Bull. Environ. Contam. Toxicol. (1987) 39:194–201

Environmental Contamination and Toxicology

Toxicity of Vanadium to Different Freshwater Organisms

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Although vanadium is as abundant as nickel and zinc in the earth's crust, it is not a common pollutant. Vanadium does not occur as the free metal, but as relatively insoluble minerals and organo-metallic complexes. Consequently, the concentration of vanadium in natural freshwaters is relatively low, usually less than 20 $\mu g.L^{-1}$ (Linstedt and Kruger 1970). A large fraction of vanadium released into the aquatic environment from natural sources originates from erosion of land surfaces by water. However, anthropogenic input has led to a significant enrichment of vanadium in the environment (Goldberg et al. 1979). Vanadium occurs in relatively high concentrations in crude oils and coals and combustion of these fuels constitutes the major source of vanadium emissions to the atmosphere. A large fraction of the anthropogenically derived vanadium-rich atmospheric particles can enter the aquatic environment as particulate fall-out or dissolved in rain. Other possible sources of vanadium contamination are effluent discharges from titanium and uranium processing plants (Jaffe and Walters 1977).

Very few vanadium toxicity tests have been conducted with invertebrates. Miramand and Unsal (1978) studied the acute toxicity of vanadium to some marine benthic species. They found 9-day LC50 values of 10, 35 and 65 ppm vanadium for *Nereis diversicolor* (worm), *Carcinus maenas* (mussel) and *Mytillus galloprovincialis* (crab) respectively. There is more information available on the toxic effects of vanadium to fishes. According to Tarzwell and Henderson (1960), 96-h LC50 values for fathead minnow and bluegill in soft and hard waters ranged from 4.8 to 55 ppm vanadium. Knudtson (1979) determined the acute toxicity of various vanadium compounds (V_2O_5, $VOSO_4$, NH_4VO_3 and $NaVO_3$) to goldfish and guppy in relatively soft water. Depending on the compound tested, the 6-day LC50 values ranged from 0.9 to 4.5 mg $V.L^{-1}$ for goldfish and 0.12 to 0.65 mg $V.L^{-1}$ for guppy. The 96-h LC50 of vanadium to adult American flagfish was 11.2 $mg.L^{-1}$ in very hard water while the threshold for chronic toxicity was judged to be about 0.08 $mg.L^{-1}$ (Holdway and Sprague 1979). Water hardness and pH had only a minor influence on

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vanadium lethality to rainbow trout (Stendahl and Sprague 1982). The 7-day LC50 values of V_2O_5 fell in a small range, from 1.9 to 6.0 mg $V.L^{-1}$.

The aim of this study is to determine the acute and subchronic toxicity of vanadium for various species of freshwater fish. The long-term toxicity and the effect of vanadium on the reproduction of *Daphnia magna* is also evaluated and compared with the toxicity of other metals.

MATERIALS AND METHODS

Sodium metavanadate is obtained from Carlo Erba Analyticals with purity > 98 %. Vanadium concentrations in the test media exceeding 2 mg.L^{-1} are determined spectrophotometrically at 400 nm using the phosphotungstate method (Snell and Snell 1949) with a Varian SuperScan 3 UV/VIS spectrophotometer. In the case of lower vanadium concentrations, graphite furnace atomic absorption spectrometry is used (Varian AA-1475 Atomic Absorption Spectrophotometer). The lower detection limit for the Atomic Absorption method is about 0.01 mg $V.L^{-1}$. In general, the measured vanadium concentrations correspond within 10 % of the initial amount of the metal added to each test container. Therefore, all LC50 and EC50 values are reported in terms of the nominal vanadium concentrations.

