations. Three groups of fish were used in this particular study: group A, control animals which were unstressed and unexposed (n = 12); group B, unstressed SCN^--exposed fish (n = 16); group C, stressed SCN^--exposed fish (n = 20). Groups B and C were exposed to a nominal concentration of 75 mg/L SCN^- for 73 h. At 72 h into the test, fish in group C were exercised as described above. The test was terminated 1 h later at which time the surviving fish from all three groups were netted from the tanks and stunned by a blow to the head. A blood sample for SCN^- determination then was collected from the caudal blood vessels of each fish.

The third study investigated the effects of water quality on accumulation of SCN^- by trout. This study used two dilution waters (dechlorinated tapwater full strength or diluted to half-strength with reverse osmosis water, see Table 1), four SCN^- concentrations spanning the nominal range of 0 to 100 mg/L SCN^-, and three levels of added NaCl (0, 1 and 2 times the molar concentration of added KSCN, equivalent to 0 to 200 mg/L NaCl). After a test period of 96 h, surviving fish were netted from the tanks and stunned by a blow to the head. Blood samples for SCN^- determination then were collected as described above.

Physicochemical analyses of the exposure solutions for SCN^-, dissolved oxygen, pH, and temperature were performed two to four times in each tank during each test. Dissolved oxygen concentration, pH, and temperature were measured with a Hydrolab Environmental Data System (Hydrolab Corporation, Austin, Texas). SCN^- in water and blood was determined, as total cyanide, by the method of Pettigrew and Fell (1972). Recovery of SCN^- from spiked samples averaged 108% (standard deviation = 6). Only measured SCN^- concentrations are reported.

The data are given as arithmetic means, with their standard errors (S.E.). Mean values were compared using Student's *t*-tests.

RESULTS AND DISCUSSION

Under the present experimental conditions, the 96-h LC50 of SCN^- for unstressed rainbow trout was greater than 94 mg/L SCN^- (Table 2). The observed mortalities of unstressed fish were sporadic and without an apparent exposure relationship. Similar exposure-response anomalies have been observed previously for SCN^- (Smith 1973, Heming *et al*. 1985), and probably are related to subtle disturbances of the test animals during routine laboratory activities (e.g., sampling of water for chemical analyses). Subtle factors, such as auditory and tactile stimuli (Smith 1973) and abrupt changes in light intensity (Heming *et al*. 1985), have been shown to increase the mortality of SCN^--exposed animals.

A single, 30-s bout of strenuous exercise at 96 h was effective to induce "sudden death" in rainbow trout exposed to $\geq$ 25 mg/L SCN^-. The syndrome signs were identical to those reported previously (Heming *et al*. 1985) and included tonic convulsions and immediate post-exercise loss of equilibrium and buoyancy. Within 2 min of the exercise bout, a total of 12 fish in the acute lethality bioassay were observed to be in distress and unable to maintain an upright posture. When the test was terminated at 1 h post-stress (97 h into the bioassay), 11 of these 12 fish were dead and the remaining individual was unable to maintain an upright posture.

The SCN^- concentrations found to induce "sudden death" in the present study were substantially greater than those previously reported (Heming *et al*. 1985) to induce the syndrome. "Sudden death" was absent in fish exposed to 10 mg/L SCN^- for 97 h and present in only 43% of fish exposed to 25 mg/L SCN^- (Table 2). In contrast, Heming *et al*. (1985) documented exercise-induced "sudden death" in 100% of rainbow trout exposed to 7.7 mg/L SCN^- for 97.25 h and in 70% of brook trout exposed to 8.0 mg/L SCN^- for 96.25 h (both groups exercised at 96 h of exposure).

Control (unexposed) fish had significant blood SCN^- concentrations; average values were 3 to 5 mg/L SCN^- (Tables 3 and 4). Dilution water was not the source of this SCN^-; repeated analyses failed to detect SCN^- in the dilution waters

Table 2. Percent mortality before and after stress of rainbow trout exposed to thiocyanate, indicating the prevalence of sudden death syndrome. Fish were stressed (chased with a dipnet) for 30 s at 96 h into the test; mortality was measured immediately pre-stress and 1 h post-stress (97 h into the test).[a]

Exposure concentration[b] (mg/L SCN^-)	96-h mortality (pre-stress)	97-h mortality (post-stress)	Mortality due to sudden death (%)
0.0	0	0	0
10 ± 0.3	37.5	37.5	0
25 ± 0.3	12.5	50	43
52 ± 1	0	25	25
94 ± 6	0	75	75

[a]Eight fish per tank. Mean values and ranges for all tanks: fish weight, 4.1 g (2.4-8.5); fork length, 7.1 cm (5.8-9.6); water temperature, 12.2 °C (11.8-12.5); pH, 8.1 (8.0-8.1); dissolved oxygen concentration, 9.7 mg/L (9.2-10.2).
[b]Mean ± S.E., N = 4.

