ACUTE AND CHRONIC TOXICITY OF VANADIUM TO FISH

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The Hon. D.J. Russell
Minister of the Environment
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Edmonton, Alberta

and

The Hon. L. Marchand
Minister of State for the Environment
Fisheries and Environment Canada
Ottawa, Ontario

Sirs:

Enclosed is the report "Acute and Chronic Toxicity of Vanadium to Fish".

This report was prepared for the Alberta Oil Sands Environmental Research Program, through its Aquatic Fauna Technical Research Committee (now part of the Water System). under the Canada-Alberta Agreement of February 1975 (amended September 1977).

Respectfully,

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ACUTE AND CHRONIC TOXICITY OF VANADIUM TO FISH

DESCRIPTIVE SUMMARY

ABSTRACT

Vanadium concentrations of 2.4 to 5.6 mg/L were lethal in 7 days to rainbow trout of wet weight 1.2-6.2 g. The LC50 varied slightly over the 12 combinations of water quality, from hardness 30 to 350 units and pH 5.5 to 8.8. The 7-day LC50 may be estimated by the following equation, which explained 91% of the variation:

$$LC50 = 14.6976 - 3.7783P + 0.1108H - 0.02137\ PH + 0.2662P^2 - 0.000073H^2 + 0.00141P^2H.$$

where H = hardness as mg/L of $CaCO_3$, and P = pH.

The response surface was slighly saddle-shaped with vanadium being somewhat more toxic in the softest water, and slightly more toxic at intermediate pH (6.6 and 7.7) than at more extreme values of pH. Two ionic species of pentavalent vanadium were the main forms present in the tests with trout, and these were of similar toxicity. No threshold of lethality was evident in an 11-day exposure.

Very young fry of American flagfish showed 28-day LC50's from 0.9 to 1.9 mg/L of vanadium, according to size and age at the start of the test. These appeared to be thresholds of lethality. Trout, flagfish, and zebrafish all appeared to be similar in resistance to lethal effects of vanadium.

In chronic exposures of flagfish, the egg-fry stage was the most sensitive one in the life cycle. Mortality of such fry was the most obvious effect. At 0.17 mg/L of vanadium, which did not cause mortality, there were marginal effects on growth of second-generation fry, but no observed sublethal effects in older fish. At 0.04 mg/L there were no deleterious effects, but a definite stimulation of growth in females and of reproductive performance. The threshold for chronic toxicity was between those two concentrations, and was judged to be about 0.08 mg/L. The "safe"-to lethal ratio

was about 0.007, close to such ratios for other pollutants. There was no evidence that vanadium had any long-term cumulative toxicity.

Overall among the metals, vanadium was of moderate non-cumulative toxicity. With respect to oil sands operations, there should be an assessment whether aerial fallout of vanadium could create undesirable levels in slow-turnover lakes.

BACKGROUND AND PERSPECTIVE

This study was authorized at the early stages of the Alberta Oil Sands Environmental Research Program. The goal of the project was to establish the thresholds for acute and chronic toxicity of vanadium for fish. Such knowledge would be necessary for an assessment of whether a vanadium problem in the aquatic environment could be caused by oil sands development activities.

Both acute and chronic effects of vanadium were to be studied. The acute toxicity tests were to serve two purposes: (1) to obtain firm numbers on vanadium lethality to allow assessment of danger in comparison with other pollutants; and (2) to obtain a quick measure of toxicity in all types of water (for example, in soft acid waters) likely to be encountered to point out any peculiarities of vanadium effects. The chronic test was designed to detect any long-term or sub-lethal effects which might affect fisheries production. The one-generation experiment with fish should show any effect, whether it results from accumulation, chronic morbidity, effects on reproduction capacity, growth, or other modes of action.

The tests were to be run using ionic forms of vanadium. However, since vanadium may be released into the aquatic environment in a variety of forms, it was recognized that there may be some limitation in directly applying the results to the AOSERP study area. Therefore, the researcher was directed to take into consideration the actual form of vanadium used in his tests.

ASSESSMENT

This project has been completed and the levels of vanadium causing acute and chronic effects in fish have been established. The report has been reviewed by scientists in Alberta Environment, Fisheries and Environment Canada, and the Alberta Agriculture Toxicology Laboratory; the censensus is that it is a well executed study. However, the conclusions of the report do not necessarily reflect the views of Alberta Environment or Fisheries and Environment Canada, and the mention of trades names for commerical products does not constitute an endorsement or recommendation for use. The Alberta Oil Sands Environmental Research Program is pleased with the efforts put forth by the researchers in this project and accepts their report, "Acute and Chronic Toxicity of Vanadium to Fish", as an important and valid document. The researchers are to be congratulated for their contribution.

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Alberta Oil Sands Environmental
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ABSTRACT

Vanadium concentrations of 2.4 to 5.6 mg/L were lethal in 7 days to rainbow trout of wet weight 1.2-6.2 g. The LC50 varied slightly over the 12 combinations of water quality, from hardness 30 to 350 units and pH 5.5 to 8.8. The 7-day LC50 may be estimated by the following equation, which explained 91% of the variation:

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where H = hardness as mg/L of $CaCO_3$, and P = pH

The response surface was slightly saddle-shaped with vanadium being somewhat more toxic in the softest water, and slightly more toxic at intermediate pH (6.6 and 7.7) than at more extreme values of pH. Two ionic species of pentavalent vanadium were the main forms present in the tests with trout, and these were of similar toxicity. No threshold of lethality was evident in an 11-day exposure.

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Overall among the metals, vanadium was of moderate non-cumulative toxicity. With respect to oil sands operations, there should be an assessment whether aerial fallout of vanadium could create undesirable levels in slow-turnover lakes.

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1. INTRODUCTION

This research was carried out to provide initial estimates of the lethal concentrations of vanadium in water for fish, and of the "safe" levels. When the project was initiated, there was little exact knowledge of toxic levels of this metal, as described in the literature review. The Athabasca Oil Sands contain some vanadium, but little of it is retained in the final product, synthetic crude oil. There was the possibility that vanadium could be released to natural surface waters during the extracting and upgrading processes. Indeed there was some public concern that this could be very dangerous, no doubt arising partly from the lack of information on toxicity. At one point, a national magazine carried an article suggesting that vanadium might be "a new mercury".

Two projects were carried out under this contract, to provide information that would allow scientists of the Alberta Oil Sands Environmental Research Program (AOSERP) to evaluate any hazard from vanadium which reached natural waters.

1. An overall picture of vanadium toxicity was provided by measuring the lethal concentrations to rainbow trout in various types of water. Because the chemistry of vanadium in water is extremely complicated, it was possible that its toxicity would vary with the type of surface water. Accordingly, lethal tests were conducted in 12 kinds of water, covering any types likely to be of interest in nature, from soft to hard, and acid to alkaline.
2. The possibility of long-term or cumulative poisoning was investigated by life-time exposure of flagfish over one reproductive cycle. This experiment was intended to provide at least an initial estimate of the "safe" concentration of vanadium in one kind of water.

The ratio of the "safe" concentration to the lethal concentration in the same kind of water provides an application factor. If the lethal level of vanadium was found to be particularly divergent in some special type of water, the application factor would provide a preliminary estimate of the "safe" level in that type of water. If this suggested the need for concern about well-being of aquatic life in such water, further research might be indicated. If the expected or measured levels of vanadium in waters surrounding oil sands operations were found to be much lower than the "safe" levels from this research, the need for concern and further research would be alleviated.

2. BACKGROUND REVIEW

2.1 TOXICITY TO AQUATIC LIFE

The lack of knowledge about vanadium as a water pollutant is indicated by general reference books. Schroeder's book (1974) on toxic metals contains only a three-sentence paragraph, ending "Vanadium contaminates but does not pollute". He considers a contaminant to be something foreign, which should not be present, but does not harm living things. The "blue book" (NAS/NAE 1974) does not consider vanadium in its section on drinking water, nor under freshwater aquatic organisms. In the section on marine organisms, there is a recommendation that vanadium concentration should not be greater than 0.05 of an unspecified lethal concentration, but no toxicity data is cited, either in the text or the technical appendix. The agricultural section cites seven references, and states that most U.S. surface waters contain less than 0.05 mg/L, and that drinking water for livestock should have an upper limit of 0.1 mg/L. The recent "red book" of U.S. government criteria for water (E.P.A. 1976) does not consider vanadium. Nor do the Prairie Provinces standards for surface water quality include this metal.

The only known tests against fish were done a couple of decades ago with bluegill sunfish and fathead minnows (Tarzwell and Henderson 1956, 1960). Vanadyl sulfate was lethal at 5 to 6 mg/L, and vanadium pentoxide at 13 mg/L, in soft water. Lethal concentrations were 4 to 9 times higher in hard water. This suggested that vanadium was similar to many other metals, changing its toxicity in different kinds of water, and possibly changing its ionic "species". These early exploratory tests were static, rather than continuous-flow and the concentrations were nominal, not measured. Therefore the estimates of lethal concentration may be open to question.

No information was found on sublethal effects to aquatic life. There have been some studies with vanadium fed to or

inhaled by mammals, most of them not too relevant. However the most toxic form with mammals appears to be pentavalent vanadium (Roshchin 1967). There is some controversy about accumulation in organisms. Schroeder and Balassa (1967) found some evidence of this in rats but the metal is also excreted readily, mostly through the urine (Dimond et al. 1963; Jaraczewska and Jakubowski 1963).

2.2 PROBABLE FORMS OF VANADIUM IN TEST-WATER

Although vanadium has four valence states, sometimes with several ionic "species" per valence, the situation would not be that complicated in our tests, or in natural surface waters. Figure 1 shows the expected distribution of forms. The picture is relatively simple in the lower central part of the figure which is of interest (concentrations of vanadium below 15 mg/L and pH between 9 and 5). Only valence state +5 would be expected, and within this, the orthovanadates would predominate. There would seem to be only a slight possibility that metavanadates or decavanadates would occur in a lethal test at pH 5.5 and 15 mg/L, the highest concentration used.

In the chronic tests, only orthovanadates would be expected. There may have been some shifting between two ionic species $H_2VO_4^-$ and HVO_4^{-2}, since test-pH was near the border between these two. We believe the chronic tests involved mostly $H_2VO_4^-$, since the pK is probably in the region pH 8.2-8.4, and average pH in our chronic tests was 8.15. In any case, preliminary bioassays indicated that the two ionic species had similar toxicity (Appendix 10.1).

Accordingly, it seems justifiable that all vanadium added in our tests was the pentavalent form, as vanadium oxide (V_2O_5). Appendix 10.2 presents a detailed consideration of the chemistry of vanadium in water, which led to the above generalizations.

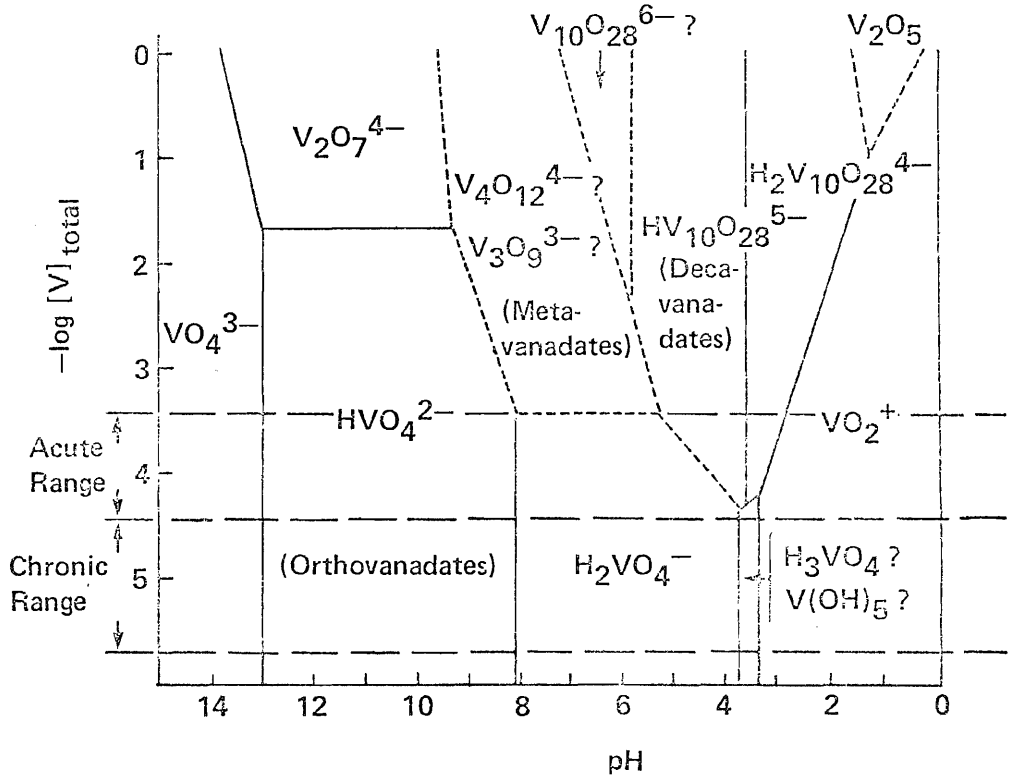

Figure 1. Approximate conditions of pH and total vanadium concentration under which given species would be major solute components of a vanadate solution at 25°C. Broken lines indicate demarcations involving considerable doubt. (From Pope and Dale 1968, with names of ion-groups added.)

2.3 FORM OF VANADIUM RELEASED FROM OIL SAND OPERATIONS

Vanadium in oil sands may be V^{+4}, perhaps as oxovanadium porphyrins (Cotton and Wilkinson 1972). One pollutional concern might be escape of vanadium from stacks, with aerial fallout to lakes with slow turnover. Any such vanadium would have passed the "boiler" used for monoxide combustion. That treatment would probably release the metal from clays and oxidize it to V^{+5}, since the process is aerobic and at a high temperature. Even if reducing conditions prevailed in the stack, any vanadyl sulphate might be changed to V_2O_5 in the plume, if it were still hot. If not, vanadyl sulphate reaching natural waters would still oxidize rapidly to the pentavalent state. Details of these likely transformations are given in Apendix 10.3.

There is less certainty about the form of vanadium in any liquid effluent such as mine-water or refinery discharge. However the amounts in any such effluents would not seem to be high enough to cause important elevations of vanadium in a flowing river as large as the Athabasca (Appendix 10.3).

3. ACUTE LETHALITY OF VANADIUM TO RAINBOW TROUT

The purpose of this experiment was to establish the lethal levels of vanadium for a Canadian salmonid fish, in types of fresh water that differed in fundamental natural characteristics. There was inadequate knowledge about the toxicity of vanadium to fish. Lethal levels for trout would be a starting-point in assessing whether oil sands operations were likely to cause problems of vanadium pollution. The experiment would also show whether vanadium was likely to be more dangerous in any particular type of surface water. It seemed possible that the diverse chemical behaviour of vanadium could result in such differential toxicity.

Different types of fresh water were simulated by three levels of water hardness (soft to hard) in all possible combinations with four levels of pH (acid to alkaline). These spanned the range of water-types likely to be of interest. Water of a given mineral content generally has a single natural value for stabilized pH, usually near-neutral but slightly acid in soft water and slightly alkaline in hard water. However in an altered environment such as one affected by pollution from mining or refining, deviant combinations of hardness and pH must be taken into account.

3.1 METHODS

3.1.1 Experimental Design and Statistical Analyses

A lethal test of vanadium was carried out at each of the twelve combinations of total water hardness 30, 100, and 360 mg/L as $CaCO_3$, and pH 5.5, 6.6, 7.7, and 8.8. Four tests were done at the "central" combination of hardness 100 and pH 7.7, to assess variability between bioassays, and allow corrections for any changes in resistance of fish. Tests were done in random order.

Median lethal concentration (LC50) was estimated by probit analysis (Finney 1971) using a computer. Standard programs (Institute for Computer Studies, University of Guelph) were followed for analysis of covariance, Bartlett's test of homogeneity of variances, and linear and multiple curvilinear regression analysis, to establish the response surface of vanadium lethality over the design region (Appendix 10.4.1).