The daphnids (*Daphnia magna*) used for acute and chronic toxicity tests are obtained from a standardized laboratory culture originally obtained from an uncontaminated pond in the vicinity of the laboratory. The stock cultures are held in 3-L beakers containing gently bubble-aerated hard water at a temperature of 19 ± 1 °C. The daphnids are fed daily with green algae (*Chlorella pyrenoidosa*). The daphnids used in all toxicity experiments are less than 24 h old. The zebrafishes (*Brachydanio rerio*) are obtained from a commercial supply house. Before testing, fishes from a single stock of similar length and age are adapted to laboratory conditions in 200-L tanks filled with hard water for at least 12 days (T = 23 ± 2°C). Guppies (*Poecilia reticulata*) are obtained from a standardized laboratory culture. The fishes are fed twice-daily with TetraMin, ceasing 24 h before the tests are initiated. The photoperiod is the same as during the tests : 14 h daylight/10 h darkness.

Dilution water used for stock cultures and tests is synthetic hard water ('Dutch standard water'), having the following chemical composition (mg.L^{-1} deionized water) : 100 mg $NaHCO_3$, 20 mg $KHCO_3$, 180 mg $MgSO_4.7H_2O$ and 200 mg $CaCl_2.2H_2O$, pH 8.2-8.4, hardness : 223 mg.L^{-1} (as $CaCO_3$ + $MgSO_4$).

Methods for the determination of the acute toxicity with daphnids and fishes generally follow guideline 84/449/EEC given by

the Commission of the European Communities (Anonymous 1984). For acute testing, 5 daphnids (< 24 h old) are used per beaker with 4 beakers per vanadium concentration. Test containers are 50-mL borosilicate glass beakers containing 50 mL of test solution. The test duration is set at 48 h and no feeding or aeration is used. However, in one test run food (3.5×10^7 cells of green algae in each test container) is added. The monitored end-points are mortality, as determined by lack of movement of antennae or post-abdominal claw on gentle prodding and complete immobilization as determined by unability of swimming for 15 seconds after gentle agitation of the test container. The temperature is maintained at 19 ± 1°C.

For acute tests with zebrafish and guppy, 5 fishes are randomly assigned to each of duplicate 1-L beakers filled with 1 L of test solution and subjected to the test conditions for 96 or 168 h. The fishes are transferred to new test medium after 48 and 96 h. No feeding is provided during the test. Mortalities are recorded at 24 h intervals. The temperature is held constant at 23 ± 2°C. Gentle bubble-aeration is used. A control and at least five vanadium concentrations are selected on the basis of a logarithmic expansion. During acute testing the measured pH of the test solutions ranged from 7.7 to 8.5 while the dissolved oxygen concentration was always above 6 $mg.L^{-1}$.

The chronic toxicity is determined from a 23-days renewal static test using 10 daphnids at each vanadium concentration. One daphnid is placed in each of ten 50-mL beakers filled with 50 mL test solution. The test medium is changed on days 2, 5, 7, 9, 12, 14, 16, 19 and 21. The food used consists of 25 µL of prepared food suspension (TetraMin extract) plus approximately 5.10^7 cells of *Clorella pyrenoidosa* following each water renewal. During the second test week, the amount of cells of green algae is raised to 1.10^8 and during the last week to 2.10^8 per beaker. No aeration is used. Reproduction is assessed by counting and discarding young produced each time the parent animals are transferred during the second week, and daily during the last week of the test. Nine vanadium concentrations are selected for testing. Adult survival, time to the first brood, young per female per day and the number of 'eggs' and newborns produced by the adult daphnids are the parameters monitored during these tests. During chronic testing the pH of the test solutions sometimes increased to about 8.9 and the dissolved oxygen concentrations sometimes exceeded saturation because of photosynthesis by algae. Nevertheless the measured pH values during chronic testing fell within the range which has no adverse effect on the physical condition of the daphnids.

The 48-h, 96-h, 168-h and 23-days LC50 and EC50 values are obtained using the graphical method of Litchfield and Wilcoxon (1949).

RESULTS AND DISCUSSION

The results of the acute toxicity tests with *Daphnia magna* are summarized in Table 1. The 48-h LC50 and 48-h EC50 values during five replicate tests all fell in a very small range (3.4-4.8 mg $V.L^{-1}$). Differences between LC50 and EC50 values are generally very small. The lowest NOEC value (No Observed Effect Concentration) from these studies is 0.8 mg $V.L^{-1}$. Feeding of the daphnids during testing seems to have little effect on the toxicity of vanadium. Zebrafish appears to be more susceptible towards vanadium than guppy (Table 2). The lowest NOLC from these studies is 0.3 mg $V.L^{-1}$, which is derived from the prolonged acute test with zebrafish.

