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Acute Toxicity of Vanadium to Two Species of Freshwater Fish

Bruce K. Knudtson*
Department of Biological Sciences, University of Southern California, Los Angeles, Calif. 90007

Recent studies have shown that vanadium, along with other less well known trace metals, is being introduced into the marine environment in quantities sufficient to allow for a significant bioaccumulation of the metal in the bodies of shellfish (PESCH et al. 1977). In addition, significant amounts are also entering freshwater and estuarine systems (DREHER 1977, JAFFE and WALTERS 1977). JERNELOV (1974), in discussing our present understanding of the environmental implications of anthropogenic inputs of vanadium to marine systems, stated "The extent of man's impact on vanadium concentrations in marine organisms is unknown and so are the biological and ecological effects of vanadium contamination" (p. 812). This same statement holds equally well for frshwater and estuarine systems.

Aside from the early, now outdated, work of PROESCHER et al (1917), the only other study of vanadium toxicity in fishes was conducted by TARZWELL and HENDERSON (1960). This study was found to be of limited use in evaluating the toxicity of this element owing to the stated "exploratory" nature of the majority of the results presented. Therefore, as a first consideration in the assessment of the aquatic toxicology of vanadium, a comparative study was undertaken to determine the acute toxicity of four vanadium compounds to two species of frwshwater fishes. The following is a brief report on the principal results of this study.

MATERIALS & METHODS

Static bioassays for determining acute toxicity were patterned after the methods recommended by the American Public Health Association (APHA 1971). Goldfish (Carassius auratus) and guppies (Lebistes reticulatus) were the two freshwater, thermophilous fishes chosen for use in this study. These species were selected for the following reasons: (1) low cost/individual, (2) ready availability, (3) ease of care and handling, (4) suspected differences between the two species in their responses to this metal, and (5) the usefulness of both as generalized models for the response of freshwater and marine fishes to vanadium.

*Present address: Oceanic Engineering Division, Interstate Electronics Corp., P.O. Box 3117, Anaheim, CA 92803.

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The C. auratus were 3-5 cm standard length and weighed (wet weight, starved) 0.3-2.9 g. The L. reticulatus were 1.5-2.5 cm standard length and weighed (wet weight, starved) 0.1-0.5 g. The fishes were maintained in a 100 liter holding tank, equipped with side and bottom filters, with water of the same temperature, pH, and chemical quality as that used in the actual tests. The fishes were held for periods ranging from 24 to 96 hours before being transferred to the test tanks. The fishes were not fed during the holding period or at any time during the tests. All experiments in this study utilized filtered, deionized (soft) water. Filtering and deionization were accomplished by passing the water through a resin ion exchange column. No experiments were conducted in hard water since previous studies have shown that water quality and the amount and form of toxicant can be sharply altered under hard water conditions. Such severe changes apparently do not occur under soft water conditions (McCARTY et al. 1978). The characteristics of the water used in this study were as follows: pH, 6.0-6.5; total hardness (as Ca), 65 ppm; carbonate hardness (as $CaCO_3$), 35 ppm; dissolved oxygen, 4 ppm; background vanadium concentration, 0.94 ± 0.39 ppb. Total and carbonate hardness were determined titrimetrically (APHA 1971); background vanadium concentration was determined colorimetrically (FISHMAN and SKOUGSTAD 1964). Artificial aeration was constantly supplied to the holding tank and each test tank during all experiments. With aeration, dissolved oxygen levels were estimated to exceed the minimum 4 ppm level by an adequate margin (APHA 1971). A Corning 610A expanded scale meter was used for pH measurements.

All toxicity tests were conducted under ambient conditions of light and temperature over a period of six months. Over the course of these tests water temperature was $22.5 \pm 3.5^{\circ}C$, but the maximum variation during any one test was less than $2.0^{\circ}C$. Photoperiod varied approximately 8 hr L:16 hr D to 12 hr L:12 hr D (winter to spring) during these experiments.

Sodium metavanadate, ammonium metavanadate, vanadyl sulfate, and vanadium pentoxide were tested separately to determine their acute toxicity to the two species of fishes. These specific compounds were chosen for the following reasons: (1) availability, (2) representative of the two major valence states (+4 & +5) of vanadium, and (3) the vanadium in each compound is not bound to an ion that is itself particularly toxic when in solution at low concentrations. Over the course of seven tests with C. auratus, approximately 780 fishes were exposed to various concentrations (range: 0.05-56.0 ppm) of each of the four compounds. The range of pH for these tests was 5.68-6.82. Over the course of seven tests with L. reticulatus, approximately 870 fishes were exposed to various concentrations (range: 0.18-7.5 ppm) of each of the four compounds. The range of pH for these tests was 5.67-7.37.

All seven tests involving C. auratus were run in 2 liter hardened glass beakers with 3 fishes/liter or approximately 2.7-3.0 g fish/liter. Six of the seven tests with L. reticulatus were run in 4 liter hardened glass beakers with 3 fishes/liter or

approximately 1.0-1.5 g fish/liter. One test with L. reticulatus was conducted in 2 liter hardened glass beakers again with 3 fishes/liter or approximately 1.0-1.5 g fish/liter.

Each tank was examined at 24 hour intervals and fishes were considered dead when they were immobile, showed no respiratory activity, and failed to respond to probing of the caudal peduncle. The raw mortality data (#dead/concentration/24 hour period) were analyzed with the aid of a toxicology data program written for a Tektronix 4051 mini-computer. The program calculates, using a modification of the method of LITCHFIELD and WILCOXON (1949), the median lethal concentration (LC50) for each 24 hour time period, the 95% confidence intervals for the LC50 values, the slope function (S) for the concentration-percent mortality log-probit regression line for each 24 hour time period, the Chi-square value for the goodness of fit of the regression line, the number of degrees of freedom allowed in the Chi-square calculations, and the significance of the Chi-square value. Only 144 hour LC50 values and their associated statistics are presented in this report.