Some difficulties were encountered in carrying out the experiments as designed. One of the "central" tests (hardness 97, pH 7.7) had to be rejected because of poor quality of water supplied to the laboratory during a University pipe-cleaning operation. The test was repeated at the end of the series. In the first test at nominal hardness 360 and pH 8.8, natural equilibrium reactions of the water resulted in a drop of hardness to an average of 242 mg/L, the loss being somewhat less severe in low concentrations of vanadium and somewhat more severe in high concentrations. This test was repeated at the end of the series with a tripled flow-rate of experimental water. This achieved an average of 335 mg/L for hardness of water, only 7% below the nominal level.

3.1.2 Apparatus

Vanadium stock-solution was pumped to a diluter (Mount and Brungs 1967) from a large reservoir where it had been controlled for temperature and pH and aged for at least five days. Dilution-water of desired hardness and temperature was pumped to a head-tank where pH was regulated by an automatic device that added acid or alkali. Dilution-water then entered the diluter. Test-water of 5 concentrations plus a control then flowed at 4 L/h to each randomly-assigned bioassay tank. These contained 10 L and had a 95% molecular replacement-time of 6 hours. The entire system was designed to hold the vanadium for at least five days in water of the hardness and pH at which it would be tested, without appreciable changes. This was to stabilize the form of

vanadium and prevent any changes during the actual bioassay.

Bioassay tanks were aerated in order to prevent a drop in pH which would otherwise have resulted from respiratory CO_2 from the fish. Light intensity was 20-30 lux at the surface during a 14-hour "day" which included gradual 15-minute periods of brightening and darkening, the same as the photoperiod for acclimation. During the 10-hour "night", a red light was used for inspecting fish so as not to disturb them. Further details are given in Appendix 10.4.2.

3.1.3 Fish and Handling in Tests

Rainbow trout (*Salmo gairdneri* Richardson) were obtained periodically from Goosens Fish Hatchery, Otterville, Ontario, and were certified free from 11 specific pathogens. The stock originally came from Edward Mcleary Trout Springs Hatchery, Washington, U.S.A. in 1963, originating from native west coast rainbow trout. Fish were fed Silvercup Feed and a Purina Mixture developed at the University of Guelph.

Fish were acclimated to within ±10% of the hardness at which they would be tested, for at least ten days, longer than is apparently necessary for them to equilibrate their physiology to hardness with respect to metal toxicity (Lloyd 1965). Acclimation temperature was 15 $\pm$ 0.2°C and oxygen was 90% of saturation or greater (Appendix 10.4.4). Values of pH during acclimation were the stabilized ones, averaging 7.32, 7.70, and 8.05 from low to high hardness. Groups of 200 fish were acclimated in green cylindrical fibreglass tanks of 1.2 m diameter. Volume and turnover-time of water (Appendix 10.4.4) were within usual guidelines (Sprague 1973). Feeding ceased 24 hours prior to a test.

At the start of an experiment, sixty fish were randomly selected and divided, 10 to each bioassay tank which was already at desired experimental conditions. Observations on mortality were done in a logarithmic fashion. Exposures continued for at least 7 days, since initial work indicated that the customary 4-day exposure would not yield a threshold of acute lethality.

Dead fish were removed from the test baths at each observation time. Criterion for death was absence of opercular movement. Wet weight and standard fork length of all fish were taken, they were washed with distilled water, freeze-dried, weighed and stored for possible later residue analysis.

Bioassay procedures were considered satisfactory. The tests utilized here had no mortality in the controls. Reasonable precision was attained within each test; 95% confidence limits differed from the LC50 by factors averaging only 1.34.

A few range-finding tests with zebrafish were carried out before the trout tests, to establish approximate lethal ranges for vanadium (Appendix 10.1).

3.1.4 Physico-chemical Conditions and Procedures

In the test-tanks, average water hardness was within 5% of the nominal levels (Table 1) and individual values were always within 10% of nominal. Two aberrant tests at nominal 360 mg/L hardness and pH 8.8 are excluded from this synopsis, and were described above. (Henceforth, the shorthand "360/8.8" etc. will be used to designate the hardness/pH levels.) Average temperature was within $\pm 0.3^{o}$ of $15^{o}C$, and dissolved oxygen was always above 90% saturation. These conditions were measured daily in each tank. The pH was measured at least once a day, usually twice, and was within $\pm$ 0.1 of desired levels except for a variation of $\pm$ 0.2 in the experiment at 29/8.8. Average measured values of the above variables are used in describing the tests. Within each bioassay, there was little or no difference from tank to tank, in average temperature, oxygen, hardness, or pH. The only exception was tank-to-tank variation of hardness of the above-mentioned aberrant tests at 242/8.75 (see footnotes of Table 1).

The highest hardness of water, provided directly by aerated well-water from the University, had typical characteristics for such water as described in Hodson and Sprague (1975). There was less than 0.01 mg/L of copper, zinc, lead, cadmium, and nickel, usually much less (Appendix 10.4.5). Lower hardnesses

Table 1. Median lethal concentrations of total dissolved vanadium for rainbow trout in one-week exposures, in water of various levels of total hardness and pH.

Average hardness	Average pH	Average wet wt. of fish,g	Date of test start	4-day exposure			7-day exposure		
				LC50, mg/L	Fiducial limits	Slope of probit line	LC50, mg/L	Fiducial limits	Slope of probit line
30	5.51	1.55	77 8 5	>10.1	- -	-	2.97	2.02-4.37	3.25
31	6.60	2.12	77 10 19	6.57	4.51-9.57	3.51	2.54	1.79-3.59	3.59
30	7.70	1.31	77 8 31	5.87	4.29-8.04	3.33	2.36	1.76-3.15	5.40
29	8.80	1.68	77 10 8	6.83	4.41-10.1	2.57	2.39	1.81-3.16	5.33
101	5.51	2.98	77 6 24	11.65	6.71-20.23	2.38	4.77	3.00-7.59	2.63
105	6.66	3.83	77 12 19	5.69	4.20-7.73	3.47	3.66	2.41-5.55	3.11
103	7.72	2.44	77 5 20	6.16	5.06-7.50	6.26	2.99	2.42-3.70	5.18
100	7.70	2.13	77 6 8	6.11	4.90-7.61	5.45	3.42	2.63-4.45	4.79
101	7.71	2.45	77 7 5	10.0	-	-	3.76	3.10-4.57	6.20
98	7.66	6.15	78 2 7	5.16	3.57-7.45	2.98	2.46	1.74-3.48	4.32
101	8.78	1.2	78 2 22	> 7.39	- -	-	5.36	4.34-6.62	5.12
357	5.50	2.93	77 11 24	>14.35	-	-	5.59	4.33-7.22	6.31
368	6.61	1.93	77 7 20	13.2	11.0 -15.9	11.3	3.56	2.97-4.26	8.81
355	7.70	1.59	77 9 21	7.21	5.14-10.12	3.06	2.80	1.95-3.40	3.57
335*	8.75	2.92	77 12 8	8.69	6.31-11.97	3.76	4.20	3.37-5.23	4.99
242**	8.83	1.18	77 8 15	>12.26	-		5.40	3.96-7.38	3.74

* Hardness increased with vanadium level, from 327 in control to 346 in 13.5 mg/L.

** Hardness increased with vanadium level, from 229 in control to 258 in 12.3 mg/L.

were obtained by mixing with deionized well-water from a reverse-osmosis softener. Deionized water retained its original chloride content of 22 mg/L, a desirable feature for health of fish (Lloyd 1961) but was satisfactorily low in other ions (Howarth 1976; Appendix 10.4.5).

Values of alkalinity and acidity are described in Appendix 10.4.6. Free carbon dioxide was never greater than 4 mg/L in the experimental tanks.

Vanadium concentrations are averages of daily measurements in each test-tank, of total dissolved vanadium as mg of Va^{+5} per litre. Nominal concentrations were from 1.0 to 15 mg/L. Concentrations were stable in test-tanks; most did not deviate more than 5% from the average, and the maximum was 9%.

Samples for determining vanadium were filtered through a 0.45 μm filter, acidified, and measured by atomic absorption spectrophotometry (Varian Techtron Model AA6). This is considered to measure ionic forms of vanadium. Total vanadium (unfiltered samples) was not detectably different. Samples stored satisfactorily without changes in vanadium concentration. Checks showed that vanadium in the stock-solution was all or almost all valence state V^{+5}, according to the titration method of Charlot and Bezier (1955; Appendix 10.4.3).

The procedure for preparing vanadium stock-solutions required five days. Enough vanadium pentoxide was added to a reservoir of water at the desired hardness to result in 30 mg/L Va^{+5}. This was vigorously mixed and aerated for two or three days to allow the metal to dissolve. A predetermined amount of sulphuric acid or sodium hydroxide was added, and pH was held for a further three days at about 0.2 units below the desired pH. This allowed stabilization of the natural ionic forms from any intermediate ions formed during the pH change. The final pH adjustment, just ahead of the bioassay tanks, always involved addition of hydroxide, since acidification can cause formation of coloured intermediates which take time to revert to orthovanadates. A

series of five reservoirs was used in succession to make up stock-solutions, and these replenished the reservoir at the base of the bioassay apparatus with properly-aged solution.

The above procedures with the stock-solution were adopted because the usual approach of using for the diluter, a very strong acidified solution of metal, was not suitable. Sudden changes in pH would have caused changes in ionic species, and continued change in form in the test-tanks, probably not attaining the final stabilized distribution during the few hours the water remained in the tanks. Further comments on chemical procedures are given in Appendix 10.4.3.

3.2 RESULTS

3.2.1 Lack of Thresholds

Thresholds of lethality were definitely not attained in 4-day exposures. In every bioassay, the 7-day lethal concentration was lower than the 4-day value (Table 1). In fact, the 7-day LC50 averaged only 42% of the 4-day LC50 (Table 2). Obviously there was a continuing lethal effect during those three extra days of exposure.

Lethal action apparently continued beyond 7 days. All five of the tests continued beyond this time showed an 8-day LC50 that was lower (Table 2). In the single bioassay continued for 11 days, the median lethal level continued to drop at 9, 10 and 11 days. The 11-day LC50 was significantly lower than that for 7 days. The toxicity curve (Figure 2) shows little evidence that it was becoming asymptotic to the time axis by 11 days. Seven-day LC50's were adopted as a reasonable basis for comparing acute lethality of vanadium in the different types of water.

Table 2. Comparison for various exposure-times, of median lethal concentrations of vanadium for rainbow trout. Each horizontal line represents a test on one group of fish.

Average hardness	Average pH	LC50, mg/L of vanadium					
		4-day	7-day	8-day	9-day	10-day	11-day
101	5.51	11.7	4.72	2.74	-	-	-
103	7.72	6.16	2.99	2.76	2.56	2.30	1.99
100	7.70	6.11	3.42	2.60	2.60	$\leq$2.09	-
101	7.71	10.0	3.76	3.26	-	-	-
368	6.61	13.2	3.56	3.35	-	-	-
Average % of 4-day LC50			42	33.8	42	38	32

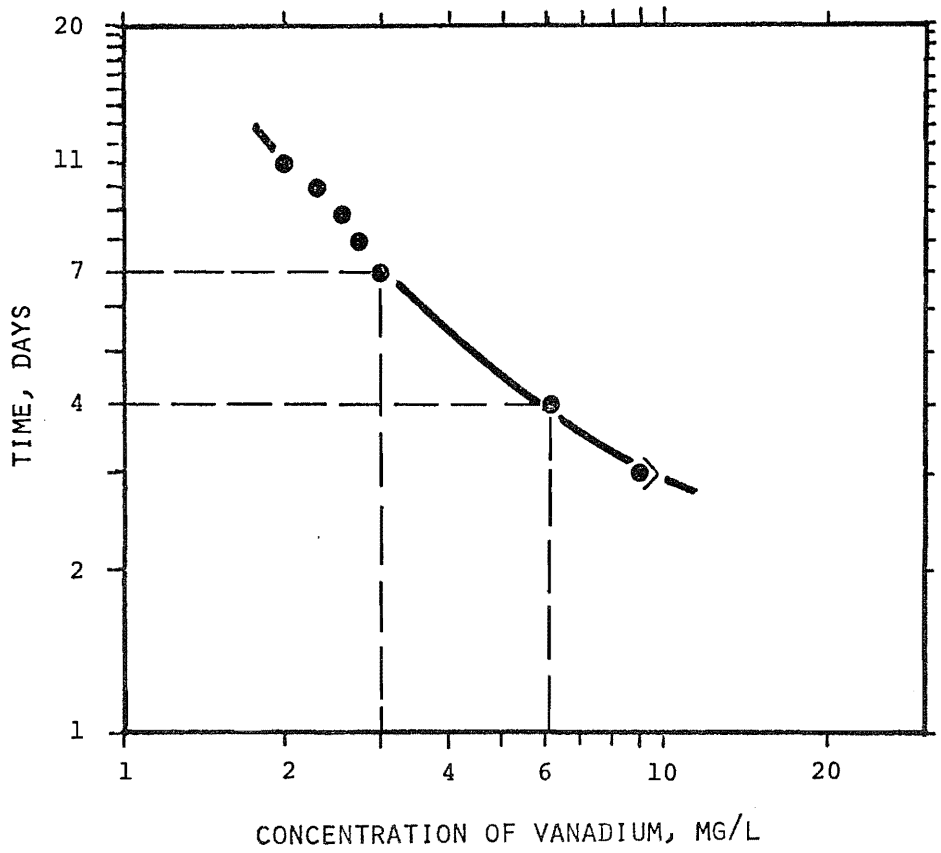

Figure 2. Lethality curve for rainbow trout exposed to vanadium in water 103 mg/L total hardness and pH 7.72.

3.2.2 Variables Other Than Hardness and pH

The responses at the central combination (100/7.7) remained constant. None of the four repeated LC50's were significantly different from another (Table 1). There was no apparent trend with time of doing the test (position in the random series, Table 1). No clear trend in LC50 was evident over the range of size used in these four tests. Although it is possible that there may be an effect of size on resistance to vanadium, the present tests do not give enough information to establish any such trend.

3.2.3 Response Surface

The following equation relates 7-day LC50 of vanadium to hardness and pH over the region of the experimental design.

$$LC50 = 14.6976 - 3.7783P + 0.1108H - 0.02137PH + 0.2662P^2 - 0.000073H^2 + 0.00141P^2H. \quad (1)$$

H = total hardness and P = pH.

The equation was calculated using the average of the four LC50's at 100/7.7, and the extra value at 240/8.8. This equation explained 91% of the variability in response. Although the equation is lengthy, it has no logarithms or other transformations, allowing straightforward calculation of a predicted LC50 for any combination of hardness and pH within the design.

The response surface has only a small amount of curvature (Figure 3); in other words the variation in LC50 is not great. For example, although there was a curved trend with pH within hardness 30 (Figure 3), this was slight and none of the four measured LC50's were significantly different from any other. Variation was somewhat greater within hardness 360. There was a "dip" at pH 6.6 and 7.7, i.e. an increase in toxicity, and those LC50's were significantly lower than the LC50 for pH 5.5. The LC50 at pH 7.7 was also significantly lower than that for pH 8.8. However, the actual differences between the LC50's were not great, those for hardness 350 being in the range 2.8 to 5.6 mg/L.

