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Influences of Hardness Constituents on the Acute Toxicity of Cadmium to Brook Trout *(Salvelinus fontinalis)*

John J. Carroll[1], Stephen J. Ellis[2], and Walter S. Oliver[2]
[1]U. S. Environmental Protection Agency, Office of Water and Waste Management Washington, D. C. 20460, and [2]EG&G, Bionomics, Wareham, Mass. 02571

The term, hardness, was originally used to quantify the ability of a water to prevent soap from foaming. This property was long used as an analytical tool for estimating the concentration of calcium plus magnesium in water. Although modern analytical techniques easily distinguish between these two ions, the term has persisted and many contemporary data have been generated without reference to specific hardness ions. Some authors have also reported alkalinity, which is a measure of buffering capacity, and which correlates reasonably well with hardness in most, but not all, natural waters. Because these parameters have dominated the literature, it has not been clear which constituents of hard water have protected aquatic life exposed to cadmium and other heavy metals.

Many workers have reported that heavy metals are less acutely toxic to aquatic organisms in hard water than in soft. KINKADE and ERDMAN (1975) showed that hard water inhibited the uptake of cadmium by several aquatic organisms. A number of workers demonstrated that hard water is protective of fish exposed to cadmium in acute studies (BROWN 1968, McCARTHY and HOUSTON 1978, PICKERING and HENDERSON 1966, and others), but few (JONES 1938, 1939, TABATA 1969) have examined the protective effects of individual hardness constituents (i.e., Ca, Mg and components of the carbonate system). HIGUCHI (1974) reported that calcium ion is protective of the protozoan, Tetrahymena pyriformis, exposed to cadmium and that magnesium is not.

The experiments reported here were designed to determine the contribution of each of several components of hardness to the protection of brook trout (Salvelinus fontinalis) against cadmium toxicity and to shed light on the question of whether it is due to detoxication by precipitation of cadmium ion.

MATERIALS AND METHODS

Five test waters were prepared in stainless steel containers by dissolving appropriate salts ($3x10^{-3}$M) in a reconstituted soft water (Recon) prepared according to U.S. EPA (1975) methods.

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The salts used were calcium carbonate, calcium sulfate, magnesium carbonate, magnesium sulfate, and sodium sulfate. A sixth test water consisted of Recon alone. Magnesium and calcium carbonate solutions were prepared by bubbling with carbon dioxide to dissolve the salts, after which the pH was adjusted to 7.3 to 7.7 by purging with air. All test waters were within this pH range when fish were introduced.

Test procedures followed were those specified in U.S. EPA (1975). The test aquaria were 19.6 liter glass jars containing 15 liters of test water. The appropriate amount of a cadmium sulfate-water stock solution was pipetted directly into each test jar and mixed thoroughly. Ten brook trout, approximately three months old with a mean (± S.D.) wet weight of 0.21 (± 0.06) g were introduced into each test jar one hour (± 1 minute) after addition of the cadmium sulfate.

The temperature of the test waters was maintained at 12 (± 1) degrees throughout testing. Dissolved oxygen concentration, total hardness, total alkalinity, and pH were measured at 0 and 96 hours in the test jars containing the highest and lowest cadmium concentrations and in controls for each test water. Water samples were collected from these same test jars at 0 and 96 hours for determination of total and "soluble" (passing a 0.45u membrane filter) cadmium. Filters used for "soluble" cadmium filtration were prewashed with nitric acid and rinsed with deionized, distilled water prior to sample filtration. The cadmium concentration of filtered and unfiltered water samples was determined by atomic absorption spectrometry according to U.S. EPA (1976) methods.

Mortality was recorded at 24, 48, 72 and 96 hours. LC50 values based on nominal concentrations were calculated by the moving average angle method (HARRIS 1959) except in the few cases for which erratic mortality data required calculation by least squares regression.

RESULTS

Table 1 presents LC50 data obtained in the different test waters. From these data it is clear that calcium ion is very protective while magnesium, sodium and sulfate ions and the carbonate system give little or no protection. The chemical characteristics of controls and test waters with highest and lowest cadmium levels for each water type are presented in Table 2. Measured hardness, alkalinity, and pH were close to the expected values and did not change greatly during the exposure period. Table 3 compares total and "soluble" cadmium concentrations at the beginning and end of each test. Total and "soluble" concentrations were comparable in most cases. However, on the average, "soluble" concentrations were higher than total, indicating the difficulty of preventing contamination of samples at such low concentrations.

TABLE 1

Acute toxicity of cadmium to brook trout (*Salvelinus fontinalis*) in various test waters

	LC50 Values (µg/ℓ)^a			
Test Water	24 hour	48 hour	72 hour	96 hour
Recon	>15	2.5 (1.9-3.3)	1.3^b (0.5-3.5)	$<1.5^c$
$CaCO_3$	>130	59 (46-77)	34 (28-42)	26 (20-34)
$CaSO_4$	>130	70 (58-80)	41 (33-54)	29 (23-54)
$MgCO_3$	>7.8	9.5^b (2.4-38)	5.3 (4.8-5.8)	3.8 (1.3-7.6)
$MgSO_4$	>15	15^b (4.2-51)	6.8 (3.8-9.1)	4.4 (3.4-6.6)
Na_2SO_4	>7.8	13^b (6.6-24)	3.0 (2.5-3.8)	2.4 (1.9-2.8)

[a] LC50 calculations based on nominal concentrations.
[b] Least squares regression.
[c] LC50 not calculable due to 100% mortality at this concentration.

