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Acute Toxicity of Cyanogen Chloride to *Daphnia magna*

Douglas W. Kononen

Department of Fisheries and Wildlife, University of Minnesota, St. Paul, Minnesota 55108

The destruction of cyanide in waste waters by chlorination has been shown to result in the formation of the extremely toxic compound, cyanogen chloride (Allen et al 1948). Industrial cyanide-containing waste waters may be treated by a batch chlorination process under highly alkaline conditions prior to being discharged into a receiving water system (Pettet and Ware 1955). Alternatively, if the concentration of cyanide is relatively low, such waste waters may be diverted to municipal waste treatment facilities where they may be subjected to a process of chlorination which may not be sufficient for the complete oxidative destruction of the available cyanide. Eden et al (1950) concluded that if a dilute solution of cyanide was allowed to react at a pH of 8.0 or less with an amount of hypochlorite only moderately over the theoretical quantity necessary for the complete conversion of cyanide to cyanogen chloride, then the resulting CNCl could persist for periods in excess of 24 h.

Although a large body of literature exists concerning the toxicity of HCN and metallic cyanide compounds to aquatic organisms, there is a comparative scarcity of information concerning cyanogen chloride toxicity. This study was designed to determine the acute toxicity of CNCl to Daphnia magna neonates under static bioassay conditions.

MATERIALS AND METHODS

Test solutions of CNCl were prepared with analytical grade sodium thiocyanate (Mallinckrodt Chemical Works, St. Louis, Missouri) and sodium hypochlorite (HILEX, 5.25% w/w NaOCl solution). To a solution containing approximately 0.47 mg/L NaSCN, a measured amount of a dilute NaOCl solution (50.0 to 54.0 mg/L NaOCl as Cl) was added to bring the total calculated concentration of hypochlorite in solution to about 1.6 mg/L (as Cl). The concentrations of the dilute hypochlorite solutions were determined as mg/L Cl per the iodometric titration Method I described in American Public Health Association (1975).

Currently with Operating Sciences Department, General Motors Research Laboratories, GM Technical Center, Warren, MI 48090-9055.

The amount of NaOCl added to the thiocyanate solution was approximately 90% of the theoretical amount necessary to convert all of the thiocyanate to CNCl according to the following equation modified from Eden and Wheatland (1950).

(1) $$NaCNS + 4HOCl \rightleftharpoons CNCl + NaHSO_4 + 3HCl$$

Concentrations of CNCl (as CN^-) were determined via a slight modification of the Colorimetric Method for determining $[CN^-]$ described in American Public Health Association (1975). In this method, CN^- is converted to CNCl by reaction with chloramine-T. After the reaction is complete, CNCl forms a red-blue dye on addition of a pyridine-barbituric acid reagent. Absorbance is read at 578 nm. Since, according to equation (1), we have already formed CNCl, addition of the chloramine-T reagent was omitted. The calibration curve used to determine CNCl concentration (as CN^-) was based on a series of standards containing from 10 to 300 μg CN^- per L.

Hydrolysis of CNCl in an alkaline solution has been described by the following relation [Price et al (1947) and Eden and Wheatland (1950)].

(2) $$CNCl + OH^- \rightleftharpoons HOCN + Cl^-$$

Price et al (1947) found that this reaction was slow at a pH of 8.0, but increased rapidly at higher pH values ($\geq$ 10.0). According to Allen et al (1948) the cyanate formed in the above reaction (equation 2) is rapidly converted to ammonia and carbon dioxide in an acidic environment:

(3) $$HOCN + H_2O \rightleftharpoons NH_3 + CO_2$$

CNCl is both pH- and time-dependent. At pH 9.0, with no excess chlorine present, CNCl may persist for 24 h (American Public Health Association 1975). CNCl hydrolysis in alkaline solution described in equation (2) can be described by the following equation:

(4) $$d[CNCl]/dt = -k[CNCl][OH^-]$$

The hydrolysis rate constant, k, has been estimated by Price et al (1947) to be approximately 600 L/mol-min at a temperature of 25° C and pH ranging from 7.70 to 10.15. To obtain an estimate of k at the conditions of the Daphnia magna acute tests (temperature: 20° C, pH: 8.50), a solution of CNCl was prepared as described above and maintained in a covered flask placed in a constant temperature water bath held at 20° C. Samples for CNCl determination were withdrawn immediately after preparation and after intervals of approximately 5, 10, 15, 20, 30, 40, 50, 60, 90, 120, and 180 min. The initial pH of the solution was 8.64.

The chemical characteristics of the well water used to prepare the CNCl solutions are described in Adelman and Smith (1972). Daphnia

magna cultures were maintained in 250 mL Pyrex beakers (with spouts) containing approximately 200 mL of well water in a constant temperature water bath held at 20±1° C. Each beaker contained up to five adults. Young were removed from the beakers three times per week at which times the adults were transferred to clean beakers containing fresh supplies of dilution water and food. The food mixture consisted of a suspension of pulverized pesticide-free trout food and yeast in well water.