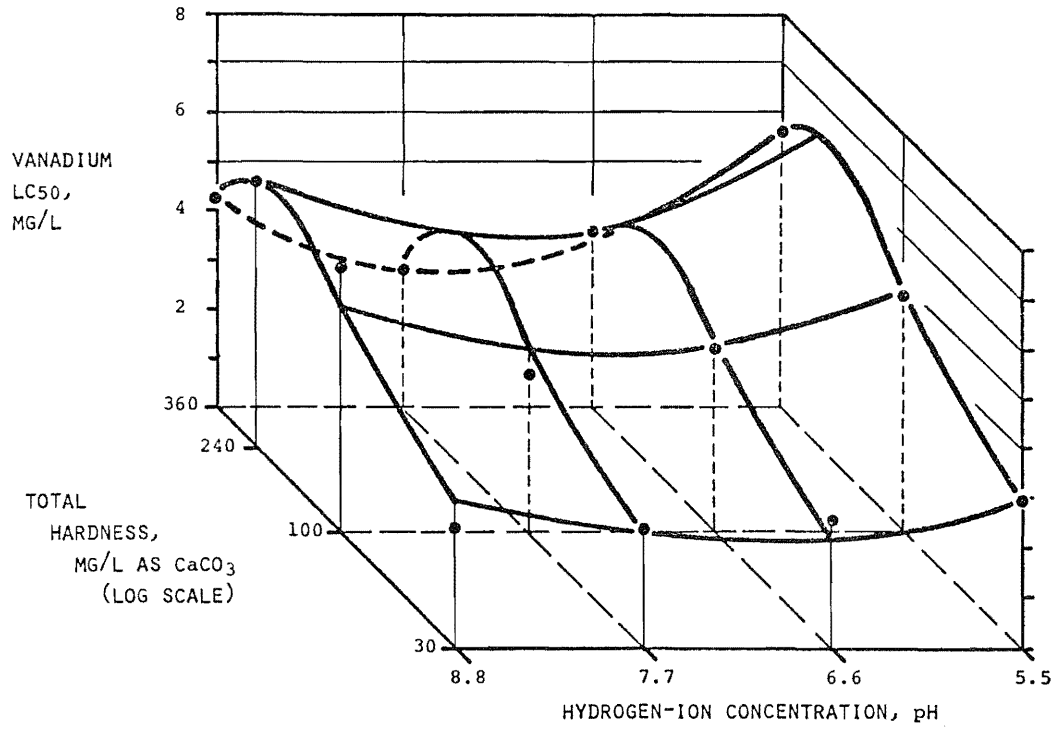

Figure 3. Lethal concentrations of total dissolved vanadium to rainbow trout for any combinations of water hardness from 30 to 360 mg/L and pH from 5.5 to 8.8. The response surface is that of equation (1), while points show actual LC50's obtained.

There was a slight trend for vanadium to be less toxic in acid waters (higher LC50's, Figure 3). However, only 35 out of 94 comparisons of LC50's between pH-values, showed significant differences (Appendix 10.5). The pH level of 8.2-8.4 would apparently divide two ionic forms of vanadium (Figure 1) but there is not a major difference between LC50's on either side of that boundary.

Hardness seemed slightly more important than pH in governing toxicity of vanadium. LC50's at hardness 30 were in the range of 2-3 mg/L (Table 1). There was a general increase in LC50's (decrease in toxicity) in harder water, at all values of pH (Figure 3). The response surface indicates a "dip" in LC50's beyond hardness 240, to the highest hardness of 360. This may be the true situation, or it could be an anomaly. The "spine" of the slightly saddle-shaped surface, at hardness 240, was probably governed by one LC50 determined at 240/8.8. It is possible that normal variation in that response may have unduly influenced the calculations for the response surface. In any case, the effect of hardness is not a major one. Comparing vanadium LC50's between hardnesses, regardless of pH, showed only 30 significant differences out of 83 comparisons (Appendix 10.5).

Some preliminary lethal tests with zebrafish demonstrated that a nominal concentration of 22 mg/L of vanadium caused 50% mortality in 25-45 hours, averaging about 1.4 days (Appendix 10.1.).

3.3 DISCUSSION

Equation (1) may be used as a predictor of vanadium lethality to trout. The equation is fairly accurate, and includes the combinations of water hardness and pH likely to be of interest in fresh waters. Any water-bodies outside the range

we tested would be unusual or polluted ones and in themselves unlikely to support healthy fish populations.

Considerable caution should be used in applying these laboratory results to field situations such as those around the Athabasca Oil Sands. Our results are for dissolved vanadium, presumably of valence +5. In particular our tests and equation (1), do not include the effects of natural or anthropogenic chelating agents which can be of great importance in reducing the toxicity of some metals.

The lethal levels for trout ranged from 2.4 to 5.6 mg/L. These values mean that vanadium is moderate in toxicity among the metals. For example, nickel is less toxic by factors of 2.5 to 10 depending on water quality. Zinc is similar to vanadium but more toxic, with LC50's of 0.8 to 3.5 mg/L (NAS/NAE 1974). Copper is one or two orders of magnitude more toxic, LC50's being 0.02 to 0.5 mg/L (Howarth and Sprague in press).

This moderate level of toxicity is much higher than present levels of vanadium around the Athbasca Oil Sands. In a series of 542 measurements supplied by AOSERP, the average concentration of vanadium was < 0.0013 mg/L. The ranges in different bodies of water were almost always < 0.001 to 0.010, usually 0.001 to 0.007 mg/L. Hardness of water in the region was variable, but was usually greater than 35 units. Values of pH were almost always in the range 6.8-8.5, usually 8.0-8.3, so that the left-hand side of the response surface in Figure 3 would be applicable. The Athabasca River should certainly have no problem of direct lethality from vanadium, if our estimate is correct that about 0.004 mg/L would be contributed by the liquid effluents of one plant (Appendix 10.2).

Absence of a threshold for acute lethality of vanadium, at least up to 11 days, is most unusual. Most toxicants show a threshold (cessation of further mortality) within four days (Sprague 1969). This should be taken into account when assessing oil sands operations. From our data on trout, levels considerably

lower than 2 mg/L could be lethal, given sufficient exposure time. However, one would expect a cessation of lethality at some point. The topic is further discussed in section 5.

The necessity of using 7-day LC50's for comparing different types of water lacks some theoretical advantages of using true thresholds of acute lethality. However, using LC50's for exposures longer than 7 days would be somewhat unrealistic. They would probably be considered as "subacute" exposures for fish, rather than acute ones.

The response surface for vanadium toxicity was fairly even over the different hardness/pH combinations. Only slight differences were found in various types of water--some of them significant but still not major. The relatively flat surface was somewhat surprising. For example such a response surface for copper is highly convoluted, with a several-fold factor between lowest and highest LC50's in different types of water (Howarth and Sprague in press).

The two ionic species of orthovanadate (HVO_4^{-2} and $H_2VO_4^-$) are about equally toxic. Each must have been present to the exclusion of the other, at different extremes of the pH-range, but there was no major convolution of the response surface. If any metavanadates or decavanadates were present at low pH, they did not greatly influence the lethal response.

The effect of water hardness on vanadium toxicity was much less than is found for some heavy metals. For example, zinc changes its toxicity by about one order of magnitude for a hardness-range similar to the one we used (NAS/NAE 1974). Our results are in good agreement with those of Anderson and Spear (in preparation) who worked under a parallel AOSERP contract. They estimated that 4.8 mg/L of vanadium was lethal to trout in 7.2 days. Assuming our central combination of 100/7.7 is a good comparison with their water (quality unstated), the average of our four 7-day LC50's was 3.16 mg/L. Thus we found vanadium to be slightly more toxic than did Anderson and Spear, our result being 66% of theirs.

Such correspondence is remarkably good; results from separate laboratories may differ by a factor approaching 10, and even within laboratories there may be a five-fold difference (Fogels and Sprague 1977).

Our findings do not agree as well with the older results of Tarzwell and Henderson (1956, 1960). Their 96-hour LC50's of 13 and 55 mg/L for fathead minnows in soft and hard water, are about an order of magnitude higher than our results (Figure 4). We found little difference in toxicity with water hardness, while they found a four-times difference. However, Tarzwell and Henderson called their vanadium tests "exploratory" ones, and they also depended on nominal concentrations. It is possible that differences in the amounts actually dissolved could account for part of this discrepancy. Tarzwell and Henderson found vanadyl sulphate lethal to fathead minnows and bluegills in a similar range of 5 to 55 mg/L, but this is of less interest here. Vanadyl sulphate would be expected to change almost immediately to the vanadate form, in the test-water.

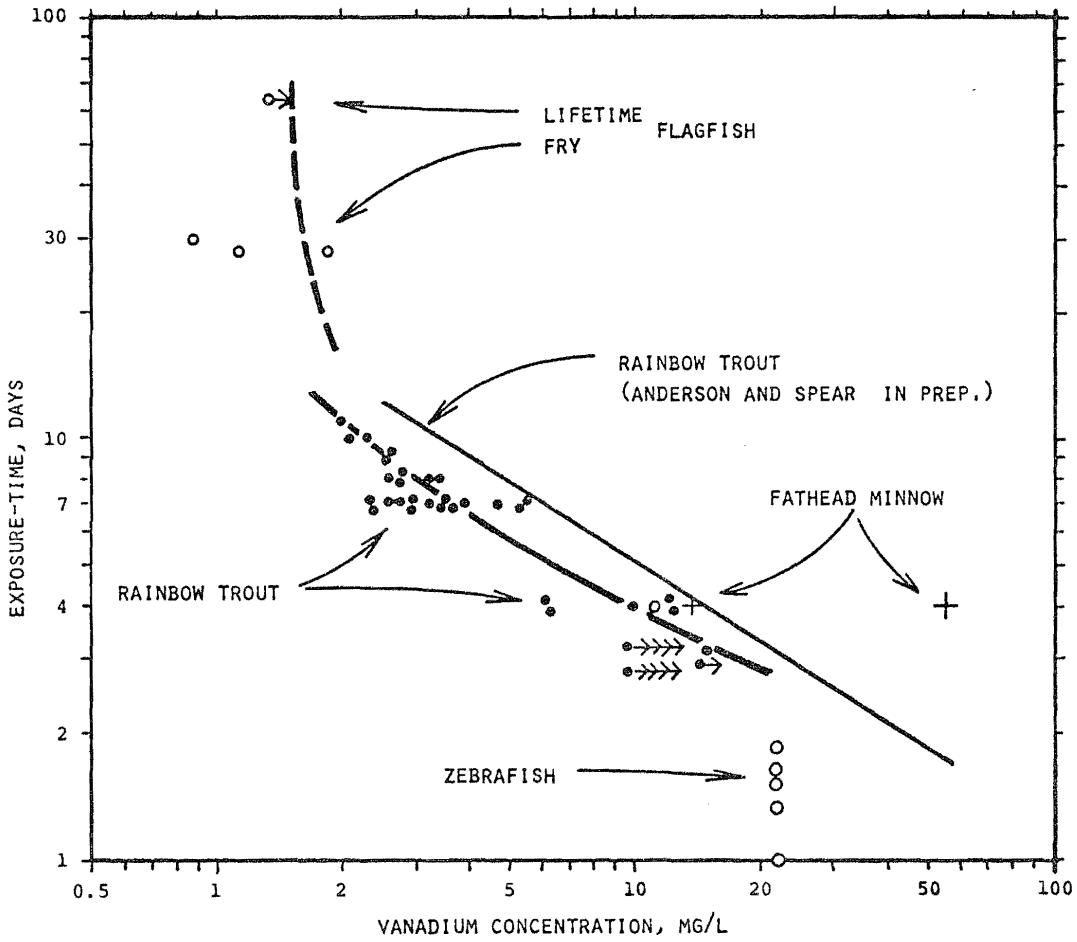

Figure 4. Overall picture of vanadium lethality to different species of fish. The graph is for general comparison only since there are many differences between the groups of tests that have been de-emphasized (see section 5.1). Tests with juvenile rainbow trout were in many kinds of water, from hardness 30-350 mg/L, and pH 5.5-8.8. Flagfish were tested in hardness/pH 350/8.15, and zebrafish in 350/7.5-9.0. Values for fathead minnows are from Tarzwell and Henderson (1956, 1960). Horizontal arrows represent median lethal concentrations that were not determined, but which would have been higher than concentration at the arrowhead.

4. CHRONIC TOXICITY TO FLAGFISH

The objective of this experiment was to determine "safe" thresholds of vanadium during one generation of exposure to continuous constant levels of the metal, including early stages of the second generation of fish. Such an experiment should reveal any appreciable sublethal effects, including major damage from cumulation of the metal.

4.1 METHODS

Procedures adhered fairly closely to the established ones for full chronic tests with flagfish *Jordanella floridae* Goode and Bean (E.P.A. no date).

4.1.1 Biological Procedures

The experiment started with one-week-old fry of American flagfish. Over a period of 3.2 months, these were reared to maturity, growth and mortality was measured at various stages, reproductive performance was assessed, and viability of the second generation of fish was followed to one month of age. Separate groups of fish were continuously exposed to control conditions and four successively-higher concentrations of vanadium, with two replicate tanks for each of the five levels. The plan of the experiment may be seen in Figure 5, and detailed description is given in Appendix 10.6.1.

A first run of the experiment used concentrations of 0, 0.051, 0.58, 3.4, and 6.3 mg/L of vanadium. The effects of vanadium were more severe than expected, the higher concentrations causing heavy mortality. This run was therefore terminated at 28 days, providing data on mortality and growth.

A second run was successfully completed at a lower geometric range of concentrations (0, 0.041, 0.17, 0.48, and 1.5 mg/L of vanadium). There were two divergences from the strict

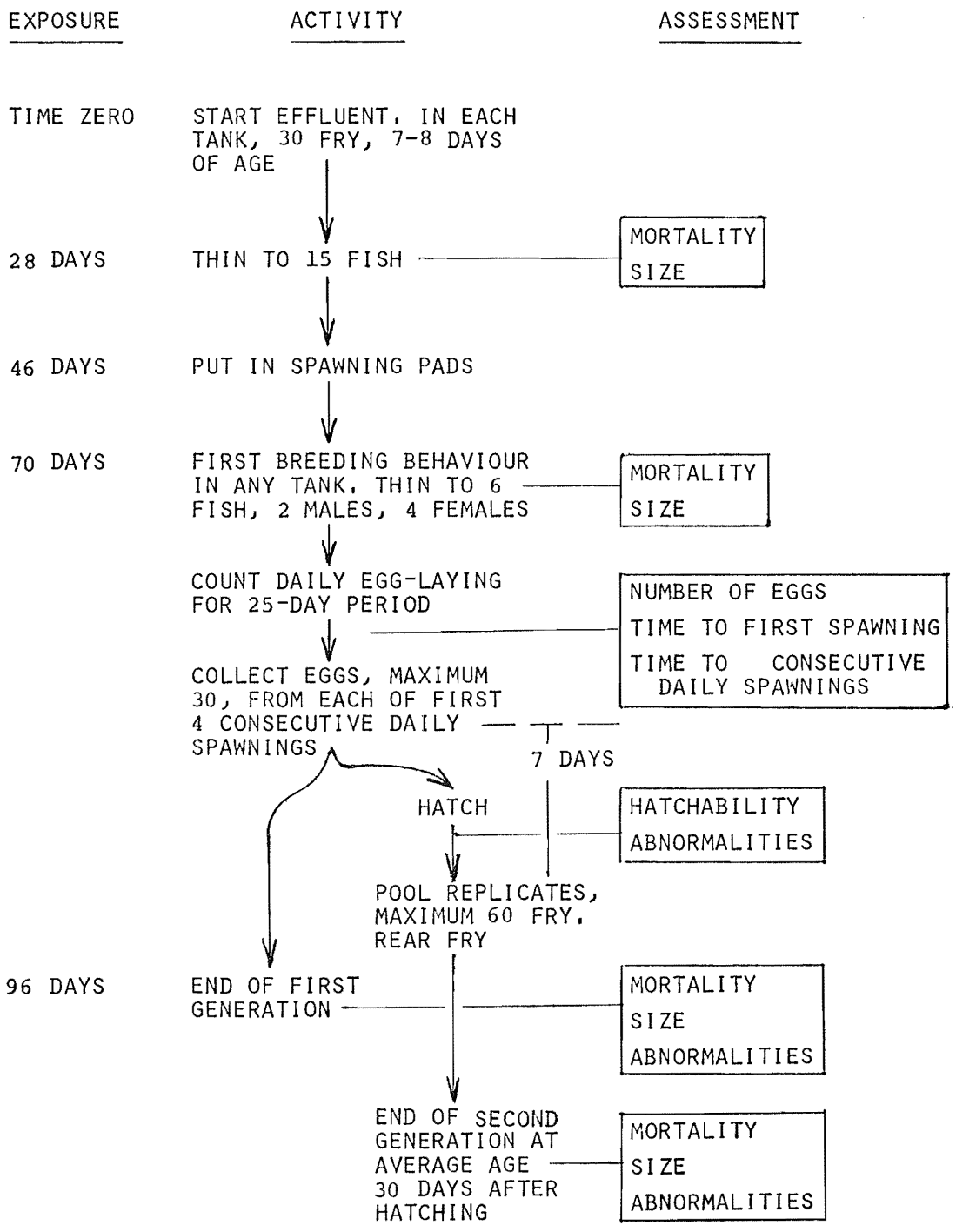

Figure 5. Schematic description of the chronic bioassay with flagfish. The boxed-in items show the observations that were made.

outline presented in Figure 5. The highest concentration caused appreciable mortality, so that in one replicate, not enough females survived to provide the desired number of four. Thus, the replicates at the highest concentration were unbalanced, and could not be included in some of the analyses of sublethal effect. This was considered satisfactory, since it was desirable to have one high concentration in the experiment giving obviously severe detrimental effects. A second divergence was a chance effect. One of the males in a replicate of 0.17 mg/L became extremely large, dominant, and aggressive. Reproductive performance in this replicate tank was noticeably inferior to that in the other replicate. Apparently this was less related to vanadium concentration than to the extreme aggression of the one fish, which interfered with mating behaviour. While a random occurrence, the spotty reproductive performance lessened the power of some analyses of egg-laying.

