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Toxicity of Vanadium to the Estuarine Mysid, *Americamysis bahia* (Molenock) (Formerly *Mysidopsis bahia*)

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Vanadium is widely distributed, occurring in a variety of minerals, coals, and petroleums. It constitutes approximately 150 ppm of the earth's crustal rock, making it the 22nd most common element (Zenz, 1980) approximately the same abundance as zinc and nickel. In seawater, vanadium occurs primarily as vanadate anions at concentrations between 0.3-3.2 µg V/L (Ünsal, 1982) and approximately 193 mg/Kg in marine sediments (Williams *et al*., 1974). Most surface waters in the United States contain less than 0.05 mg V/L (Holdway and Sprague, 1979). Municipal drinking water in the United States averages approximately 1-6 µg V/L (Beliles, 1979; A.P.H.A., 1992).

Anthropogenic sources of vanadium originate from the processing of a variety of mineral ores, burning of fossil fuels, and refining of petroleum products (Holdway and Sprague, 1979; Giles and Klaverkamp, 1982; Ünsal, 1982; Stendahl and Sprague, 1982). Vanadium compounds are used in a variety of industrial processes including the production of steel tools, and corrosion and temperature resistant alloys; refining of iron and steel; manufacturing of pigments, printing inks, paints, and glass; and in industrial catalysts (Alessio *et al*., 1988).

The aquatic toxicity of vanadium is not well characterized. A review of the toxicity data in the U.S. EPA's Aquire database (August, 2003) show that data for estuarine and marine species using standardized test methods are noticeably lacking. Three marine fish species have been tested using acute methods and vanadium. Dorfman (1977) reported 96-hr LC50s of 13.5 and 17.5 mg V/L as NH_4VO_3 for the mummichog, *Fundulus heteroclitus,* respectively. The threespined stickleback, *Gasterosteus aculeatus* demonstrated similar sensitivity in studies reported by Dorn (1992). *G. aculeatus* were exposed to vanadium as $VOSO_4$ and NH_4VO_3 in four tests (one test using $VOSO_4$ and three tests using NH_4VO_3). The stickleback 96-hr LC50 of $VOSO_4$ was 15.8 mg V/L and the three 96-hr LC50s of NH_4VO_3 were 6, 14, and >9.7 mg V/L. Vanadium LC50s for tigerfish, *Therapon jarbua* were reported by Krishnakumari *et al*., to be 1 mg V/L (24 hr), 0.97 mg/L (48 hr), 0.80 mg/L (72 hr) and 0.62 mg/L (96 hr). Larvae of three marine invertebrate species, the brine shrimp, *Artemia salina*; the oyster, *Crassoatrea gigas*; and the sea urchin, *Paracentrotus lividus* were tested by Fichet and Miramand (1998) and sub-lethal

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toxicity reported at 0.250 mg V/L (8 d), 0.050 mg V/L (48 hr) and 0.100 mg V/L (48 hr), respectively.

The purpose of this research was to develop information about the toxicity of vanadium using a commonly used test species the estuarine mysid, *Americamysis bahia* (formerly *Mysidopsis bahia*) and accepted test guidelines.

MATERIALS AND METHODS

Organisms were cultured in a 1:1 mixture of natural and aged artificial seawater. Natural seawater was collected from Manasquan Inlet, NJ; a New Jersey Department of Environmental Protection and Energy's (NJDEPE) designated collection site of natural seawater used in New Jersey Pollution Discharge Elimination System toxicity testing. Artificial seawater was formulated using reverse osmosis water and Instant Ocean^R seasalts to 25‰ and aged for at least 14 d before use. Juvenile mysids (<24 hr old) were acclimated to test conditions for 6 d. No mortality was observed during the acclimation period. The test laboratory has successfully cultured and acclimated *A. bahia* for years using these materials and methods.