Initial range finding acute toxicity tests utilized neonates between 1 to 5 days old. Neonates used in the acute toxicity bioassay were ≤ 24 h old. Test organisms were not fed during the testing period. One control plus six toxicant concentrations with an approximate 60% dilution factor were run in duplicate. Ten neonates in 200 mL of test solution were used for an initial range finding test. The acute experiment utilized 10 neonates per concentration replicate. The initial cohorts from which the test organisms were selected consisted of two to three times the total number of daphnids actually required. Test animals were introduced into the test solutions with a pipette, beginning with the controls and continuing up through successively increasing treatment concentrations. Lighting over the constant temperature water bath in which the test chambers were situated was provided by four 40-Watt Vita-Lite fluorescent tubes located approximately 50 cm above the tops of the test chambers. The day length was set at 16 h of daylight with 8 h of total darkness.

Measurements of water temperature, dissolved oxygen, pH, and toxicant concentration from each treatment level were made at the beginning and end of the 48-h toxicity bioassay. Dissolved oxygen, pH, and water temperature were determined for each treatment replicate. A combined sample consisting of 10 mL from each of two replicates per treatment level was used to determine [CNCl]. Dissolved oxygen was measured with a galvanic-type membrane electrode meter (Yellow Springs Instrument Co., Yellow Springs, Ohio) and pH was determined with a Beckman Zeromatic pH Meter (Beckman Instruments, Inc., Fullerton, California). Each test chamber was covered with a glass plate. This arrangement did not provide a complete seal since spouted beakers were utilized. Since the tests were conducted at 20° C, an unknown, but potentially significant quantity of CNCl was lost via volatilization (b.p. = 12.7° C). Computerized probit analysis (SAS Institute 1985) was used to calculate LC50s.

RESULTS AND DISCUSSION

Table 1 lists the results of the CNCl hydrolysis experiment. Regressing the natural log of CNCl [as CN^- (mol/L)] versus time results in the following equation.

$$(5) \qquad \log_e[CNCl]_t = \log_e[CNCl]_{to} - k'(t)$$

$$= -11.9446 - 0.0022(t); \; r^2 = 0.95$$

The high coefficient of determination ($r^2 = 0.95$) indicates that the hydrolysis of CNCl in a mildly alkaline solution (pH = 8.64) is a first order reaction. The pseudo first order hydrolysis rate constant, k', divided by the hydroxyl ion concentration gives the true hydrolysis rate constant, k. The bottom of Table 1 shows the calculated values for the hydrolysis rate (504 L/mol-min) and half life (315 min) at a pH and temperature of 8.64 and 20° C, respectively. The volatility of CNCl indicates the necessity for a closed system when working with this compound to prevent excessive loss via volatization. The half life of a CNCl under the described conditions can be expected to be less than 315 min if the test chambers are freely exposed to the atmosphere. Since a 48-h test period contains 2880/315 or 9.1 half lives, an estimate of [CNCl] remaining after 48 h is obtained by multiplying the initial concentration by $(0.5)^{9.1}$ or 0.0018. In the absence of direct measurements of [CNCl], this method can be used to estimate the toxicant concentration at any time after the beginning of a test.

Table 1. Hydrolysis of CNCl at 20° C (pH = 8.64)

Time (min)	[CNCl as CN^-] (μg/L)	[CNCl as CN_6] (Mol/L x 10^6)
0	165	6.341
5	162	6.226
12	163	6.264
18	162	6.226
23	161	6.188
35	157	6.034
43	158	6.072
53	154	5.919
63	150	5.765
93	146	5.611
128	123	4.727
190	110	4.228

$$d[CNCl]/dt = -k[OH^-][CNCl] = k'[CNCl]$$

$$\log_e [CNCl]_t = -11.9446 - 0.0022(t); \; r^2 = 0.95$$

$$k = k'/[OH^-] = 504 \text{ L/mol-min}$$

$$t_{1/2} = \log_e 2/k' = 315 \text{ min}$$

Table 2 lists the results of the 48-h range finding test using daphnid neonates up to 5 days old. Approximate 95% confidence interval estimates for the 24- and 48-h LC50s are given in parentheses after the LC50 values. Test organisms were considered dead when no movement was detected after gentle stimulation with a small glass probe. Within minutes after their introduction to the test chambers, daphnids in the highest test concentrations (i.e., 154 and 262 μg/L) swarmed to the surface and swam in erratic spiraling bursts. This agitated swimming behavior was not

observed in either the controls or in the lower treatment levels. The calculated 48-h LC50 value (65 μg/L) is an overestimate of the "true" LC50 since it is based on observed treatment concentrations at the beginning of the experiment. Different values for the LC50 might be obtained from a continuous flow, constant concentration experimental design.