4.1.2 Dosing Apparatus

Dilution-water was a constant flow of well-water, aerated and temperature-controlled. Toxicant came from one of two large reservoirs, used alternately so that the vanadium had at least 48 hours to equilibrate with the well-water in which it was dissolved. The two streams of water were regulated to the desired concentrations of vanadium by a fail-safe diluter (Mount and Brungs 1967). Each delivered concentration was split to flow into three aquaria, two replicates for the first generation and one for eggs and fry of the second generation. The flow of 120 L/**d** provided 95% molecular replacement of aquarium water in 13 hours. Other details are given in Appendix 10.6.2.

4.1.3 Water Chemistry

Vanadium was measured by atomic absorption spectrophotometry (Varian CRA 90 carbon-rod). All concentrations are stated as mg of vanadium per litre. Average concentrations were used to characterize exposure-levels. In the first experimental run, terminated at 28 days, averages were 0, 0.051, 0.58, 3.4, and

6.3 mg/L. These measured concentrations were in reasonable ratios to each other, compared with the expected ratios from the nominal concentrations (Table 3).

For the second or complete experimental run, a lower series of concentrations was selected. Exposures for any observation-period were adequately represented by the overall averages for the experiment: 0, 0.41, 0.17, 0.48, and 1.5 mg/L. No significant differences were found between measured concentrations in the replicate tanks, nor among the various exposure-periods used in making observations, with one minor exception (Appendix 10.6.3). However, for calculating LC50's the actual averages during the 28 or 30-day exposures were used, instead of the averages during the entire experiment.

Average temperature during the 95-day exposure, was 25.4^{o}C, pH 8.15, dissolved oxygen 7.4 mg/L, and hardness 347 mg/L. These general characteristics of the water did not change greatly from tank to tank or over the course of the exposure (Table 4). Temperature and pH were measured daily in each tank, and DO every fourth day, and any upward or downward trends tended to occur simultaneously in all tanks. Because of the role of pH in governing forms of vanadium, average values for various concentrations of the metal are shown in Table 5. There seemed to be a tendency for slightly lower pH in the tanks with 0.041 mg/L of vanadium. However, the differences were not great, and the important thing is that all averages were below the range 8.2-8.4, thought to be critical in the change of ionic species of vanadium.

The procedure of using well-water at its stabilized pH was checked before the experiment by lethality tests at pH 7.5, 8.2, 8.8, and 9.0. The purpose was to see whether there was any great difference in toxicity of vanadium species, above and below the "boundary" of pH 8.2-8.4. These lethal tests using zebrafish, did not detect any such general tendency (Appendix 10.1); in other words the two ionic species appeared to be equally toxic. It was concluded that the method described here, using unmodified well-water

Table 3. Mortality and size of flagfish fry in the first experimental run, at the end of 28 days of exposure of flagfish to vanadium. A and B are replicates.

Concentration of vanadium, mg/L			Mortality			Average wet weight mg, of survivors		
Nominal	Measured average	No. of measurements	Replicates, out of 30 starting		Combined			
			A	B		A	B	Combined
0	0	6	3	1	4	58	53	55
0.056	0.051	3	2	1	3	74	79	76
0.56	0.58	3	5	1	6	69	53	61
3.14	3.4	18	21	26	47	70	56	60
5.6	6.3	12	30	30	60	-	-	-

Table 4. Basic characteristics of test-water during the 96-day exposure of flagfish.

	Temperature degrees C	pH	Dissolved oxygen mg/L	Total hardness mg/L as $CaCO_3$
Overall average	25.4	8.15	7.45	348
Averages, individual tanks	25.3-25.5	8.12-8.16	7.4 -7.5	332-355
Standard deviations, individual tanks	0.21-0.34	0.07-0.09	0.35-0.45	4-8
Extremes recorded in experiment	24.4-26.4	7.90-8.31	5.8 -7.8	319-368

Figure 5. Average pH values for various concentrations of vanadium. First experimental run terminated at 96 days. A and B are replicates, with their averages underlined.

First experimental run.				Second experimental run.					
Concentration	A	B	Average	Concentration	First generation A	B	Average	Second generation	Overall average
0	8.14	8.12	8.13	0	8.14	8.16	8.15	8.21	8.16
0.051	8.13	8.14	8.14	0.041	8.12	8.12	8.12	8.10	8.12
0.58	8.14	8.17	8.16	0.17	8.16	8.16	8.16	8.11	8.15
3.4	8.15	8.15	8.15	0.48	8.16	8.15	8.16	8.16	8.16
6.3	8.24	8.24	8.24	1.5	8.14	8.17	8.16	8.21	8.16

in the chronic tests, should be satisfactory even if the pH fluctuated around or through the "boundary".

Hardness was measured 7-12 times per concentration during the 96-day exposure. The two higher concentrations of vanadium apparently resulted in slightly reduced hardness or else interfered with the titration (EDTA) (Table 6). The average hardness in 1.5 mg/L tanks was significantly lower than in all other concentrations, and that in 0.48 mg/L was lower than in the control and 0.17 mg/L. However this is not considered a major factor since the difference between highest and lowest hardness was only 6% and hardness did not seem to be a major factor in vanadium toxicity, as judged by lethal concentrations reported in section 3.2.

4.1.4 Statistical Analysis

Results were analysed by computer using APL-PLUS, and standard programs from the library of the Institute for Computer Studies, University of Guelph. One-way or two-way analysis of variance was generally used to initiate comparison. Frequently, replicate observations were pooled and tested by one-way analysis. Although two-way, with the replicates kept separate, would have been somewhat more powerful, many of the replicates were not balanced in number of observations. No program for unequal numbers was available without the considerable labor and expense of card-punching for another language. It is believed that pooling replicates was a conservative approach, i.e. only substantial differences between concentrations would show up as significant.

Follow-up tests were usually Duncan's New Multiple-Range Test to distinguish differences between means of various groups, or Dunnett's test when it was only desired to find which concentrations had means that were different from the control.

Lethal concentrations were estimated by probit analysis (Finney 1971) using a computer.

Table 6. Average hardness of water for each test-concentration of vanadium during the 96-day exposure of flagfish.

Vanadium concentration	Average hardness
0	355
0.041	350
0.17	352
0.48	345
1.5	335

4.1.5 Acute Lethality

One set of lethal tests with larger flagfish was not part of the chronic experiment. It was carried out in order to obtain an "application factor", i.e., the ratio between the sub-lethally "safe" level and the acutely lethal level of vanadium. Such an application factor could be of practical interest when applied to native fish of the Athabasca region. In using such a factor, the acute test is customarily based on immature fish of the "fingerling" stage. Accordingly, we used immature flagfish of average wet weight 0.13 g (SD - 0.037).

This was a simple 4-day static test, with test-water renewed daily. There were five fish at each of the four concentrations utilized. Total hardness of water averaged 275 mg/L, temperature 24.0°C, pH 8.17 and dissolved oxygen 8.4 mg/L.

4.2 RESULTS

4.2.1 Lethality in Chronic Experiment

In the first experimental run, the two higher concentrations of vanadium caused complete or severe mortality of flagfish (Table 3). The 28-day LC50 was 1.88 mg/L, with confidence limits of 1.54 and 2.68, and a slope of 2.68 for the probit line.

In the second experimental run, the first 28 days of exposure of similar fry to a lower range of concentrations, also yielded appreciable mortality as shown in Table 7. The 28-day LC50 was 1.13 mg/L (c.l. 0.858, 1.47; slope 4.66). The similar exposure of second-generation fry yielded the results shown in Table 8. The 30-day LC50 was estimated as 0.891 mg/L (c.l. 0.122, 6.49; slope 0.478). Slight differences in this third test should be noted. Fish were exposed for 30 days instead of 28. The eggs from which they hatched had been exposed to the same concentrations. Exposure started at age zero instead of age one-week, and fry were 30 days old at the end of the exposure, instead of 35-36 days as the preceding tests. In addition,

Table 7. Mortality of flagfish fry at the end of the first 28 days of exposure in the second experimental run. A and B are replicates.

Measured vanadium, mg/L	Number of dead fry out of 30 per replicate			Combined as %
	A	B	Combined	
0	8	7	15	25
0.039	6	9	15	25
0.14	6	3	9	15
0.47	8	9	17	28
1.5	22	26	48	80

Table 8. Mortality of second-generation flagfish fry at the end of 30 days exposure to vanadium.

Measured vanadium, mg/L	No. of fry starting exposure	No. of dead fry	% mortality
0	55	2	3.6
0.055	60	21	35
0.18	28	4	14
0.47	60	29	48
1.5	28	17	61

of course, the parents had been exposed to similar levels of vanadium. These fry averaged 25 mg wet weight at the end of the test.

Comparing these lethal levels, the 28-day LC50 for the first run was significantly higher than the same value in the second run (Standard Error of the Difference). However, fish were more than three times larger in the first run, because of larger amounts of food given (average wet weight 45.5 mg compared to 13.6 mg). The second-generation LC50 is not significantly different from either of the first-generation LC50's. The wide confidence limits, because of the variable response, obscured any differences which might exist.

In 70-day and 96-day exposures to vanadium, there was no further mortality among flagfish which survived the initial 28 days. Most fry died at about two weeks, and a threshold was thus attained in the 28-day exposure.

The heavy initial mortality in the highest concentration (1.5 mg/L) meant that no additional thinning was necessary in these tanks. In replicate A, two males and four females survived, exactly the numbers required for spawning, in the experimental design. In replicate B, three males and one female survived. This tank could therefore not provide information for evaluation of spawning. The fish in replicate B were allowed to continue within the standard regime, to measure their growth at 96 days.

4.2.2 Effects on Growth

Vanadium concentrations up to 3.4 mg/L had no detrimental effect on size of fry which survived the 28-day exposure in the first experimental run. In fact, average wet weights were higher in vanadium concentrations than in the control (Table 3). The heaviest fish were produced in the low concentration (0.051 mg/L), and their average weight of 76 mg was significantly heavier than the 55 mg of the control. There were no other significant differences. This analysis was based on pooled replicates, since

separate analysis had shown no differences in weight between any replicate and its companion. Standard deviations of the groups of fry weights were fairly large, from 22 to 35 mg. This probably reflects differences between sexes, which could not be distinguished at that stage.

For the initial 28-day exposure of the second experimental run, again there were no deleterious effects of vanadium on size, up to 0.47 mg/L. The largest fish were those in the two lowest concentrations (Table 9). Fry from 0.041 mg/L were significantly larger than the controls in wet and dry weights and length. Fry from 0.17 mg/L were larger than the controls in wet weight and length. The only other difference, with replicates pooled, was greater dry weight of fry from 0.041 mg/L, compared to those from 0.48 mg/L. There were some differences between pairs of replicates when analyses kept them separate: none for lengths, one out of four for wet weight, and 2 out of 4 for dry weights (Appendix 10.6.4). These differences have not been considered since the overall analysis of pooled replicates was considered more useful.

In the similar exposure of second-generation fry, fish from 0.041 mg/L were again significantly larger than the control, for all three methods of measurement. However, some detrimental effects of vanadium occurred. The three higher concentrations of vanadium (0.17, 0.48, and 1.5 mg/L) were associated with lower dry weights of fry, significantly lower than the control (Table 10). Fry from 0.48 mg/L were smaller than the control in length. There were no other significant differences.

For 70 days of exposure, sexes could be distinguished and must be treated separately because males were larger than females (Table 11). Again there were no deleterious effects of vanadium up to 0.48 mg/L. Among males, there were no significant differences in size, by any method of measurement, between any concentrations. Females from 0.041 mg/L were larger in wet and dry weights, and length, compared to the control. They were also heavier in dry

Table 9. Size of flagfish fry at the end of the first 28 days of exposure in the second experimental run. A and B are replicates, and the underlined values are for pooled replicates.

Concentration of vanadium, mg/L		Average size								
		Wet wt., mg			Dry wt., mg			Length, mm		
Nominal	Measured	A	B		A	B		A	B	
0	0	7	9	8	1.3	1.9	1.6	7.1	7.6	7.3
0.050	0.041	19	13	16	4.7	1.5	3.4	9.8	8.3	9.2
0.158	0.17	11	20	17	1.7	3.2	2.6	8.5	9.4	9.0
0.500	0.48	14	10	12	2.2	1.6	1.9	8.5	8.2	8.4
1.58	1.5	-	-	-*	-	-	-*	-	-	-*

* No fish removed because of high mortality

Table 10. Sizes of flagfish of the second generation, following 30 days exposure as fry, plus the egg stage, to the stated concentrations of vanadium.

Vanadium concentration mg/L	Wet wt., mg	Dry wt., mg	Length, mm
0	24	4.8	11.5
0.041	34	6.5	13.3
0.17	22	3.5	10.6
0.48	22	3.7	9.9
1.5	21	3.1	10.9

Table 11. Sizes of flagfish removed from various concentrations of vanadium after 70 days of exposure. A and B are averages for replicate tanks, underlined values are averages for pooled replicates.

Sex	Vanadium concentration, mg/L	Total no. of fish	Wet weight,mg			Dry weight,mg			Length, mm		
			A	B		A	B		A	B	
Male	0	10	446	343	395	117	86	101	29	26	28
	0.041	10	455	327	416	90	124	100	30	26	29
	0.17	17	332	428	382	86	105	96	26	29	28
	0.48	12	429	308	378	111	74	96	28	26	27
	1.5	0	-	-	-	-	-	-	-	-	-
Female	0	8	328	226	276	84	50	67	26	24	25
	0.041	8	354	351	352	101	106	105	28	27	27
	0.17	1	303	-	303	79	-	79	26	-	26
	0.48	6	301	258	272	80	66	71	26	24	24
	1.5	0	-	-	-	-	-	-	-	-	-

weight than females from 0.48 mg/L. Females from 0.17 mg/L were larger than the controls in wet weight and length. The above analyses on pooled replicates does not consider differences between replicates, which in this case were not important for general conclusions (Appendix 10.6.4).

For 96 days of exposure of the first generation, differences in size were extremely clear-cut (Table 12). Up to 0.48 mg/L, males showed no significant differences in size between any concentrations (2-way analyses of variance). Females from 0.041 mg/L were larger than all other groups up to 0.48 mg/L in wet and dry weights, and longer than the control and 0.48 mg/L. Because of unbalanced number of fish, the highest concentration (1.5 mg/L) was compared with the others by pooled one-way analysis. As previously, these high-concentration fish were not smaller than the control, but females were smaller by all three methods of measurement, than females from 0.041 mg/L. Males from 1.5 mg/L were shorter than those from 0.041 and 0.17 mg/L but weights were not different.

4.2.3 Reproduction

Vanadium did not have a deleterious effect on average daily egg production, up to 1.5 mg/L. In fact, controls had lower production than any vanadium level, during the 25-day period (Table 13). Fish in 0.48 mg/L of vanadium had significantly higher average production than any lower concentration including the control (two-way analyses, replicates considered separately). Similarly, fish in 0.041 mg/L had higher production than the control and 0.17 mg/L. Only one tank of fish could be evaluated for 1.5 mg/L, by analysis based on individual replicates. This showed that average egg production in 1.5 mg/L was not different from that in either control tank (Appendix 10.6.4).

Fish started spawning in all tanks almost immediately after 70 days, when the first spawning activity was observed and thinning to six fish was carried out. Times to observed initial

Table 12. Sizes of adult flagfish of the first generation, at the termination of experiment, following 96 days of exposure to the stated concentration of vanadium. A and B are averages for replicate tanks, and underlined values are overall averages.