All 144 hour LC50 values are reported in terms of the initial amount of the metal added to each tank. Since solubility of the vanadium compounds is possibly influenced by the character of the test water and, after the compound dissolves, some quantity of the metal is removed either by adsorption to particles or plating to the sides of the container, the concentrations of the metal to which the fishes were exposed are not definitely known; the amount may have been somewhat less than the calculated LC50 values indicate (McCARTY et al. 1978, PICKERING and HENDERSON 1966).

RESULTS & DISCUSSION

The calculated 144 hour LC50 values and their 95% confidence intervals, together with their respective slope (S) functions, Chi-square values, degrees of freedom, and significant heterogeneity indicators are given in Table 1. One feature of this study which sets it apart from all other published toxicity studies is the reporting of Chi-square values obtained for each of the percent mortality-concentration log-probit regression lines. The Chi-square value is an estimate of the goodness of fit of the regression line and serves as a direct of expression of the degree of variability inherent in the data. The method used here for determining and assessing the significance of the Chi-square values is the most conservative and restrictive possible, thus the tendency is toward an overestimation of the amount of heterogeneity in the data. Although calculated LC50 values become less meaningful as heterogeneity increases, significant heterogeneity in the data does not invalidate its usefulness but instead enhances it by revealing that the fishes are not responding in as uniform a manner as one would suppose from neatly fitted toxicity curves.

Consideration will now be given to the specific differences in toxicity between the four compounds:

TABLE 1
TOXICITY OF VANADIUM AS INDICATED BY THE 144 HOUR LC50 (ppm)

Species/ Parameter	V_2O_5	$VOSO_4$	NH_4VO_3	$NaVO_3$
C. auratus				
LC50	8.08	2.95	3.82	2.45
95% C.I. (-)	5.14	2.44	2.83	1.73
(+)	13.01	3.54	5.68	3.49
S	1.41	3.34	1.50	1.53
degrees freedom	3	4	9	6
Chi^2	12.00	30.14	+100	28.56
heterogeneity	yes	yes	yes	yes
L. reticulatus				
LC50	1.05	0.37	1.49	0.49
95% C.I. (-)	0.85	0.24	0.87	0.36
(+)	1.26	0.47	18.17	0.57
S	3.94	3.15	1.82	3.00
degrees freedom	2	3	1	4
Chi^2	9.40	6.91	58.71	10.68
heterogeneity	yes	no	yes	yes

Vanadyl sulfate: In C. auratus, the 144 hour LC50 of this compound (2.95 ppm) was approximately the same as that for sodium metavanadate (2.45 ppm) which suggests similar degrees of toxicity. However, the S value for vanadyl sulfate is higher than that for sodium metavanadate. A higher S value indicates that more of the latter compound would be required to initiate a toxic response. Thus, even though there is a close similarity in their respective LC50 values, vanadyl sulfate is the more toxic of the two. In L. reticulatus, vanadyl sulfate gave the lowest LC50 value but while the S value is high, it is not the highest. This suggests a greater uniformity of toxic response to the different vanadium compounds by L. reticulatus than C. auratus.

Sodium metavanadate: There was an approximate five-fold difference between the 144 hour LC50 values for C. auratus (2.45 ppm) and L. reticulatus (0.47 ppm) and the slope value for L. reticulatus (3.00) is twice that for C. auratus (1.53). Thus, sodium metavanadate is more toxic to guppies than to goldfish. The 144 hour LC50 values for vanadyl sulfate (0.37 ppm) and sodium metavanadate (0.47 ppm) in L. reticulatus are very similar, as are the S values. This suggests that for this species, vanadium (IV) ions may be equally toxic as vanadium (V) ions.

Vanadium pentoxide: The 144 hour LC50 value for C. auratus (8.08 ppm) was the highest of any of the values. This high value can be explained as follows: (1) vanadium pentoxide is only moderately soluble (0.8 g/100 ml @ 20°C) in water, so more of the compound must be added to produce vanadium metal levels equal to

those produced by adding lesser amounts of the more soluble compounds. Since the LC50 values are based on the amount of vanadium added (nominal value) and not on the actual amount of vanadium metal in the water (absolute value), having to add large amounts of vanadium as vanadium pentoxide would tend to drive the nominal LC50 value upward. However, because only a percentage of the vanadium metal is going into solution, the absolute LC50 value is approximately the same as for the more soluble compounds, or (2) C. auratus is substantially more resistant to vanadium pentoxide than L. reticulatus, as indicated by the lower 144 hour LC50 and higher S value for the latter. The pentoxide, when dissolved in water, forms hypovanadic acid which lowers the pH of the test solution. There may therefore be a differential sensitivity between C. auratus and L. reticulatus that is due to their respective abilities to tolerate acid conditions as well as the presence of vanadium ions.

Ammonium metavanadate: The 144 hour LC50 value for this compound in both C. auratus and L. reticulatus are relatively close (3.82 ppm vs 1.49 ppm) and this is the only instance in which the 95% confidence interval for the LC50 of one species overlaps that of the other. In addition, the S values are similar (1.50 vs 1.82). This suggests that this compound is of approximately equal toxicity to both species.

In general, these results indicate that, with the possible exception of ammonium metavanadate, there is an obvious differential sensitivity to vanadium compounds between the two species. In addition, they suggest that intraspecific sensitivity to the various compounds is substantially less that interspecific sensitivity.

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