Toxicity tests were performed using natural seawater collected from Manasquan Inlet, NJ. Salinity of the seawater was adjusted with reverse osmosis water to 25‰ to meet test requirements.

A nominal 40 mg V/L stock solution (41 mg V/L measured) was prepared by dissolving 98% pure $NaVO_3$ (Sigma Chemical Co., 40.9% V) in natural seawater (25‰ salinity) in a polypropylene vessel. The stock solution was stored at room temperature until used. Each treatment was prepared daily by adding appropriate amount of stock solution to natural seawater (25‰ salinity) in a polypropylene volumetric flask to obtain the desired test concentration. Replicate test solutions were prepared by splitting this solution into eight 200 ml aliquots in polypropylene beakers.

A reference toxicity test was also performed using cadmium to assess the health of the organisms used for the vanadium test. A nominal 3.065 mg Cd/L stock solution (2.929 mg Cd/L measured) was prepared by dissolving 99.2% pure anhydrous $CdCl_2$ (J.T. Baker Inc., 61.3% Cd) in natural seawater (25‰ salinity) in a polypropylene volumetric flask. The stock solution was refrigerated until used. Each treatment was prepared daily by adding the appropriate amount of stock solution to natural seawater (25‰ salinity) in a polypropylene volumetric flask to obtain the desired test concentration. Replicate test solutions were prepared by splitting this solution into eight 200 ml aliquots in polypropylene beakers.

The vanadium stock solution and treatments were analyzed by inductively coupled argon plasma emission spectrophotometry using U.S. EPA Method 200.7 to determine exposure concentrations (Table 1). The stock solution was sampled on Day 0. Treatment solutions were sampled on Days 0, 1, 4, and 7. All vanadium results presented in this paper are based on the arithmetic mean of analytically verified total

Table 1. Vanadium concentrations measured in the test solutions.

Nominal Concentration mg V/L	Day	Solution Analyzed New/Old	Measured Concentration mg V/L	Mean mg V/L	Standard Deviation mg V/L
Control	0	New	<0.05	<0.05	–
	1	Old	<0.05		
	4	New	<0.05		
	7	Old	0.05		
0.875	0	New	0.71	0.75	0.04
	1	Old	0.71		
	4	New	0.80		
	7	Old	0.76		
1.75	0	New	1.53	1.56	0.08
	1	Old	1.46		
	4	New	1.63		
	7	Old	1.6		
3.5	0	New	3.13	3.18	0.13
	1	Old	3.04		
	4	New	3.23		
	7	Old	3.33		
7	0	New	6.47	6.45	0.38
	1	Old	6.03		
	4	New	6.95		
	7	Old	6.36		
14	0	New	14.0	13.65	0.35
	1	Old	13.3		
	4	New	13.9		
	7	Old	13.4		
20	0	New	18.3	18.55	0.35
	1	Old	18.8		

No survival by end of day three in 20 mg V/L treatment.

vanadium concentrations in each treatment.

Total cadmium in the stock solution was analyzed by flame atomic absorption spectrophotometry using U.S. EPA Method 7130. Treatment solutions were not analyzed, thus cadmium results presented in this paper are based on nominal dilutions of the analytically verified stock concentration.

This study was conducted in accordance with U.S. EPA (1988) and NJDEPE (1989) guidelines and was performed to comply with U.S. EPA Good Laboratory Practices Standards (U.S. EPA, 1989). Table 2 summarizes conditions under which the tests were performed.

Table 2. Test conditions for the vanadium short-term chronic estimator test with *Americamysis bahia*.