Sex	Vanadium concentration mg/L	Total no. of fish	Wet weight, mg			Dry weight, mg			Length, mm		
			A	B		A	B		A	B	
Male	0	4	677	662	670	200	178	189	33	33	33
	0.041	4	779	932	856	218	264	241	35.5	37	36.3
	0.17	4	924	807	865	261	210	235	36.5	34.5	33
	0.48	4	747	558	652	208	157	182	34.5	31.5	33
	1.5	5	768	464	585	220	126	163	33.5	29.7	31.2
Female	0	8	584	505	544	158	136	147	31.5	30.5	31
	0.041	8	732	615	674	206	175	190	33.5	32.5	33
	0.17	8	577	532	555	156	141	149	31.8	31.5	31.6
	0.48	8	551	599	575	149	166	158	30.8	31.3	31.0
	1.5	5	506	477	500	141	131	139	30.5	29	30.2

Table 13. Average daily and total egg production during the 25-day observation period. A and B are replicates, pooled averages are underlined.

Vanadium Concentration mg/L	Egg production average daily			Total		
	A	B		A	B	
0	11.1	4.4	7.7	278	109	194
0.041	17.5	28.7	23.1	438	718	578
0.17	4.1	17.0	10.5	102	425	264
0.48	46.4	24.6	35.5	1161	616	889
1.5	12.2	-	12.2	305	-	305

spawnings in the various tanks are listed in Table 14. It seems possible that some spawning had occurred before 71 days, at times when fish were not being observed. In any case, no significant differences can be shown for time to first spawning.

A more meaningful comparison of spawning-time might be termed "time to steady spawning", defined here as the days of exposure until there were 20 or more eggs produced on four consecutive days. This is entirely arbitrary, but useful as an assessment of regular laying of appreciable numbers of eggs. Days of exposure to this end-point appear in Table 15. There was apparently stimulation at 0.041 mg/L of vanadium, since both replicates completed "steady spawning" in the shortest possible time after counting was begun. Time at this concentration was significantly shorter than in the control (2-way analysis of variance and Duncan's test, 1.5 mg/L excluded and > 95 taken to be 100). There were no other differences. It is seen that 1.5 mg/L of vanadium did not have a deleterious effect on "time to steady spawning", compared to the control.

Hatchability of eggs may be considered an aspect of spawning, since vanadium might have caused the production of sterile eggs. There were no significant differences between concentrations in hatching success, as judged 7 days after collecting the eggs. (Most eggs hatched in 4 days.) The variation shown in Table 16 is apparently random. Replicates were pooled for one-way analysis, in the three cases where they were available, since this was one of the unbalanced sets of data. Only one replicate at 0.17 mg/L produced enough eggs on four consecutive days, to be kept and hatched. The other replicate contained the large aggressive male, which may have resulted in less spawning activity. Again, the highest concentration had only one replicate with the proper number of males and females.

Table 14. Times to observed initial spawning of flagfish in various concentrations of vanadium.

Concentration	Days of exposure
Control	71, 73
0.041	71, 71
0.17	71, 72
0.48	71, 71
1.5	71, -

Table 15. Days of exposure until "steady spawning" occurred, i.e. 20 or more eggs were produced on four consecutive days.

Concentration	Days of exposure
0	> 95, 95
0.041	74, 74
0.17	> 95, 84
0.48	76, 92
1.5	93, -

Table 16. Hatching success of flagfish eggs, 7 days after egg collection, from various concentrations of vanadium.

Concentration	% hatching success	No. of egg-cups assessed
0	29.9	8
0.041	38.9	8
0.17	25.0	4
0.48	35.4	8
1.5	35.4	4

4.2.4 Abnormalities

No obvious abnormalities were noted among the first generation of fish. In the second generation, two abnormal conditions were seen when fry were assessed at 7 days after egg-collection (about 3-4 days after hatching). Some fry were obviously "slow developers", being much smaller in size. A few fry were "deformed", i.e. had obviously abnormal spinal curvature. Table 17 shows that slow developers were prevalent at the two highest concentrations, while deformed larvae were uncommon in all concentrations.

Statistical analysis gave general confirmation of these impressions. Proportion of slow developers was significantly higher in 0.48 mg/L than in the control and at any lower concentration. The proportion of slow developers in 1.5 mg/L, although high, was not significantly different from the proportion in any other concentration, perhaps because there was only one replicate and considerable variation of response. Perhaps the most important findings are that 0.48 mg/L had more slow developers than the control, while the two lower concentrations did not. The distribution of deformed larvae did not show any significant differences between concentrations.

4.2.5 Acute Lethality

In the static tests of acute lethality to immature flagfish, the LC50 was 11.2 mg/L. Confidence limits were 9.7 and 13 mg/L, with a slope of 11.0.

4.3 DISCUSSION

The experiment assessed some 22 items of response, as summarized in Table 18. Clearly some items are more important than others but there is no obvious way to weight for importance of effect.

Table 17. Abnormalities in flagfish fry of the second generation exposed to vanadium, at 5 days after hatching. A and B are replicates, comb. is for combined replicates. Fractional representation is affected fry/total hatched.

Vanadium concentration mg/L	"Slow developers"				Deformed			
	A	B	Comb.	Comb.%	A	B	Comb.	Comb.%
0	1/35	0/23	1/58	1.7	0/32	1/23	1/55	1.8
0.041	0/49	2/42	2/91	2.2	1/49	2/42	3/91	3.3
0.17	*	0/28	0/28	0	*	1/53	1/53	1.9
0.48	11/98	1/12	12/110	10.9	2/65	0/12	2/77	2.6
1.5	3/29	*	3/29	10.3	0/29	*	0/29	0

* No eggs collected for hatching, since spawning did not meet the requirements of the experiment.

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	A	B	Comb.	Comb.%	A	B	Comb.	Comb.%
0	1/35	0/23	1/58	1.7	0/32	1/23	1/55	1.8
0.041	0/49	2/42	2/91	2.2	1/49	2/42	3/91	3.3
0.17	*	0/28	0/28	0	*	1/53	1/53	1.9
0.48	11/98	1/12	12/110	10.9	2/65	0/12	2/77	2.6
1.5	3/29	*	3/29	10.3	0/29	*	0/29	0

* No eggs collected for hatching, since spawning did not meet the requirements of the experiment.

Table 18. Summary of effects of vanadium on flagfish during one-generation exposure.

Response assessed	Significant effect of stated concentration compared to control*					
	0.041	0.17	0.48	1.5	3.4	6.3
Lethal to fry, 28 days. (1st generation)	No	No	No	Yes (to >50%)	Yes (to most)	Yes (to all)
Lethal to older fish, 28-96 days exposure	No	No	No	No	-	-
Lethal to fry of 2nd generation, 1st 30 days	No	No	No	Yes (to >50%)	-	-
Size at 28 days (1st gen.)						
-wet weight	(Yes+)	(Yes+)	No	-	-	-
-dry weight	(Yes+)	No	No	-	-	-
-length	(Yes+)	(Yes+)	No	-	-	-
Size at 70 days	♂ ♀	♂ ♀	♂ ♀			
-wet weight	No (Yes+)	No (Yes+)	No No	-	-	-
-dry weight	No (Yes+)	No No	No No	-	-	-
-length	No (Yes+)	No (Yes+)	No No	-	-	-
Size at 96 days	♂ ♀	♂ ♀	♂ ♀	♂ ♀		
-wet weight	No (Yes+)	No No	No No	No No	-	-
-dry weight	No (Yes+)	No No	No No	No No	-	-
-length	No (Yes+)	No No	No No	No No	-	-
Size at 1 month, 2nd generation						
-wet weight	(Yes+)	No	No	No	-	-
-dry weight	(Yes+)	Yes	Yes	Yes	-	-
-length	(Yes+)	No	Yes	No	-	-
Spawning performance						
-Time to first spawning	No	No	No	No	-	-
-Time to "steady spawning"	(Yes+)	No	No	No	-	-
-Daily number of eggs	(Yes+)	No	(Yes+)	No	-	-
-Hatchability of eggs	No	No	No	No	-	-
Abnormalities						
-First generation	No	No	No	No	-	-
-"Slow" recently-hatched, 2nd gen.	No	No	Yes	No	-	-
-Deformed, recently hatched, 2nd gen.	No	No	No	No	-	-

No = not significantly different in response than control

Yes = significantly deleterious compared to control

(Yes+) = a positive response, significantly "better" than the control

4.3.1 Lethality

Sub-acute lethality to flagfish fry was of over-riding importance in this experiment. If fish survived the first month of exposure, they suffered relatively few significant sublethal effects during the remainder of the life-cycle. This is true, for example, at 1.5 mg/L of vanadium. Thus the early mortality seems to be about the most sensitive clear-cut response.

4.3.2 Cumulation

There is little evidence that vanadium was acting as a cumulative poison. If it were, we might expect the first generation of fish to sicken and die as they accumulated the metal during their lifetime. There was no evidence of this. No further mortality occurred after the first 28-day exposure. The final size and spawning performance of fish in 1.5 mg/L of vanadium gave no evidence that they were becoming more sickly, although that concentration had killed more than half of the fry.

Nor did the second generation provide any strong evidence of cumulative poisoning. The lethal concentration did not prove to be different from that of the first generation. Size of the second generation at one month may or may not indicate cumulative poisoning. Some criteria, notably dry weight, showed deleterious effects on size at 0.17 and 0.48 mg/L, concentrations which had not adversely affected size of the first generation. However there are other possible explanations for this effect. More importantly, second-generation fish started their vanadium exposure immediately upon hatching (as well as during the egg stage), whereas first-generation fish did not start until they were about a week old. Smaller fish are more sensitive, judging by the two lethal tests in the first generation. It is noteworthy that 0.17 mg/L of vanadium had a stimulatory effect on growth in the first generation, for wet weight and length, but no effect for the same parameters in the second generation. This concentration had no stimulatory effect on dry weight of the first generation,

but a deleterious effect on dry weight of the second generation. It looks very much as if there were a balance in the second generation, between the stimulating effects of this level of vanadium, and the deleterious effects of starting exposure earlier. All in all, it is difficult to create a case for cumulative effects of vanadium on flagfish.

This fits with recent work showing that vanadium is excreted fairly readily, at least among mammals. For example, when vanadium was injected into rats, four days later there had been excretion of 46% of it in the urine and a further 9% in the faeces (Hopkins and Tilton 1966).

4.3.3 Positive Effects on Growth and Spawning

Stimulatory effect of low concentrations of vanadium is another major finding. A few cases occurred at 0.17 mg/L of vanadium, but there were very consistent positive effects at 0.041 mg/L. This level was always associated with increased size of females, at any stage of life measured, in both generations, by any of the three parameters used to measure size. There was no effect on size of males. This lowest concentration also resulted in rapid and prolific spawning, much better than in the control tanks.

No firm answer can be given for the reason or mechanism of this stimulatory effect. It would seem unwise to attribute it entirely to the "need" for such concentrations of vanadium, to supply a necessary trace element. For one thing, there was apparently no benefit to males.

Some speculation may be made about the physiological processes behind the stimulatory effects. It is not impossible that flagfish suffered vanadium deficiency under control conditions, which was relieved at the lowest concentration. Vanadium is known to be an essential element for some animals. For example, a deficiency in chicks reduced the blood cholesterol and growth of feathers (Hopkins and Mohr 1971).

Aside from the simple but unlikely reason mentioned

above, there is little explanation of the females-only increase in growth, with improved spawning. Inhibition of spawning could have been explained. For example, increased vanadium levels in mammals inhibit the production of cysteine, co-enzyme A, and cholesterol, precursors in the chain leading to formation of female sex hormones. Vanadium-increased synthesis of cholesterol in rats (Curran and Burch 1967) was the only literature reference which might explain stimulation of female reproductive pathways.

It is possible that the apparent stimulatory effect of low concentrations of vanadium was merely a manifestation of "a little stress improves the performance of animals". Pickering and Gast (1972) found an almost exactly parallel stimulation in a chronic experiment with cadmium. McLeay and Brown (1974) documented such growth stimulation for salmon in low levels of pulp mill waste. The general topic of improved performance of animals under slight stress has been reviewed by Smyth (1967). It seems unwarranted to extrapolate such laboratory stimulation to field conditions, such as waters around the oil sands operations, but at least the stimulatory concentrations cannot be considered harmful.

4.3.4 Threshold of Sublethal Effect

The "safe" level of vanadium for flagfish may be assessed from the overall view in Table 18. Concentrations of 3.4 and 6.3 mg/L had severely lethal effects on young fish, and are obviously unsafe. Vanadium at 1.5 mg/L killed more than half of flagfish fry, and thus is also severely detrimental. Considering the lowest concentration of 0.041 mg/L, there was not a single deleterious effect during the experiment, so this must be considered a "safe" concentration. Thus a threshold for chronic or sublethal responses is clearly somewhere between 0.041 and 1.5 mg/L.

Examining the two concentrations within this range, 0.48 mg/L showed a detrimental effect on size of the second generation. This was found at five days of age, as judged by eye,

and was also found at one month of age. No evidence of growth stimulation was found for this concentration, at any stage of the life cycle. Although the effects of 0.48 mg/L may not be severe, we consider that this concentration does have measurable harmful effects on flagfish, upon long-term exposure. These are not balanced by any "beneficial" effects. Thus the sublethal threshold must be within the narrower range of 0.041 to 0.48 mg/L.

Vanadium at 0.17 mg/L caused one deleterious effect, a decrease in dry weight of second generation fry. This concentration did not cause mortality, and if the fry had been allowed to grow older, they might have obtained benefit from the stimulatory effect noted at 70 days. Nevertheless the decrease in weight at 0.17 mg/L must be considered a deleterious effect. We consider that the sublethal threshold for toxicity of vanadium would approximate the logarithmic average of 0.17 and 0.041 mg/L, which is 0.08 mg/L.

The "safe"-to-lethal ratio should be estimated, since it is useful in predicting sublethal thresholds from lethal tests done under other circumstances. The 96-hour LC50 of 11.2 mg/L is appropriate for use in the calculation. It is based on immature flagfish, and such routine lethal tests are customarily done with such reasonable-sized fish. Thus:

$$\frac{\text{"safe" threshold}}{\text{threshold LC50}} = \frac{0.08}{11.2} = 0.007$$

This is near the usual range of 0.01 to 0.1 for such ratios (Sprague 1971). The value of 0.007 may be used in practical situations, as a multiplier of the LC50 for native fish under local conditions, to predict the "safe" level of vanadium.

One may question the applicability of these sublethal tests with a tropical fish, for protection of Canadian species. We do not feel this is a problem. The bulk of evidence is that most fish species are not greatly different in their response to pollutants, either for lethal levels (Fogels and Sprague 1977) or for sublethal levels (McKim 1977).

5. GENERAL DISCUSSION

5.1 LETHAL RESPONSES

The overall picture for vanadium is a remarkably consistent one. Trout had 7-day LC50's from 2.4 to 5.6 mg/L, with fairly small differences resulting from type of testwater. An 11-day LC50 of 2 mg/L was found for one kind of water, and there was a suggestion that a threshold of lethality would be approached at somewhat lower concentration and longer time. All these findings group together on a toxicity curve (Figure 4) along with those observed at shorter times. Results obtained in another laboratory (Anderson and Spear in preparation) are similar (Figure 4).

Longer exposures with flagfish fit the same pattern fairly well (Figure 4). Week-old fry showed 28-day LC50's of 1.1 and 1.9 mg/L, depending on size. This was apparently a threshold since general observations in the tanks indicated little mortality after about two weeks. Larger flagfish, survivors of the fry mortality, suffered no mortality in a further 68 days of exposure to 1.5 mg/L of vanadium. Considering the 4-day LC50 of 11.2 for larger flagfish, this species is only slightly more resistant than trout.

Zebrafish tested at 22 mg/L in a variety of waters were somewhat less resistant than trout, judging from an extrapolation of the trout toxicity curve. Fathead minnows were similar in resistance to our trout, or more resistant by a factor of about five, depending on type of water. However these are not great differences, considering usual variation in such bioassays, up to five-fold within laboratories and approaching ten-fold between laboratories (Fogels and Sprague 1977). Results for zebrafish and fathead minnows may be less precise since they are based on nominal concentrations of vanadium.