Table 1. Summary of the results of the acute toxicity tests with *Daphnia magna* on vanadium (all concentrations in mg $V.L^{-1}$).

Run	48-h LC50 (CI)	48-h EC50 (CI)	48-h NOLC	48-h NOEC
1	4.8 (4.4-5.3)	3.8+	2.6	2.6
2	3.9 (3.1-4.9)	2.9 (2.3-3.7)	0.8	0.8
3	4.3 (3.5-5.3)	3.9 (3.3-4.6)	2.1	2.1
4	3.9 (2.7-5.6)	3.6 (3.0-4.2)	1.3	1.3
5	3.4 (2.7-4.3)	3.3 (2.6-4.0)	1.3	1.3
6*	4.2 (3.7-4.8)	4.0+	2.1	2.1

NOLC = No Observed Lethal Concentration
NOEC = No Observed Effect Concentration with
E = complete immobilization
CI = 95 % confidence intervals
\+ No CI calculated
* Food added during testing

In the 23-days exposure test, the survival and reproduction of *Daphnia magna* exposed to varying vanadium concentrations is studied. Preliminary chronic tests indicated that concentrations up to 1.6 mg $V.L^{-1}$ did not inhibit the reproduction of the daphnids. The results of the life-cycle reproduction test with *Daphnia magna* exposed to vanadium are summarized in Table 3. During chronic testing control mortality did not exceed 10 %. The 23-days LC50 and 23-days NOLC values as well as the MATC (Maximum Acceptable Toxicant Concentration) based on reproduction are presented in Table 4.

Table 2. Median lethal and No Observed Lethal Concentrations of vanadium for *Brachydanio rerio* and *Poecilia reticulata* (all concentrations in mg $V.L^{-1}$).

Species	LC50 (CI)			NOLC		
	48-h	96-h	168-h	48-h	96-h	168-h
Brachydanio rerio	8.0 (5.5-11.7)	2.9 (2.1-3.4)	-	1.6	0.8	-
	14.2 (8.2-24)	4.1 (2.6-6.4)	-	1.0	0.6	-
	19.4 (11.3-34)	5.3 (3.4-8.4)	2.3 (1.5-3.5)	1.1	0.7[a]	0.3[a]
Poecilia reticulata	14.2 (10.4-19.6)	6.1 (4.9-7.5)	-	6.3	3.0[a]	-
	17.1 (15.0-19.5)	10.2 (7.4-14.2)	3.3 (2.3-4.7)	9.4	2.6	0.8[a]

NOLC = No Observed Lethal Concentration
CI = 95 % confidence intervals
[a] Extrapolated value; since mortality occurs at the lowest test concentration in the experiment, the LC5 value is considered als NOLC

The 23-days LC50 is only about half of the 48-h LC(EC)50 values which suggests that vanadium has no long-term toxic effect on the daphnids. The addition of food during testing does not significantly alter the LC(EC)50 and NOL(E)C values during acute testing. By contrast, Biesinger and Christensen (1972) found that median lethal concentrations (48-h LC50) for other metals such as cobalt, copper, nickel and zinc were higher with than without food addition.

From the life-cycle test with *Daphnia magna*, it appears that vanadium induced mortality rather impairment of reproduction (Table 3). The similarity between the NOLC values derived from the chronic and acute tests (Tables 1 and 4) points to a direct toxic action of vanadium on daphnids, rather than by accumulation. A "safe" level of vanadium for *Daphnia magna* is estimated to be about 1.6 mg $V.L^{-1}$. This level is much higher than the vanadium concentrations normally found in freshwater

Table 3. Summary of *Daphnia magna* life-cycle test results for vanadium.