Test Parameter	Test Conditions
Test type	Static renewal
Photoperiod	16 hr light/8 hr dark with phase period
Light quality	Wide spectrum fluorescent
Test chamber	250 mL polypropylene beakers
Test solution volume	200 mL
Renewal of solutions	Daily
Age of organisms	7 d
Number of organisms per chamber	5
Number of chambers per treatment	8
Food source	*Artemia sp.* nauplii
Feeding regime	0.15-0.20 mL/chamber, twice daily
Dilution water	Natural seawater
Test duration	168 hr (7 d)
Test end points	Survival, growth, egg development
Test concentrations (measured, mg V/L)	0.74, 1.56, 3.2, 6.4, 13.6, 18.6
Salinity	24-25‰
Test temperature	26.0-26.9 °C
pH	7.8-8.4
Dissolved oxygen	5.6-7.7 mg/L
Number of treatments	6 and a control

Organisms were randomly distributed into test chambers and chamber placement was determined by a computer generated randomization schedule. All test chambers were covered to reduce evaporation. Observations of survival, abnormal behavior, and physical appearance of the mysids were made at 24-hr intervals ($\pm$1 hr). Water quality measurements (dissolved oxygen, pH, salinity, and temperature) were performed on each fresh solution at the beginning of the 24-hr period. Dissolved oxygen was also measured in the old solution at the end of each 24-hr period.

After each 24 hr period of the 7-d exposure, survival in each chamber was recorded. At test termination, surviving organisms were examined microscopically to determine sex and egg development. Mysids were then rinsed, dried in an oven at 60 °C ($\pm$5 °C) for 24 hr, and cooled in a desicator. Mysids in each treatment replicate were weighed to the nearest 0.001 mg. The total weight for each replicate was divided by the number of mysids weighed to yield the average mysid weight per replicate.

The no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) were determined using U.S. EPA (1988) methods and TOXSTAT, Version 3.3 (Gulley *et al.*, 1990) computer software ($\propto$ = 0.05). Lethal concentration (LC) values were determined using the Probit procedure (Finney, 1971) and the Statistical Analysis System (SAS Institute, Inc., Cary, North Carolina) or using the trimmed Spearman-Karber method (Hamilton *et al.*, 1977).

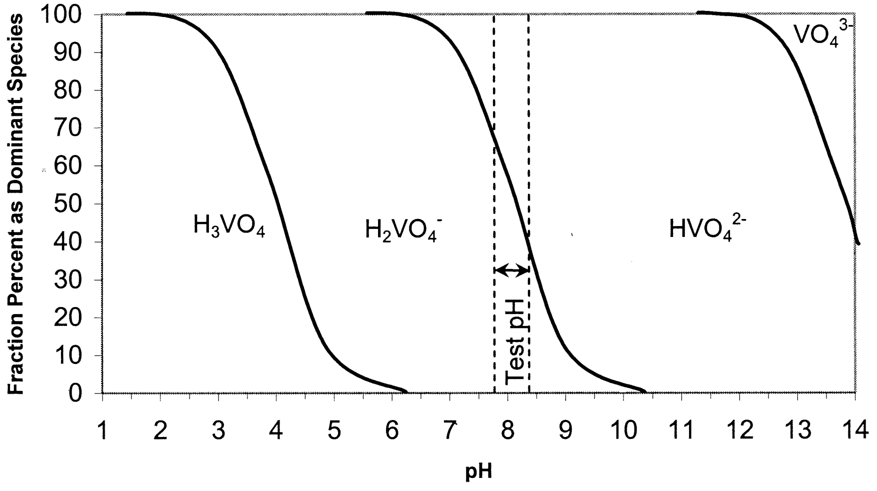

Figure 1. Equilibrium diagram of vanadate species (Kustin and Macara, 1982) as a function of pH and vanadium concentration. Predicted speciation under the test conditions is indicated.