The similarities of this general comparison between species should not be over-emphasized in view of the many differences such as size and life-stage of fish, water quality,

and measured versus nominal concentrations. Nevertheless, from the overall pattern one might generalize that the lethal threshold of vanadium for fish could be in the vicinity of 1 or 2 mg/L, and this threshold might perhaps occur in 2 or 3 weeks.

The absence of a lethal threshold in short exposures is an unusual and important feature of vanadium toxicity. It has been mentioned that most toxicants show such a threshold within four days.

5.2 CUMULATIVE TOXICITY

The lack of a rapid acute threshold might lead one to suspect that there was a continuing accumulation of vanadium in fish tissues, with consequent slowly-increasing toxicity to the fish. This may or may not be true in short exposures of a few weeks. Some other toxicants, such as chlorine, show a similar absence of acute threshold without being cumulative in the tissues.

In longer exposures with flagfish, there was no evidence of long-term cumulative poisoning (section 4.3.2). This fits with findings in the literature that vanadium is readily excreted, at least in mammals. It would appear that cumulative poisoning of fish by vanadium should not be regarded as a problem.

5.3 CHRONIC THRESHOLD OF TOXICITY

The major factor in long-term toxicity to flagfish was mortality of very young fry that had been exposed as eggs and from hatching onward. This resulted in death of half the fry at 0.9 mg/L of vanadium. Fish surviving a higher concentration of 1.5 mg/L showed few effects on growth and none on reproduction. Such a finding is in line with recent general summarization that the egg-fry stage is the most sensitive in life-time exposures to many toxicants (McKim 1977).

It seems safe to assume that our estimate of about 0.08 mg/L as the threshold of long-term toxicity of vanadium, is

a valid one for flagfish. From the general correspondence between lethal responses of different species of fish (section 5.1), this chronic threshold may approximate the "safe" level for many species of native Canadian fish. Such "safe" levels for native fish would best be estimated by using the application factor of 0.007 on a 4-day LC50 determined for the species of concern.

6. CONCLUSIONS

1. The purpose of this research was to establish thresholds for acute and chronic toxicity of vanadium to fish. This would allow AOSERP to determine whether or not there was a pollution problem with this metal.

2. Vanadium in the pentavalent state is likely to be the main form reaching natural waters as fallout from stack emissions.

3. Rainbow trout were killed in seven days by 2.4 to 5.6 mg/L of vanadium. The range resulted from tests in 12 different types of water.

4. Mortality of trout had not ceased by 11 days of exposure, i.e. no threshold of acute lethality was found. A threshold in the vicinity of 1-2 mg/L was considered likely in an exposure of several weeks.

5. For combinations of water characteristics from hardness 30 to 360 units, and pH 5.5 to 8.8, there was a slightly saddle-shaped response surface for vanadium lethality to trout. However, the water quality did not have a major effect on vanadium toxicity compared to that for other metals.

6. The two ionic "species" of orthovanadate present in the trout tests, seemed to be about equally toxic.

7. The 7-day median lethal concentration for rainbow trout can be estimated for any usual type of freshwater, by an equation which explains 91% of the variation:

$$LC50 = 14.6976 - 3.7783P + 0.1108H - 0.02137PH + 0.2662P^2 - 0.000073H^2 + 0.00141P^2H$$

8. The 4-day LC50 for immature but nearly full-grown flagfish was 11.2 mg/L of vanadium

9. In continuous exposure over one reproductive cycle of American flagfish, mortality and growth in the egg-fry stage was the most sensitive response to vanadium toxicity.

10. The 28-day LC50 for fry of flagfish, one week old

at the start of the test, was 1.1 to 1.9 mg/L of vanadium, depending on size of the fry as governed by feeding rate. For newly-hatched fry from the second generation, also exposed as eggs, the LC50 was 0.89 mg/L, not significantly lower than the values for week-old fry. These seemed to be thresholds of lethality, since mortality had ceased before the end of the exposure.

11. Vanadium at 0.48 mg/L had marginal effects on growth of fry, but did not cause mortality, or sublethal effects in older fish.

12. Vanadium at 0.041 mg/L had no deleterious effects on flagfish, as assessed by 22 measurements of growth, reproduction, abonormality, and mortality during the life-cycle exposure. This level had a stimulatory effect on growth of females, measurable at all stages of life, and was associated with improved spawning performance. This experimental finding should not be construed to mean that such a level of vanadium would be beneficial to fish, if added to natural waters.

13. The chronically "safe" concentration of vanadium for flagfish was between the two above-mentioned levels, and was judged to be in the vicinity of 0.08 mg/L.

14. The "safe"-to-lethal ratio for flagfish was about 0.007, near the usual range for such ratios. This "application factor" can be used as a multiplier of the lethal levels of vanadium for native fish, to predict "safe" levels for them. The "safe" levels for trout would be predicted as 0.017 to 0.039 mg/L depending on water quality. These are similar to the actual "safe" level for flagfish.

15. Trout and flagfish, and to a lesser extent zebrafish and fathead minnows, had generally similar lethal levels of vanadium. It appears that the metal's toxicity is much the same for different species of fish.

16. There was no evidence in the flagfish experiment, that vanadium was acting as a cumulative poison. There should be

minimal concern about this as a potential problem in the region of the Athbasca Oil Sands. There need be little expenditure of effort on surveys of vanadium residues in aquatic organisms, unless exploratory surveys detect anomalies in such residue levels.

17. Vanadium in liquid effluents from oil sands operations would not seem to create a toxicity problem in the Athabasca River, according to available figures. There should be a safety factor of about 10 for a single such operation.

18. Fallout of vanadium emitted from stacks could conceivably cause a build-up in any slow-turnover lakes of the region. The likelihood of attaining deleterious concentrations could be assessed.

7. IMPLICATIONS AND RECOMMENDATIONS

This study was originally undertaken because almost nothing was known about the toxicity of vanadium to aquatic organisms. There was some concern among public and professional groups, that vanadium released from oil sands operations would prove to be dangerous, perhaps "another mercury". Our ignorance on vanadium toxicity is somewhat relieved by the two studies in this report. They give a fairly complete initial assessment of such toxicity to fish. One study shows lethal levels of vanadium in any type of fresh water likely to be of interest, and such information is a good starting-point for assessing potential danger. The other study reports a "safe" level for lifetime exposure of a fish, including reproduction and early viability of the second generation.

The authors have been mildly surprised by the report. Before starting, we thought that vanadium might prove to be relatively non-toxic. The results of lethal tests place it as a metal of moderate aquatic toxicity. However, this lethal action does not vary greatly with type of water; in particular, vanadium is not much more toxic in soft and/or acid water, as are some metals. There was also some concern that vanadium might be a cumulative poison upon long exposure. The chronic test showed this was not so. In fact, there were very few side-effects, beyond the major direct effect on survival and growth of newly-hatched fish.

What the experiments show is that vanadium is a straightforward non-cumulative pollutant, of moderate toxicity among the metals, whose probable effects can be evaluated using normal procedures.

The next step is to evaluate present concentrations of vanadium, and potential future ones, in waters that might be affected by vanadium from oil sands operations. We particularly recommend predictive modelling of likely levels of vanadium in slow-turnover lakes, which might be affected by fallout originating

in refinery stacks. Comparison of such predicted levels with our estimate of "safe" levels will show whether there are likely to be problems. We suggest that baseline studies of vanadium residues in fish should not be over-done. Initial surveys on a small scale should already have indicated whether there are anomalies.

It might be argued that our laboratory studies using "clean" water may not represent vanadium toxicity in surface waters which contain organic matter and other things originating from runoff. We do not feel this is a problem. The dissolved, ionic vanadium that we tested is likely to be the most toxic form. With most metals, presence of humic acids and similar binding agents will reduce toxicity of the metal (Geckler et al. 1976). Therefore any assessment of problems, based on our results, will tend to err on the conservative or safe side, if anything.

8. NEED FOR FURTHER STUDY

As mentioned in section 3.3, toxicity from vanadium seems unlikely in flowing water near oil sands operations. However there could be a build-up in lakes that had a slow turnover of water. Probably there should be some preliminary tabulation of sources of vanadium in the operations, with predictive modelling of its fate in nature. This should include likely concentrations to be caused in surrounding waters. This could be largely a "desk" study based on current knowledge, but should be carried out by a competent chemist or a broader team. Such assessment combined with our results should indicate whether there is likely to be a toxicity problem. If probable levels are far below the "safe" level, future monitoring operations on vanadium could drop to a low degree of effort.

We do not feel that additional work on toxicity of vanadium is warranted at this time, since it had neither violent nor cumulative toxic effects. The present report gives an adequate first estimate of toxicity. One other area of concern is already being covered by an AOSERP contract--the possibility that vanadium may have unusual or dangerous interactions with other pollutants.

We understand that initial programs have included the measurement of vanadium residues in fish from the AOSERP study area. If those initial surveys did not show anomalous high residues, this topic could be down-graded in importance, since vanadium does not seem likely to be a toxicant that accumulates upwards in food-chains. Chemical analyses in such a program can be expensive, especially if field collectors routinely send in large samples for analysis without a clear-cut objective in mind.

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10. APPENDICES

10.1 ASSESSMENT OF RELATIVE TOXICITY OF VANADIUM SPECIES ABOVE AND BELOW pH 8.2-8.4, AS A CHECK OF METHOD TO BE FOLLOWED IN A CHRONIC TEST

Before the chronic test with flagfish was undertaken, some preliminary lethal tests were carried out. The purpose was to find out whether small fluctuations in pH would affect results greatly. Using Guelph well-water, pH in the chronic experiment was expected to be in the vicinity of 8.0-8.2. (In fact, average pH of the chronic test was 8.15.) This seemed to be just below the pH of 8.2-8.4, at which we would expect vanadium to change between two orthovanadate species (from $H_2VO_4^-$ to HVO_4^{-2} as pH rises).

Each test was done with 10 zebrafish (*Brachydanio rerio*) at a nominal concentration of 22 mg/L vanadium, in water hardness 350 mg/L and temperature 25°C, the same as in the chronic experiment. The fish were about 4 months old and weighed about 0.29 gm. Static tests were done at controlled levels of pH, to determine the lethal times of the adult zebrafish at a fixed concentration of vanadium. The form of vanadium was stabilized for at least a week before the tests, at each of the pH values. It was assumed that if the lethal tests showed no great effect of pH, then there would be no major effect in chronic exposures.

Two tests were run at pH 7.5, and one each at pH 8.2, 8.8 and 9.0. There were two sets of tests at different times. These pH values would guarantee the presence of only one species of orthovanadate at the high and low ranges. The middle value of pH was about that of the chronic test, allowing comparison with high- and low-pH tests. Table 19 gives median survival-times in hours (and fiducial limits).

Resistance-times were the same for pH 8.2, 8.8 and 9.0 (tested by standard error of the difference). This relieved one area of concern; any slight elevations of pH from that of the chronic test, through the "boundary" of orthovanadate species, would apparently not affect toxicity.

Table 19. Results of lethal tests with vanadium, using zebrafish, at various pH levels. There were two series of simultaneous tests, A and B. Slope is for the probit line relating mortality to time.

pH	Series	Median Survival Time (hours)	Fiducial Limits	Slope
7.5	A	23.5	21-26	6.5
	B	45	38-52	5.1
8.2	B	32	26-37	4.6
8.8	B	39	29-55	2.6
9.0	A	37	25-51	2.1

The two tests at pH 7.5 were somewhat anomalous. Survival-time in Test A was shorter than in all the others, including the other pH 7.5. Test B showed a longer survival-time compared to pH 8.2 and 9.0, but not compared to 8.8. The variation between the two tests at pH 7.5 was greater than their differences from higher-pH tests. The differences of the two tests at pH 7.5 tend to balance out, overall, any differences between 7.5 and the higher pH values. The average survival time at pH 7.5 was 34 hours, about the same as those at higher pH.

Slopes of the probit lines relating mortality of zebrafish to time, appeared to change with pH. At high pH there was a low slope, and at lower pH there was high slope (Table 19). The slope at pH 8.2 was more similar to those at pH 7.5. If this is related to the metal toxicity per se, it would appear that pH 8.2 and 7.5 go together, and that at pH 8.2 we are dealing with the $H_2VO_4^-$ species of orthovanadate, as predicted from theory. This is reassuring, since pH 8.2 showed the same survival-time as occurred at high pH with the other species of orthovanadate.

Results were somewhat contradictory because of the divergent survival-times at pH 7.5. However, toxicity differences were actually rather small, despite statistical significance of some. All median survival-times fell within the relatively narrow range 23.5 - 45 hours. It was concluded that there was no large difference in acute lethality at divergent levels of pH, hence no reason to suspect a difference in sublethal toxicity, and that the chronic tests should therefore go ahead at the natural pH of well-water. Probably the vanadium would be in the form $H_2VO_4^-$. There did not seem to be sufficient evidence of differential toxicity at the expected pH range, to warrant the delay and expense of installing pH control to a higher or lower value than the natural one.

10.2 PROBABLE VALENCE AND IONIC SPECIES OF VANADIUM IN TEST-WATER AND NATURAL WATERS

Vanadium has four valence states, but it appears that only V^{+5} is stable to any extent under usual water conditions. Other valence-states are likely to change to V^{+5}, and fairly rapidly. Figure 1 shows the expected distribution of V^{+5} forms of vanadium in normal water. Some of the detailed evidence is presented in the following paragraphs.

One authority (P.H. Rieger, Dep. of Chemistry, Brown University, Providence, R.I. Telephone communication, 26 January 1977) said that the "solubility of V^{+4} is very slight at neutral pH". At pH 12 to 14 it is soluble only to about 2 millimoles, and below pH 3, to similarly small amounts. Above pH 3, it is oxidized in a matter of hours. He further suggested that even if a salt of V^{+4} went into the water, it would remain there as a solid, until oxidized.

According to Chau and Chan (1970), V^{+5} (vanadate) is thermodynamically the most probable valence in natural aerobic waters. Vanadyl (V^{+4}) has been detected at about 1% of total vanadium. V^{+3} exists in extremely low concentrations in natural waters, except for unusual ones with very high acidity.

Similarly, Szalay and Szilagyi (1967) consider vanadates the most stable mobile forms in natural waters. Quadrivalent vanadium cannot be a significant mobile form since it is readily hydrolyzed above pH 3. Trivalent ions could exist only in strongly acidic or reducing environments. The V^{+2} cation is very unstable. Only tetravalent and pentavalent forms are likely to be soluble (Kunz et al. 1976). Even if solutions of vanadyl sulphate are made up, raising the pH to 8 will create the hypovanadic ion ($V_4O_9^{-2}$) which is rapidly oxidized toward the pentavalent state (R.G. Kunz, Air Products and Chemicals, Inc., Allentown, Pennsylvania, Telephone communication, 24 January 1977). In toxicity studies with wastewater Kunz used sodium metavanadate because any tetravalent salt solution would soon be oxidized to the pentavalent state. Thus oxidation proceeds rapidly at $15^{o}C$ in alkaline solutions

(0.006 - 3.8 N NaOH; Dean and Herringshaw 1963). VO^{+2} also oxidized rapidly in strong and moderately strong solutions, unless pH was below 2.45 (loc. cit.). Solutions of vanadyl salts oxidize in the air, if pH is above 2 (Charlot and Bezier 1957). Gustavson and Knudson (1922) found it impossible to get reproducible results with solutions of reduced vanadium, unless they were kept under carbon dioxide to prevent oxidation.

The aqueous equilibrium diagram of Evans and Garrels (1958) shows that V^{+5} predominates in the pH range 5-9 and natural oxidation levels. In seven surface waters of California that were well-oxygenated, the oxidation potentials were in the range for existence of V^{+5}, except for one (E_h 4.2; Breck 1971). R. M. Garrels (Geological Sciences, Northwestern University, Evanstone, Illinois, telephone communication, 25 January 1977) believes that oxidation of lower mineral oxides of vanadium to valence 5 is inevitable. He believes this is likely to occur rapidly in solution although it may take months or years for crystalline minerals of vanadium.

10.2.1 Ionic Species of Vanadium in Natural Waters

This situation is apparently not very complex at low concentrations of vanadium. Many papers on this topic deal with relatively high concentrations of vanadium, greater than 10^{-3} M or 50 mg/L. At these concentrations there are different equilibria, and more forms such as complex polyanions, than at the concentrations of 0.05-15 mg/L that we used. Figure 1 shows the forms of vanadium that are stable in water. The added horizontal lines indicate the concentration ranges of probable interest, in the lower portion of the diagram.