Table 3. Effect of a single bout of exercise on mortality and blood thiocyanate concentrations of rainbow trout exposed to thiocyanate (average water concentration, 65 ± 1 mg/L SCN^-, ± S.E., N = 35) for 73 h. Fish in group C were stressed (chased with a dipnet) for 30 s at 72 h into the test.[a]

Group	Mortality (%)		Blood [SCN^-] at 73 h[b] (mg/L)
	72 h	73 h	
A, control (unstressed unexposed)	0	0	5 ± 1
B, unstressed exposed	25	25	208 ± 26
C, stressed exposed	30	40	72 ± 5

[a]Four fish per tank. Mean values and ranges for all tanks: fish weight, 29.5 g (14.5-60.5); fork length, 13.4 cm (10.5-17.0); water temperature, 10.8 °C (10.1-11.7); pH, 7.6 (7.3-7.8); dissolved oxygen concentration, 10.2 mg/L (9.5-10.6).
[b]Mean values ± S.E. (N = 12).

Table 4. Effect of water quality on accumulation of thiocyanate in the blood of rainbow trout over a 96-h test period.[a] Chemical characteristics of full and half-strength tapwaters are summarized in Table 1. Mean values ± S.E. (N = 4-5).

	Water SCN^- concentration (mg/L)							
	Half-strength tapwater				Full strength tapwater			
Molar ratio $Cl^-:SCN^-$	0	6±1	14±1	100±4	0	7±1	15±1	100±5
	Blood SCN^- concentration (mg/L)							
0:1	3±0.4	35±2	53±8	349±12	3±0.5	40±3	66±2	323±18
1:1	ND	8±1	13±1	93±10	ND	7±2	16±5	76±14
2:1	ND	5±1	5±1	23±3	ND	5±2	5±1	20±2

[a]Five fish per tank. Mean values and ranges for all tanks: fish weight, 14.5 g (5.1-36.6); fork length, 10.6 cm (8.1-14.4); water temperature, 10.7 °C (9.0-11.8); pH, 7.4 (6.9-7.7); dissolved oxygen concentration, 9.9 mg/L (7.0-11.4).
ND, not determined.

(<0.1 mg/L SCN^-). Cross contamination was an unlikely source because, during each study, there was no transfer of equipment (e.g., dipnets, water chemistry electrodes) from toxicant tanks to control tanks, while, between studies, all equipment was thoroughly cleaned and tank liners replaced. Finally, the presence of SCN^- in the blood of control fish did not appear to be an artifact of the measurement technique because previous analyses using the same method with rainbow trout maintained on a different diet (Rangen Incorporated, Buhl, Idaho) yielded blood SCN^- concentrations that were not significantly different from zero (0.4 ± 0.4 mg/L SCN^-, N = 12). Thus, dietary precursors (Haynes and Murad 1985) were considered to be a pausible source of SCN^- for animals in the present studies.

Fish were able to accumulate SCN^- against its diffusion gradient (Tables 3 and 4). The blood SCN^- concentration of exposed fish exceeded the water SCN^- concentration by as much as 5.8 times, dependent upon the exposure concentration and duration. Blood SCN^- concentrations were also influenced by exercise. After identical SCN^- exposures, the average blood SCN^- concentration of fish that survived an exercise bout was 65% lower than the blood SCN^- concentration of unstressed fish (Table 3). In other words, while the blood SCN^- concentration of unstressed exposed fish (Group B) exceeded the water SCN^- concentration by about three times, the blood SCN^- concentration of stressed exposed fish (i.e., fish that survived the exercise bout, Group C) was approximately equal to the water SCN^- concentration. Gill ion

permeability is hormonally modulated in fish; passive permeability increases in response to stress hormones, such as epinephrine and norepinephrine (Eddy 1981). Thus, the observed decrease in blood SCN^- concentration following an exercise bout probably was attributable to a stress-induced hormone-mediated increase in gill passive permeability and a resultant increase in leakage of SCN^- down its diffusion gradient from blood to water. Exercise-induced reductions in blood SCN^- concentration would explain our previous observation that, at a given water SCN^- concentration, the severity of exercise-induced "sudden death" is inversely related to the frequency of the exercise bouts (Heming et al. 1985).

Effects of ambient water quality on blood SCN^- concentrations are summarized in Table 4. Exercise stress was not involved in this particular test and all animals survived the 96-h test period. At any given water SCN^- concentration, the 96-h blood SCN^- concentration was inversely related to the water Cl^- concentration. SCN^- competes with other anions, particularly chloride, for uptake across the fish gill (Epstein et al. 1973, 1975, Heming et al. 1985). In the present study, Cl^- at concentrations $\geq$ 23 mg/L (two times the molar equivalent of SCN^-) abolished SCN^- accumulation at exposure concentrations $\leq$ 15 mg/L SCN^-. The average blood SCN^- concentrations of fish under such conditions were not significantly different from that of control (unexposed) fish. At 100 mg/L SCN^-, Cl^- at concentrations $\geq$ 125 mg/L (two times the molar equivalent of SCN^-) reduced SCN^- accumulation by almost 95%. Thus, one would reasonably expect ambient Cl^- to protect fish from SCN^- toxicity, including "sudden death syndrome".

No significant effect of dilution water (half-strength tapwater versus full strength tapwater) was seen with respect to 96-h blood SCN^- concentrations (Table 4). This indicates that water hardness in the range of 86 to 152 mg/L (as $CaCO_3$) was not a determinant factor for SCN^- accumulation. The absence of a hardness effect on SCN^- accumulation suggests that any decrease in the rate of SCN^- leak from blood to water due to reduced gill passive permeability at the higher water hardness level was offset by a decrease in the rate of SCN^- uptake due to a general reduction in ion influx.

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