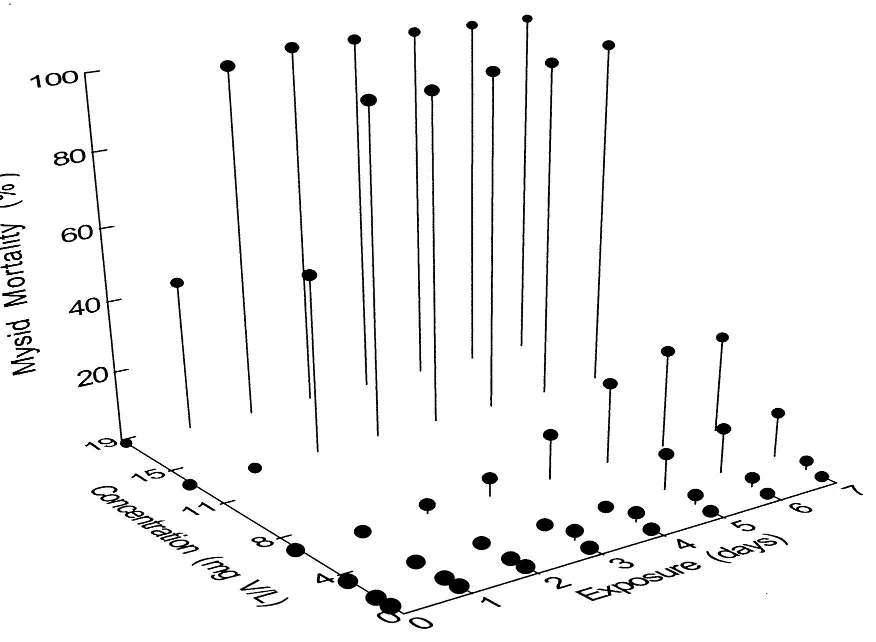

Figure 2. Effects of vanadium concentration and exposure duration on survival of mysids.

Table 3. Summary of vanadium test survival, growth and fecundity data.

Test Concentration (mg V/L)	Replicate	Gravid Females (%)	Mean Weight (mg)	Surviva
Control (<0.05)	1	67	0.341	4
	2	100	0.393	5
	3	100	0.481	4
	4	0	0.328	4
	5	100	0.371	4
	6	50	0.316	4
	7	100	0.383	4
	8	100	0.352	5
0.875 (0.75)	1	67	0.347	5
	2	100	0.371	4
	3	100	0.348	5
	4	100	0.406	4
	5	100	0.336	5
	6	50	0.346	5
	7	100	0.347	5
	8	100	0.369	5
1.75 (1.56)	1	100	0.353	5
	2	100	0.393	5
	3	67	0.383	4
	4	100	0.362	5
	5	100	0.424	5
	6	100	0.426	5
	7	100	0.403	5
	8	100	0.375	5
3.5 (3.18)	1	NF	0.356	5
	2	0	0.358	2
	3	0	0.320	4
	4	100	0.459	5
	5	100	0.424	5
	6	100	0.329	5
	7	100	0.421	5
	8	100	0.329	4
7 (6.45)	1	NF	0.428	4
	2	100	0.314	5
	3	100	0.405	3
	4	50	0.379	3
	5	NF	0.352	4
	6	50	0.324	5
	7	NF	0.384	1
	8	100	0.388	4
14 (13.65)	7	0	0.332	1
20 (18.55)		–	–	0

Measured vanadium in ( ). NF indicates no females observed in replicate.

RESULTS AND DISCUSSION

Results of positive and negative controls indicate organism health and methods used were acceptable. Survival in the natural seawater control of the cadmium and vanadium tests were 100% and 90%, respectively, and within the required level of $\geq$80% survival (U.S. EPA, 1988 and NJDEPE, 1989). The 7-d no observed effects concentration (NOEC) for cadmium was 7.6 µg Cd/L based upon a statistically significant reduction in mysid survival after the 7-d exposure period (arc sine square root transformation, Steel's many-one rank test, $\propto$=0.05, 1 tailed). This NOEC was within an acceptable range of historical NOECs measured in reference tests conducted on site using cadmium as cadmium chloride.