From Pope and Dale's review (1968), it would seem that there were only three groups of ions in our stock- and test-solutions, at our pH range of 5.5-8.8 which would cover most surface waters:

1. the orthovanadates $H_2VO_4^-$ and HVO_4^{-2};
2. the metavanadates $V_3O_9^{-3}$ and perhaps $V_4O_{12}^{-4}$; and
3. the decavanadates $HV_{10}O_{28}^{-3}$

These formulae are the simplest ones and do not necessarily indicate the degree of hydration (Cotton and Wilkinson 1972). For example HVO_4^{-2} [$=VO_3(OH)^{-2}$] might be $VO_2(OH)_3^{-2}$, etc.

At concentrations below about 10^{-4} gram-atoms per litre (5.4 mg/L), probably there were only relatively simple colourless orthovanadates. The ions do not, according to mass action laws, encounter each other frequently enough to form complicated orange-coloured polymers such as might be found in high concentrations below pH 6.5 (Ingri and Brito 1959; Pope and Dale 1968; Rieger 1973).

At these low concentrations of interest at the lethal levels and in the chronic test, the two orthovanadate ions would change rapidly from one to another depending on the pH. However, the borderline pH is not fixed. The pK value may vary from 7.88 to 8.45 depending on the type of water (Pope and Dale 1968; Rieger 1973). As ionic strength of the water increases, HVO_4^{-2} is stabilized and the pK value falls. In our water and almost all surface waters, ionic strength is low, about 0.01 M. Thus the higher pK values apply, and a change between the two orthovanadates might be anticipated in the region of pH 8.2-8.4. Hence the possible change between ionic species when running chronic tests at the natural pH of 8.0-8.2.

In stock-solutions, it is possible that the added vanadium pentoxide formed colourless metavanadate ions. However, when the stock-solution was diluted into the test-tanks, they would have converted back to orthovanadates in milliseconds (P.H. Rieger, Dept. of Chemistry, Brown University,Providence, R.I., telephone communication, 21 January 1977). Dr. Rieger does not believe that any metavanadate would exist at our test-concentrations.

As an aside, there has been a dispute for many years whether the polymerized metavanadates are $V_3O_9^{-3}$ or $V_4O_{12}^{-4}$ (Dullberg 1903). It now appears that they co-exist, but the former predominates by an order of magnitude at low concentrations (Brito et al. 1964; Copely et al. 1965; Rieger 1973).

Above 5.4 mg/L, $H_2VO_4^-$ polymerizes, and in acidic solution, will co-exist with decavanadate. We may have had some formation of decavanadate in stock solutions at pH 5.5. The change from ortho to deca form is slow, over a few days. We allowed at least 72 hours of continuous stirring in the vats of stock-solution, to allow for this equilibrium. The 72-hour period would be more than adequate at higher pH values. Whittaker et al. (1966) similarly allowed several days for such an equilibrium.

Upon dilution of the stock solution in low-pH bioassays, decavanadate would have tended to change back to orthovanadate. We are uncertain whether this is a fast or slow reaction. Corigliano and DePasquale (1975) studied decomposition of millimolar concentrations of decavanadates. After 24 hours, they observed orthovanadates only as intermediate products, but this may have been because of the low ratio of OH^- to V (5.6 vs. 7.0). Since decavanadate is yellow-orange, its proportion can be monitored by spectroscopy, at key concentrations.

10.3 PROBABLE FORM OF VANADIUM ESCAPING FROM OIL SANDS OPERATIONS

Pentavalent vanadium seems to be the most likely form to be of concern from oil sands operations. Ash from the stack of such an operation may contain 1.5 to 4.0% vanadium, measured as ignited dry weight (W.L. Cary, Co-ordinator--Environment Conservation, telephone communication, 23 June 1976). The potential problem of greatest concern may be fallout of such vanadium into waters of the area. There could be a build-up of concentration in lakes over the years, especially those having low turnover of water.

Vanadium occurs naturally associated with the bitumen of the Athabasca Oil Sands at concentrations from 150-290 ppm, probably as oxovanadium (IV) porphyrins (Cotton and Wilkinson 1972). During oil sands processing, vanadium escaping from the stack could have its final source in the "CO boiler" used for monoxide combustion. Since this is an aerobic process taking place at high temperature, it is likely that vanadium emerging from this source would be oxidized to the valence state 5, as pentoxide (Tullar and Suffet 1975). According to Tullar and Suffet, the lower oxides of vanadium rapidly oxidize to V_2O_5 when heated in excess air, although this process is slow at room temperature.

Considering the appreciable SO_2 emissions in the area, the following reactions are likely (D.B. Carlisle, Advisor, Water Quality Research, Environment Canada, Ottawa, telephone communication, 28 June 1976).

1. the V_2O_5 acts as a catalyst for conversion of SO_2 to SO_3.
2. $H_2O + SO_3$ form H_2SO_4, which dissolves V_2O_5 off the clay particles on which it has been adsorbed.
3. excess SO_2 then forms vanadyl sulphate.

If reducing conditions prevailed after the boiler, the end product would likely remain the same.

1. In the presence of SO_2, there would be production of $VO_2 + SO_3$.
2. $VO_2 + H_2O + SO_2$ would form vanadyl sulphate.

The vanadium, however, would not necessarily remain as vanadyl sulphate in the stack gases, if they were still hot and exposed to the atmosphere. Flood and Kleppa (1947) state that $VOSO_4$, when heated in air to 530°C, normally decomposes into V_2O_5, SO_2 and SO_3 according to the following equation:

$$2\ VOSO_4 = V_2O_5 + SO_2 + SO_3$$

When the partial pressure of SO_2 exceeds a certain limit the decomposition would proceed as follows:

$$2\ VOSO_4 = V_2O_4 + 2\ SO_3$$

In both cases, oxides of vanadium are formed. It is probable then, that any vanadium would fall out as V_2O_5.

Since V_2O_5 is soluble in water to at least 392 mgV/L 25°C (Meyer and Aulich 1930) and probably to higher concentrations according to other sources, air-borne vanadium would likely dissolve and ionize in raindrops or fall out directly into natural waters. As discussed above, the metal would stay in the stable pentavalent state, mostly as orthovanadates, in the range of conditions expected in natural waters.

If vanadyl sulphate failed to reach its decomposition temperature in the plume and entered the atmosphere, upon reaching natural waters it would dissolve (very soluble [Weast 1976]) then ionize in water and oxidize to valence 5. The vanadyl cation (VO^{+2}) is not a significant mobile form of vanadium at pH greater than 3 (Szalay and Szilagyi 1967).

The presence of particulate H_2SO_4 aerosols would increase the rate of deposition of vanadium from air (Tullar and Suffet 1975). The use of electrostatic precipitators for removal of particulates may, by the catalytic action of the corona discharge, increase the conversion of SO_2 to SO_3 and thus also decrease the vanadium residence time in the air (Matteson et al. 1972). Amounts coming from the stack would vary with the particular plant and the use of electrostatic precipitators. One estimate for a large plant would be about 2.8 kg of vanadium per day (Martin Bik, modified from paper at AOSERP technical seminar, 14 November 1975).

Liquid effluents from oil sands operations may contain up to 0.3 to 0.4 mg/L of vanadium (W.L. Cary, Co-ordinator-Environment Conservation, Great Canadian Oil Sands, Ltd., telephone communication, 23 June 1976). Liquid discharge may be of the order of 36 million litres per day. This leads to a maximum estimate of 13 kg of vanadium discharged per day, which upon entering natural waters would likely also persist in the V^{+5} valence (Szalay and Szilagyi 1967) as $H_3H_2O_7^-$ and $H_2VO_4^-$. It may also exist with porphyrins or other bound forms. Apparently

there is little knowledge at present about the form in liquid effluents. Release of vanadium in liquid effluents may in the future be reduced by recycling of tailing water in plants. On a concentration basis, it is doubtful that vanadium in liquid wastes would pose a problem. A large plant would probably not use more than 1% of the minimum monthly flow of the Athabasca River (Shewchuk 1975). Even assuming dilution of effluent at that rate, river concentrations from a single plant would be of the order of 0.004 mg/L which is not high. Indeed it is similar to natural levels or below them (NAS/NAE 1974).

10.4 DETAILS OF METHODS FOR ACUTE LETHALITY TO TROUT

10.4.1 Statistical Analysis

After considerable discussion with statisticians, we abandoned the rotatable designs they suggested. Although these have the advantage of a constant error factor within the design, they neglect the extreme "corners" of our combinations (e.g. hardness 30, pH 5.5) and predict LC50's for some combinations within the design instead of actually measuring them. Such a design may be satisfactory for fairly regular responses, but the possibility of irregular peaks and valleys of response to vanadium led us to test all twelve combinations of hardness/pH. The response was described by relatively routine but satisfactory methods of multiple regression analysis. Statistically this is less powerful, but practically there is less likelihood of making an incorrect prediction for any combination.

10.4.2 Apparatus

Figure 6 shows the general experimental set-up. The hardness of the dilution-water was regulated by proportional mixing of well-water and deionized water, preheated to slightly above $15^{o}C$ in aerated head-tanks. The stream of water then passed to another head-tank above the bioassay apparatus, again aerated

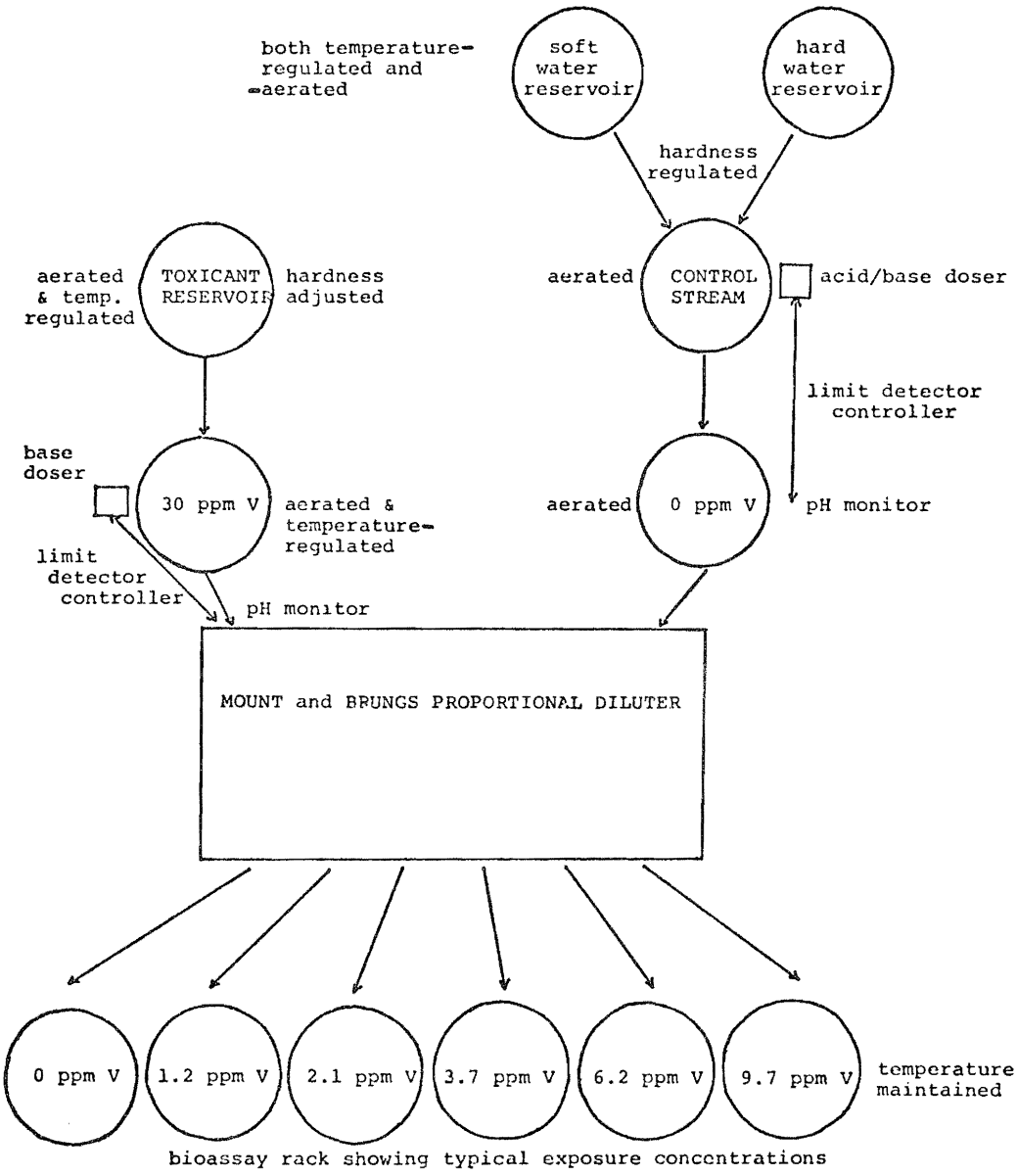

Figure 6. Schematic flowchart of the bioassay assembly.

and from which a continuous flow passed to the diluter. In the second head-tank, the pH of dilution water was regulated by dosing of acid or base through a solenoid, controlled by a pH meter and limit detector.

The vanadium solution was contained in a 330-L, uncoloured polyethylene reservoir, controlled to 15 ± 0.2°C. It had been manually controlled to 0.1-0.2 pH units below the desired level for the previous five days. It was pumped to a head tank above the diluter, where an automatic controller raised pH the slight amount to the final level desired. This dosing with base had the advantage of polishing the pH without incurring change in vanadium species. Dosing with acid would have resulted in formation of coloured ions which require several days for equilibration.

The two streams of water mixed and flowed by gravity to the bioassay tanks which were immersed in a temperature-control bath. Tanks were of dark green polyethylene, 12 cm deep and 33 cm in diameter. Black plastic curtains surrounded the tanks, to avoid visual disturbance of the fish.

10.4.3 Chemical Procedures with Vanadium

The method of preparing stock-solutions of vanadium was developed by measuring how long it took to dissolve, and by following convenient colour changes between ionic species after certain changes in pH. Measurements showed that vanadium dissolved in well-water within one day, so the 2-3 days allowed was ample. The slowest change between ionic species is thought to apply to low pH. Metavanadates may be present at the concentration of the stock-solution, and upon acidification, take some time to change to decavanadates. Any decavanadate in stock-solution would quickly change to orthovanadate ahead of bioassays, visible as a colour change from orange to clear.

Vanadium was relatively stable in sample-bottles. Tests showed no loss during two weeks, regardless of whether or not there had been acidification. Samples were therefore acidified, stockpiled,

and analyzed in batches. Chau and Chan (1970) also found no appreciable loss in vanadium in filtered lake water samples when stored in polyethylene containers at the natural pH of about 8, and also at pH 2.

Vanadium measurements by atomic absorption were found to be satisfactorily accurate. Standards gave a slightly curvilinear relationship which was allowed for. No refractory problems developed with flameless determination of low concentration although this could have been a problem (Barringer Research Associates, Toronto). Only one minor quirk was found. There was a slight "signal enhancement" in water of high hardness, when measuring high vanadium concentrations. Thus standard solutions were always made up in water having the same hardness as the samples.

Valence state of vanadium was checked during the experiment. Vanadate was reduced to vanadyl by adding acid, followed by an indicator and titration as described below. Batches showed an average of 94% of vanadium to be present as V^{5+}, with the minimum being 89%. The lower values probably represent error in measurement caused by improper standardization of titrant.

1. 100 ml sample of vanadium solution;
2. Add 50 ml of 9N sulphuric acid;
3. Add 15 ml of concentrated H_3PO_4;
4. Add 5 drops of 0.5% aqueous barium diphenylamine sulphonate indicator; and
5. Titrate with 0.1N ferrous ammonium sulphate in 1N sulphuric acid, until violet colour disappears.

Within valence state 5, no completely satisfactory method of assessing ionic species was found. Pulse polarography is one recommended method, but off-campus analyses would have involved considerable expensive start-up time (personal communications, Y.K. Chau and Barringer Associates, Toronto). Raman spectrophotometry did not prove satisfactory. A series of 13 samples was analyzed at University of Waterloo. Only the highest concentration of 1000 mg/L could be measured, and this is far above the levels

of interest. Ultra-violet spectroscopy would probably be of use mainly for coloured species, not the two orthovanadates which are of most interest in our bioassays.