Vanadium concentration (mg $V.L^{-1}$)	Total number of mortalities after 23 days	Cumulative number of living neonates/female daphnid (% of control value)
0.63	1	99.9
0.76	0	102.0
0.91	0	106.5
1.07	0	94.1
1.29	0	101.2
1.58	0	95.9
1.89	2	102.6
2.27	8	64.0
2.71	10	0

Table 4. Median Lethal Concentration (LC50), No Observed Lethal Concentration (NOLC) and Maximum Acceptable Toxicant Concentration (MATC) for *Daphnia magna* chronic toxicity test.

Parameter	Concentration (mg $V.L^{-1}$)
23-days LC50 (CI)	2.0 (1.9-2.2)
23-days NOLC	1.6
MATC*	1.9

* This is the MATC value based on reproduction

(0.1-1.0 $\mu g.L^{-1}$). During acute tests with fishes, zebrafish seems to be more sensitive to vanadium than guppy. The 96-h median lethal concentrations for zebrafish are comparable to those obtained for *Daphnia magna*. However, mortality continued during fish tests when the exposure time was prolonged to 7 days. This points to a slow rate of vanadium uptake by fish (Bell et al. 1980) and a delay in the expression of toxicity (Holdway et al. 1983). Prolongation of the exposure time to 7 days resulted in substantially lower LC50 values of 2 to 3 mg $V.L^{-1}$, similar to the results of Stendahl and Sprague (1982) for rainbow trout. Regarding the slope of the toxicity curve (Figure 1) longer exposure times would not significantly decrease the NOLC values. The 168-h NOLC values of 0.2 and 0.5 mg $V.L^{-1}$ for zebrafish and guppy respectively (Table 2) represent fairly good estimates of the toxicity threshold of vana-

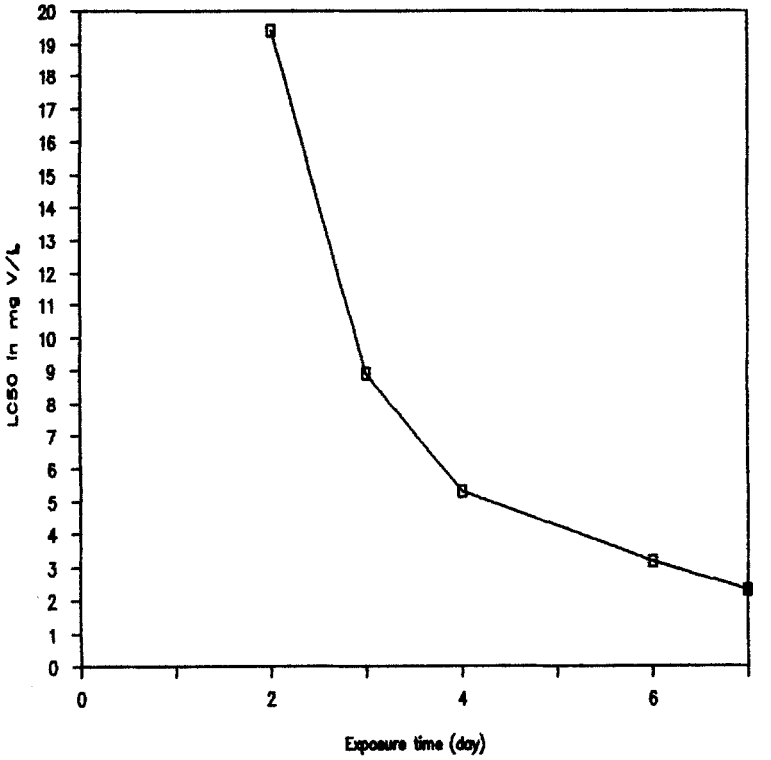

Figure 1. Toxicity curve of vanadium for zebrafish.

dium to fish. Canton and Slooff (1979) proposed the lowest NOLC x LC25/LC50 value as a criterion for water quality. For vanadium this value would be 0.16 $mg.L^{-1}$ based upon the 7-days test with zebrafish using mortality as criterion. According to Holdway and Sprague (1979) the sublethal threshold for vanadium toxicity to flagfish is 0.08 $mg.L^{-1}$, a value very similar to ours.

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Received November 20, 1986; accepted April 22, 1987.