Based on pH measured during the test and vanadium distribution diagrams presented by Kustin and Macara (1982), H_2VO4^- and HVO_4^{2-} were likely the dominant vanadate species present (Figure 1). Three pH measurements were <8.1, all others ranged between 8.1-8.4. The exact proportions of various species were neither identified nor predicted, nor was there an attempt to identify any possible organic ligand bound species, thus the speciation presented here is only an approximation.

The toxicity of vanadium to A. *bahia* is similar to most of the species previously reported in the literature. Survival was the most sensitive test endpoint measured, producing statistically significant 7-d NOEC and LOEC at 6.4 and 13.6 mg V/L, respectively. The selected exposure concentrations resulted in treatments with near zero, partial and complete mortality (Table 3). Though only two treatments (18.55 and 13.65 mg V/L) resulted in mortality rates statistically significantly higher than the controls, there was a monotonic increase in mortality in treatments $\geq$0.75 mg V/L.

Much of the mortality at higher concentrations occurred in the first 72 hr. Acute toxicity (LC50) was estimated after each 24-hr exposure period (Table 4). LC50s decreased substantially during the first 72 hr of exposure and decreased only slightly during the next 96 hr. Based on this, an estimate of the concentration at which *A. bahia* detoxify vanadium at the same rate of chemical reactions that produce 50% lethality is approximately 7-9 mg V/L.

Vanadium is considerably less toxic than cadmium and a variety of other metals. Based on the result of the concurrent and historical reference toxicity tests conducted in our laboratory, vanadium is approximately three orders of magnitude less toxic than cadmium. Lussier *et al.* (1985) conducted acute tests with nine metals and cyanide using *A. bahia*. Their 96-hr LC50 data obtained using acute methods and the 96-hr LC50 obtained in this study using chronic estimator methods are presented in Table 5. Vanadium appears much less toxic than the other metals and cyanide and the sensitivity of *A. bahia* to vanadium is similar to most species previously reported.

Table 4. LC10s, LC50s[1] and 95% confidence intervals (in parentheses) for each 24 hr exposure.

Test Day	LC10 (mg V/L)	LC50[1] (mg V/L)
1[2]	>18.6	>18.6
2	9.3 (7.1-10.7)	13.3 (12.2 – 14.3)
3	NA[3]	9.2 (8.5 – 9.9)
4	5.2 (1.6-7.4)	9.4 (7.2 – 12.6)
5	3.9 (2.8-4.8)	8.3 (7.4 – 9.5)
6	3.1 (0.57-4.7)	8.0 (6.2 – 10.5)
7	2.8 (<2.8-5.0)	7.7 (5.5 – 11.6)

[1] All except day 3 calculated using Probit method. Day 3 calculated using Spearman-Karber method and no confidence interval was calculated.
[2] Day 1 LC vales could not be calculated. Mortality in 18.6 mg V/L = 0%.
[3] LC10 could not be calculated using the Spearman-Karber method.
Results are based on measured concentrations of vanadium.

Table 5. Summary of acute effects of vanadium, nine other metals and cyanide.

Substance	96 hr LC50 mg/L	Acute Rank	Saltwater Criteria Maximum Concentration[1] mg/L
Mercury	0.0035 (0.0027-0.0048)	1	0.0018
Cadmium	0.110 (0.102-0.118)	2	0.042
Cyanide	0.113 (0.096-0.137)	3	0.001
Copper	0.181 (0.146-0.250)	4	0.0048
Silver	0.249 (0.220-0.283)	5	0.0019
Lead	0.330 (.235-Infinity)	6	0.210
Zinc	0.499 (0.350-0.600)	7	0.090
Nickel	0.508 (0.387-0.635)	8	0.074
Arsenic	1.740 (1.390-2.260)	9	0.069
Chromium	2.030 (1.560-2.450)	10	1.1
Vanadium	9.400 (7.200-12.600)	11	–

[1] From U.S. EPA (1999). Values are 96-hr LC50s with 95% confidence intervals in parentheses. The vanadium LC50 is from this study. Data for other substances are from Lussier *et al.* (1985).

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