10.4.4 Physico-chemical conditions during acclimation of rainbow trout

The values represent averages of conditions preceding 19 experiments on toxicity, and extreme ranges during any period.

Condition	Average	Range
Days of acclimation	51	14 - 140
95% molecular replacement of water in tank, hours	6.8	4 - 9
Temperature, Celsius	15.0	14.8 - 15.2
Hydrogen-ion concentration, pH		
Experiments at hardness 30	7.32	6.94 - 7.52
Experiments at hardness 100	7.71	7.40 - 7.95
Experiments at hardness 350	8.06	7.76 - 8.44
Total hardness, mg/L $CaCO_3$		
Experiments at hardness 30	28.5	26 - 33
Experiments at hardness 100	99.0	90 -110
Experiments at hardness 350	370	352 -388

10.4.5 Ancillary Physico-chemical Conditions in Test-Water* (Values in mg/L unless otherwise stated)

	Deionized water	Well water
Specific conductance microsiemens	172	550
Ca	0.4	64.35
Mg	0.003	50.19
Na	23.0	46.0
K	3.91	7.82
Zn	0.003	0.008
Cu	0.0012	0.001
Fe	0.0008	0.011
Cd	0.0003	0.00008
Cr	not det.	-
Ni	0.0015	0.0002
Pb	0.0029	0.0016

* courtesy of Ms. E. Thomas, Dept. of Zoology, and Mr. D. Tel, Dept. of Land Resource Science, University of Guelph. February 1977.

10.4.6 Ancillary Chemical Measurements in Bioassays with Rainbow Trout

Total alkalinity, as mg/L of $CaCO_3$, was calculated according to the formula developed for our laboratory water (Howarth and Sprague, in press). Some measurements were checked in the present experiment, and agreed reasonably.

Test	Alkalinity	
	Calculated	Measured
30/5.5	0.4	0.4
30/6.6	6.0	-
30/7.7	22	-
30/8.8	45	-
100/5.5	0.6	-
100/6.6	8.8	4.5
100/7.7	38	35
100/8.8	99	95
350/5.5	1.2	-
350/6.6	17	-
350/7.7	88	-
350/8.8	280	-

Total acidity was zero at pH 8.8, and ranged upwards to values, at pH 5.5, of 2.7 mg/L in water of hardness 30 units, 4.0 mg/L in hardness 100, and 8.0 mg/L in hardness 350.

10.5 Tests of statistically significant differences between 7-day LC50's of vanadium for rainbow trout.

Hardness		30	31	30	29	101	105	103	100	101	97**	98	100	357	368	355	335	242
	pH	5.5	6.6	7.7	8.8	5.5	6.6	7.7	7.7	7.7	7.7	7.7	8.8	5.5	6.6	7.7	8.8	8.8
30	5.5		·	·	·	·	·	·	·	·	–	·	·	·	·	·	·	·
31	6.6	NS		·	·	·	·	·	·	·	–	·	·	·	·	·	·	·
30	7.7	NS	NS		·	·	·	·	·	·	–	·	·	·	·	·	·	·
29	8.8	NS	NS	NS		·	·	·	·	·	–	·	·	·	·	·	·	·
101	5.5	NS	*	*	*		·	·	·	·	–	·	·	·	·	·	·	·
105	6.6	NS	NS	NS	NS	NS		·	·	·	–	·	·	·	·	·	·	·
103	7.7	NS	NS	NS	NS	NS	NS		·	·	–	·	·	·	·	·	·	·
100	7.7	NS	NS	NS	NS	NS	NS	NS		·	–	·	·	·	·	·	·	·
101	7.7	NS	NS	*	*	NS	NS	NS	NS		–	·	·	·	·	·	·	·
97	7.7**	–	–	–	–	–	–	–	–	–		–	·	·	·	·	·	·
98	7.7	NS	NS	NS	NS	*	NS	NS	NS	NS	–		·	·	·	·	·	·
100	8.8	*	*	NS	*	NS	NS	*	*	*	–	*		·	·	·	·	·
357	5.5	*	*	*	*	NS	NS	*	*	NS	–	*	NS		·	·	·	·
368	6.6	NS	NS	NS	NS	NS	NS	NS	NS	NS	–	NS	*	*		·	·	·
355	7.7	NS	NS	NS	NS	*	NS	NS	NS	*	–	NS	*	*	NS		·	·
335	8.8	NS	*	*	*	NS	NS	NS	NS	NS	–	*	NS	NS	NS	*		·
242	8.8	*	*	*	*	NS	NS	*	*	NS	–	*	NS	NS	*	*	NS	

* = significant difference.

NS = not significant.

** = Test rejected because of poor water quality during cleaning of pipes.

10.6 DETAILED METHODOLOGY IN THE CHRONIC TEST WITH FLAGFISH

10.6.1 Biological Procedures

To start the experiment, eggs were obtained from adult flagfish held for 4 weeks at 25°C in continuously-flowing well water. Eggs came from four tanks, each containing two males and four females. Eggs were pooled and hatched and 300 larvae, 7-8 days old were obtained. Thirty of these were randomly assigned to each of the ten test tanks. They were fed five times daily throughout the experiment, one of four diets, depending on their age.

Age, days	Feedings	Diet
2 - 12	3	Live nauplii of brine shrimp
	2	"Liquidfry #1 for egg layers"
12 - 32	2	Live nauplii
	3	"Baby tropical fish food." Jungle brand
32 - 46	3	"Silvercup #2" trout pellets, finely ground
	1	Frozen brine shrimp
	1	Live nauplii
46 +	2	Flaked food. San Francisco brand.
	3	Frozen brine shrimp.

After 28 days of exposure, these fry of the first generation, now 35-36 days old, were thinned to 15 fish per tank. The remaining fish were killed with carbon dioxide and measured for wet weight. Mortalities among the original 30 fish were tabulated.

Three weeks later, on day 46, breeding mats were placed at the front of each tank. Each mat was made by wrapping green orlon wool around a glass plate 8 cm x 20 cm.

At 70 days of exposure, the first observation of an attempt at spawning was noted, shortly after sexes could be clearly

distinguished. Fish were thinned on that day to 6 adults, 2 males including the dominant one, and 4 females. In one replicate at the highest concentration only 4 fish remained alive, 3 males and 1 female, so these fish were allowed to continue. Size and mortality was again assessed at this thinning.

Subsequently in the experiment the spawning pads were inspected for eggs each afternoon at the same time, and eggs counted. Collection of eggs for rearing began in each tank when 20 or more were present on a given day. Collection ended with the 4th consecutive day of 20+ eggs, up to 30 per day being saved for rearing. Eggs were placed in small cups of nylon net with a surrounding styrofoam float. These cups were floated in the separate egg-fry tanks at the same vanadium concentrations from which they had arisen. A daily 5-minute treatment in 4 mg/L of Malachite Green was given to prevent fungus. This was discontinued at the first sign of hatching, generally at 4 days.

Hatchability was measured for each group of eggs 7 days after collection. Mortality was measured and obvious deformities and size variations were tabulated. Eight days after the fourth egg collection from each tank, all surviving larvae which averaged 6 days old, were grouped together. If 3 or fewer days separated the time to first egg collection from the two replicates, then up to 30 larvae were selected from each replicate and pooled in that egg-fry test-tank. If more than 3 days separated the time to first egg collection from replicates, then up to 60 larvae were selected from the first replicate to spawn.

The second generation fry were raised for an additional 24 days after being pooled. At an average age of 30 days, they were killed with CO_2 and mortality, size, and abnormalities were assessed.

Egg collection from the first generation was continued for 25 days after the first spawning in any tank. At the end of this period, 96 days total exposure, the adult fish were killed with carbon dioxide and the usual assessments made.

10.6.2 Experimental Apparatus

A continuous flow of well water was heated in a commercial glass-lined hot water heater. This flowed through a filter and aeration column into a 140-L uncoloured polyethylene reservoir where it was aerated. Water was then pumped through the diluter.

Stock solutions of vanadium were prepared by adding vanadium pentoxide to well water in 340-L uncoloured polyethylene reservoirs. These stock solutions were held at 25^{o}C and aerated at least 48 hours before pumping to the diluter was commenced.

The test-tanks were all-glass aquaria 50 cm x 24.5 cm, filled to a depth of 22.5 cm with approximately 28 L. Water entered at the top and back of each aquarium and left by means of a constant level siphon at the bottom front. Each aquarium received a pulse of 167 mL of water every 2 minutes. Daily flow thus equalled 120 L for a 95% molecular replacement of water in about 13 hours.

Equal lighting was supplied to each test tank from a row of eight 7.5-watt incandescent light bulbs. Light intensity at the water surface was 20 lux at the back and 40 lux at the front. Photoperiod was 16 hours of light with 1/2 hour of gradually increasing and decreasing light included.

10.6.3 Measured Concentrations of Vanadium

In the first experimental run of 28 days, average concentrations have been used to characterize exposure-levels, without differentiating between replicates. Averages were 0, 0.051, 0.58, 3.4 and 6.3 mg/L. Most of the 42 measurements were made at the high concentrations, and these showed reasonable consistency. However, the unbalanced set was not suitable for analysis of variance to test for significant differences between all replicate tanks.

In the second, or complete, experimental run, concentration

was measured in each tank every second or third day. Averages have been tabulated for the various exposure-periods in Table 20, and it may be seen that averages remained much the same throughout the experiment. Some variations at low concentrations are probably due in large part to analytical error, since the variation was not reflected at high concentrations at which more accurate measurements were possible.

Individual measurements in the replicates and various time-periods were compared by analyses of variance and Duncan's test. In general, these showed no differences. For example, grouping by A and B replicates, and by 70 days, 96 days, and the 30-day exposure in the second-generation tanks, showed no significant differences among the six averages within each concentration. The only difference found in the experiment was replicate A of the highest concentration (1.5 mg/L), for the period 0-28 days. This average was higher than that of its companion 28-day replicate B, and that of the 70-day replicate A. It was decided that this difference was not an important one. This concentration had an obviously severe effect, killing many of the first generation. In fact not enough spawners remained to provide replicated observations on most of the sublethal effects. It was the lower concentrations, which showed no differences, which were useful in the analyses to decide on "safe" levels, the main objective of the experiment. Accordingly, the average concentrations over the duration of the experiment were considered adequate to represent any time-period within it, for assessing sublethal effects.

10.6.4 Differences Between Replicates

This appendix gives details of statistically significant differences in response between replicate tanks, for cases that are not fully described in the text.

Table 20. Average concentration for replicates A and B, and together.

Exposure-period yielding measurements	Control			0.041			0.17			0.48			1.5		
	A	B	$\bar{X}$	A	B	$\bar{X}$	A	B	$\bar{X}$	A	B	$\bar{X}$	A	B	$\bar{X}$
0 - 28 days	0	0	0	.037	.042	.039	.14	.14	.14	.48	.47	.47	1.6	1.5	1.5
0 - 70 days	0	0	0	.035	.037	.036	.16	.16	.16	.48	.48	.48	1.5	1.5	1.5
0 - 96 days	0	0	0	.038	.039	.039	.16	.16	.16	.48	.47	.48	1.5	1.5	1.5
30-days, 2nd generation tanks			0*			.055*			.18*			.47*			1.5*
Overall $\bar{x}$	0	0	0			.041			.17			.48			1.5
SD			0			.015			.028			.044			.081
Minimum			0			.017			.11			.40			1.4
Maximum			0			.071			.22			.61			1.8
No. of measurements			47			41			44			44			41

* No replicates, pooled in separate aquarium

10.6.4.1 Sizes. Initial 28-day exposure of second experimental run

Wet weight	.17A ≠ .17B
Dry weight	control A ≠ control B
	.041A ≠ .041B

70-day exposure

♂♂	.041A ≠ .041B in wet, dry, length
	.48 A ≠ .48 B in wet, dry, length
	control A ≠ control B in dry weight
♀♀	control A ≠ control B in wet and dry weight

10.6.4.2 Average daily egg production. Analysis of this, with replicates kept separate, gave the following result from Duncan's test. Those exposures joined by underlining are statistically the same.

0.17	0	0	1.5	0.17	0.041	0.48	0.041	0.48
A	B	A	A	B	A	B	B	A

The major conclusion is that 1.5 mg/L is the same as both controls. Other conclusions of less interest:

1. Most replicates differ from each other, although controls are the same.
2. Both 0.48 are greater than both controls
3. One 0.041 is greater than both controls.
4. The other 0.041 is greater than one control.

11. AOSERP RESEARCH REPORTS

1.		AOSERP First Annual Report, 1975
2.	AF 4.1.1	Walleye and Goldeye Fisheries Investigations in the Peace-Athabasca Delta--1975
3.	HE 1.1.1	Structure of a Traditional Baseline Data System
4.	VE 2.2	A Preliminary Vegetation Survey of the Alberta Oil Sands Environmental Research Program Study Area
5.	HY 3.1	The Evaluation of Wastewaters from an Oil Sand Extraction Plant
6.		Housing for the North--The Stackwall System
7.	AF 3.1.1	A Synopsis of the Physical and Biological Limnology and Fisheries Programs within the Alberta Oil Sands Area
8.	AF 1.2.1	The Impact of Saline Waters upon Freshwater Biota (A Literature Review and Bibliography)
9.	ME 3.3	Preliminary Investigations into the Magnitude of Fog Occurrence and Associated Problems in the Oil Sands Area
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11.	AF 2.2.1	Life Cycles of Some Common Aquatic Insects of the Athabasca River, Alberta
12.	ME 1.7	Very High Resolution Meteorological Satellite Study of Oil Sands Weather: "a Feasibility Study"
13.	ME 2.3.1	Plume Dispersion Measurements from an Oil Sands Extraction Plant, March 1976
14.	HE 2.4	Athabasca Oil Sands Historical Research Design (3 Volumes)
15.	ME 3.4	A Climatology of Low Level Air Trajectories in the Alberta Oil Sands Area
16.	ME 1.6	The Feasibility of a Weather Radar near Fort McMurray, Alberta
17.	AF 2.1.1	A Survey of Baseline Levels of Contaminants in Aquatic Biota of the AOSERP Study Area
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19.	ME 4.1	Calculations of Annual Averaged Sulphur Dioxide Concentrations at Ground Level in the AOSERP Study Area
20.	HY 3.1.1	Characterization of Organic Constituents in Waters and Wastewaters of the Athabasca Oil Sands Mining Area

21.		AOSERP Second Annual Report, 1976-77
22.	HE 2.3	Maximization of Technical Training and Involvement of Area Manpower
23.	AF 1.1.2	Acute Lethality of Mine Depressurization Water on Trout Perch and Rainbow Trout
24.	ME 4.2.1	Review of Dispersion Models and Possible Applications in the Alberta Oil Sands Area
25.	ME 3.5.1	Review of Pollutant Transformation Processes Relevant to the Alberta Oil Sands Area
26.	AF 4.5.1	Interim Report on an Intensive Study of the Fish Fauna of the Muskeg River Watershed of Northeastern Alberta
27.	ME 1.5.1	Meteorology and Air Quality Winter Field Study in the AOSERP Study Area, March 1976
28.	VE 2.1	Interim Report on a Soils Inventory in the Athabasca Oil Sands Area
29.	ME 2.2	An Inventory System for Atmospheric Emissions in the AOSERP Study Area
30.	ME 2.1	Ambient Air Quality in the AOSERP Study Area, 1977
31.	VE 2.3	Ecological Habitat Mapping of the AOSERP Study Area: Phase I
32.		AOSERP Third Annual Report, 1977-78
33.	TF 1.2	Relationships Between Habitats, Forages, and Carrying Capacity of Moose Range in northern Alberta. Part I: Moose Preferences for Habitat Strata and Forages.
34.	HY 2.4	Heavy Metals in Bottom Sediments of the Mainstem Athabasca River System in the AOSERP Study Area
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39.	ME 1.0	The Climatology of the Alberta Oil Sands Environmental Research Program Study Area
40.	VE 7.1	Interim Report on Reclamation for Afforestation by Suitable Native and Introduced Tree and Shrub Species
41.	AF 3.5.1	Acute and Chronic Toxicity of Vanadium to Fish

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