Table 2. Daphnia magna **neonate 48-h range finding LC50**

Starting Test Conditions				Survival[1]	
CNCl as CN (μg/L)	Temp (°C)	pH	D.O. (mg/L)	24 h S/N	48 h S/N
Control	20.5	8.41	7.9	10/10	10/10
18	20.6	8.46	7.9	10/10	10/10
31	20.6	8.47	7.8	10/10	10/10
53	20.6	8.47	7.8	9/10	7/10
88	20.6	8.50	7.8	3/10	2/10
154	20.6	8.50	7.8	2/10	0/10
262	20.6	8.51	7.9	0/10	0/10

[1]S/N = No. surviving/total number tested
24-h LC50 = 86 μg/L; 95% conf int: (66,111); χ^2_2 = 2.709 (p=0.608)
48-h LC50 = 65 μg/L; 95% conf int: (52,81) ; χ^2 = 0.275 (p=0.991)

Table 3 summarizes the 48-h acute test using neonates ≤ 24 h old. Calculated 24- and 48-h LC50 values are approximately one half as large as those obtained using slightly older organisms (cf. Table 2). The last two columns in Table 3 are predicted CNCl concentrations at 24 and 48 hours, respectively. These predicted concentrations were calculated using the half life data obtained in

Table 3. Daphnia magna **neonate 48-h LC50**

Starting Test Conditions					Ending Test Conditions			Survival[1]		Predicted CNCl
CNCl as CN (μg/L)	Replicate	Temp (°C)	pH	D.O. (mg/L)	Temp (°C)	pH	D.O. (mg/L)	24-h S/N	48-h S/N	(as μg/L CN) 24-h	48-h
Control	1	20.8	8.46	7.6	21.1	8.50	7.7	10/10	10/10	-	-
	2	20.6	8.52	7.6	21.3	8.56	7.7	10/10	10/10	-	-
8	1	20.8	8.49	7.5	21.3	8.51	7.6	10/10	10/10	0.3	0.01
	2	20.8	8.50	7.5	21.1	8.50	7.7	10/10	10/10	-	-
16	1	20.8	8.51	7.6	21.3	8.56	7.6	10/10	10/10	0.7	0.03
	2	20.7	8.50	7.7	21.1	8.60	7.5	10/10	10/10	-	-
28	1	20.8	8.50	7.6	21.0	8.63	7.7	9/9	9/9	1.2	0.05
	2	20.8	8.51	7.5	21.0	8.63	7.7	8/10	1/10	-	-
48	1	20.8	8.50	7.4	21.0	8.61	7.5	7/10	1/10	2.0	0.09
	2	20.8	8.49	7.4	21.0	8.54	7.6	0/10	0/10	-	-
77	1	20.8	8.50	7.4	20.6	8.56	8.1	0/10	0/10	3.2	0.1
	2	20.8	8.50	7.4	20.5	8.57	-	0/10	0/10	-	-
126	1	20.8	8.49	7.3	20.8	8.58	8.2	0/10	0/10	5.3	0.2
	2	20.8	8.50	7.3	20.7	8.60	-	0/10	0/10	-	-

[1]S/N = No. surviving/total number tested
24-h LC50 = 40 μg/L; 95% conf int: (35,46); χ^2_2 = 0.990 (p=0.911)
48-h LC50 = 29 μg/L; 95% conf int: (25,34); χ^2 = 0.275 (p=0.969)

the CNCl hydolysis experiment (Table 1). At the end of the 48-h lethality tests summarized in Tables 2 and 3, the highest treatment levels were analyzed for [CNCl]. As predicted by the last column in Table 3, no CNCl was detected.

On the basis of this test (Table 3), a concentration of 29 μg/L CNCl (as CN^-) is sufficient to result in 50% mortality of a Daphnia magna neonate population exposed to similar conditions. This is markedly below the 3.4 mg/L free cyanide 48-h LC50 estimate reported by Meinck et al (1956) for this same species. Broderius et al (1977) reported 0.12 and 0.11 mg/L 96-h free cyanide LC50s for fathead minnows (Pimephales promelas) at pH values of 8.29 and 8.67, respectively. These latter estimates are close to the LC50 estimates calculated for Table 2 data, suggesting that CNCl may be at least as toxic to aquatic organisms as HCN.

Strictly speaking, the LC50 values derived from Tables 2 and 3 do not pertain to pure CNCl since this compound undergoes considerable hydrolysis at alkaline pH (equation 2). The observed lethality could be attributed to a combined effect of CNCl and its hydrolysis by-products (i.e. Cl^-, NH_3, CO_2, etc.).

The importance of cyanogen chloride as an environmental contaminant depends largely on its environmental persistence. Bailey and Bishop (1970) reported half life values for CNCl ranging from 1 min at 45° C to 10 h at 5° C. Toxic concentrations of this compound may, therefore, be present in a given waste receiving water for a period of time sufficient to result in acute toxicity to populations of aquatic organisms.

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