TABLE 2

Chemical characteristics of test waters measured at 0 hour and after 96 hours.

Test[a] Water	Nominal Cd. conc. (µg/ℓ)	Total Hardness (mg/ℓ as $CaCO_3$)		Total Alkalinity (mg/ℓ as $CaCO_3$)		pH	
		0 h	96 h	0 h	96 h	0 h	96 h
Recon	0	44	40	30	32	7.4	7.0
	1.5	42	40	30	32	7.3	7.1
	15	42	42	28	30	7.3	7.2
$CaCO_3$	0	344	340	325	328	7.4	7.7
	6.0	320	340	326	332	7.3	7.7
	130	340	344	328	327	7.3	7.5
$CaSO_4$	0	356	332	30	30	7.3	7.0
	6.0	352	348	30	30	7.3	7.1
	130	348	344	30	28	7.3	7.1
$MgCO_3$	0	356	348	312	314	7.6	7.8
	1.1	356	352	323	324	7.7	8.0
	7.8	352	360	319	317	7.7	7.9
$MgSO_4$	0	322	332	32	32	7.4	7.1
	1.5	324	336	30	27	7.3	6.7
	15	336	324	29	30	7.3	6.9
Na_2SO_4	0	44	46	29	27	7.4	7.0
	0.5	42	44	32	34	7.4	7.1
	7.8	46	44	29	29	7.4	7.0

[a] Calcium, magnesium and sodium salts added to Recon at concentration of 3×10^{-3}M.

TABLE 3

Total and soluble[a] cadmium concentrations

Test Water	Nominal Cd. Conc. (μg/ℓ)	Measured Concentrations			
		Total Cd. Conc. (μg/ℓ)		Soluble Cd. Conc. (μg/ℓ)	
		0 h	96 h	0 h	96 h
Recon	0	0.30	2.9	0.06	2.7
	1.5	1.6	2.0	3.3	2.7
	15	14	13	18	14
$CaCO_3$	0	0.19	1.5	0.42	2.6
	6	5.2	8.6	7.9	8.1
	130	190	180	140	120
$CaSO_4$	0	0.32	0.70	0.38	1.6
	6	7.2	7.0	6.9	8.1
	130	190	180	120	140
$MgCO_3$	0	0.58	0.50	0.72	0.47
	1.1	1.3	1.7	1.4	1.0
	7.8	9.4	12	10	8.6
$MgSO_4$	0	0.12	0.08	4.8	0.23
	1.5	1.4	1.6	1.6	1.7
	15	18	18	19	16
Na_2SO_4	0	0.063	0.16	0.20	0.29
	0.5	0.81	1.1	0.72	0.83
	7.8	9.3	10	11	10

[a] Soluble defined as passing 0.45μ membrane filter.

DISCUSSION

Since hard water normally contains more dissolved carbon dioxide than soft water, it has a higher concentration of carbonate ion at a given pH and may precipitate cadmium carbonate. If carbon dioxide levels fall (as during aeration) solution pH rises, precipitating cadmium carbonate and perhaps the hydrous oxide. In tests reported here, every effort was made to avoid precipitation, so that any protective effect observed could be attributed to other mechanisms.

KINKADE and ERDMAN (1975) and McCARTHY et al. (1977) noted a rapid disappearance of cadmium from solution. However, both groups worked with much higher levels than those reported here. There was no indication of toxicant loss from solution during this study except at two of the highest concentrations tested ($CaCO_3$ and $CaSO_4$ test waters with a nominal cadmium concentration of 130 ug/l) (Table 3). While precipitation may have influenced LC50 values slightly in these two tests, the influence could not have been great because:

(a) Precipitation amounted to no more than about 40% of the cadmium present.

(b) No precipitation was detected at 15 ug/l nominal concentration, a level at which 90 to 100% mortality was observed in waters low in calcium, but at which little or no mortality was observed in waters high in calcium.

Any precipitation that occurred must have quickly reached equilibrium since cadmium concentrations in filtered samples did not decline over the test period.

One hundred percent mortality at the lowest concentration tested made it impossible to estimate a 96-hour LC50 for cadmium in Recon. Although measured total cadmium concentration in the Recon control at 96 hours was greater than the LC50, control survival was 100 percent. This may have been an analytical error. Alternatively, the cadmium concentration may have risen too late in the test to cause mortality. Measured total cadmium concentrations in the controls increased by an average of about 0.7 ug/l over the test period. (Table 3). Since this is a large fraction of the 96-hour LC50 for brook trout in very soft water (our 96-hour LC50 value is <1.5 ug/l), it could be expected to generate erratic results and bias the observed LC50 toward a lower than true value. For more tolerant species, or for brook trout in waters of higher calcium content, this effect might be negligible, but in this case it puts the precise 96-hour LC50 in doubt. Future testing with such sensitive species will require more stringent control of cadmium contamination.

CONCLUSIONS

These data show that of the three major constituents characteristic of most hard waters (i.e., Ca^{2+}, Mg^{2+}, and the CO_2 system) calcium ion, introduced either as sulfate or carbonate, is the major source of protection against cadmium toxicity. This protective effect is observed in the absence of significant cadmium precipitation. Magnesium ion, sulfate ion, sodium ion and the carbonate system provide little or no protection.

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