THE ACUTE TOXICITY OF SALINE GROUNDWATER AND OF VANADIUM TO FISH AND AQUATIC INVERTEBRATES

by

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for

ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM

Project AF 3.2.1

June 1979

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ABSTRACT

The 10 d LC50 of saline groundwater to juvenile rainbow trout (*Salmo gairdneri*) varied from 20 to 35% of full strength, although median survival times of trout in groundwater varied substantially. Histopathological lesions were observed in the secondary gill lamellae and kidneys of juvenile trout exposed to groundwater concentrations of 6.25 to 50%. No measurable changes in heart rate, metabolic rate, buccal pressure, or ventilation rate were observed in adult trout exposed to 10 to 100% saline groundwater. *Gammarus lacustris* exhibited a highly significant avoidance reaction to groundwater concentrations of 1.0 to 25%, whereas whitefish (*Coregonus clupeaformis*) neither avoided nor preferred these concentrations. Various responses of five species of aquatic invertebrates to acute exposure of saline groundwater were investigated and a ranking of increasing tolerance of *Daphnia magna*<<*Chironomus tentans*<*Hexagenia rigida*<<*Orconectes virilis*<*Artemia salina* was observed. No significant differences in the toxicity of saline groundwater were observed when Winnipeg city or Athabasca River water was employed as diluent. Substantial changes in the chemical composition were observed during transport and storage of the effluent, ethereby complicating the interpretation of the toxicity studies.

Vanadium was moderately toxic to juvenile rainbown trout and whitefish (96 h LC50, 6.4 and 17.4 mg/L, respectively) and the toxicity increased slightly with decreasing pH. Pronounced histopathological lesions were observed in the gills and kidneys of trout exposed to sublethal concentrations of vanadium and the extent of damage increased with time of exposure. Eyed eggs of trout were 250 to 300 times more resistant to vanadium than fingerlings and the toxicant did not appear to induse histopathological lesions in the developing embryos. Acute exposure to vanadium did not produce significant changes in nine cardiovascular/respiratory parameters

or liver catalase and xanthine oxidase of adult trout. Juvenile whitefish actively avoided vanadium at concentrations $\geq$0.5 mg/L.

The acute toxicity of copper, which was studied as a reference for vanadium, was observed to be in general agreement with observations from other studies in the literature.

ACKNOWLEDGEMENTS

This research project AF 3.2.1 was funded by the Alberta Oil Sands Environmental Research Program, a joint Alberta-Canada research program established to fund, direct, and co-ordinate environmental research in the Athabasca Oil Sands area of northeastern Alberta.

1. INTRODUCTION

As a part of a toxicological program for the Alberta Oil Sands Environmental Research Program (AOSERP), the Industrial Toxicology and Biological Methodology projects of the Freshwater Institute (FWI) undertook to assess the acute toxicity of saline groundwater and certain inorganic components from sites in the AOSERP study area. Open-pit mining techniques are employed in extracting the bitumen-bearing sands. Saline groundwater discharges arise as a result of depressurization procedures required to maintain the water table below the level of the mine. At present, this waste water enters the Athabasca River via surface drainage through tributary streams. The groundwater is characterized by extremely high chloride, bicarbonate, and sodium ion concentrations and contains variable amounts of many other organic and inorganic components.

It is well established that acute lethality criteria of toxicity are often insufficient to assess the impact of a toxicant or an effluent upon an aquatic ecosystem and that this deficiency is probably more serious when a chemically complex effluent is tested. In an attempt to circumvent these objections, the approach employed in the present studies was to examine the toxicity of the saline groundwater and selected components using a variety of increasingly sensitive acute responses ranging from lethality to sublethal physiological, biochemical, embryological, behavioural, and histopathological changes in fish and invertebrates. Although distinct toxic responses were not necessarily expected in all parameters tested, the application of this broad toxicological screening system to the whole saline groundwater effluent, and subsequently to selected components, was designed to provide insight into their possible impact on the aquatic ecosystem in the AOSERP study area.

Vanadium and copper were selected for testing as individual components. Vanadium concentrations in the bituminous strata of the Athabasca Oil Sands range between 150 and 290 ppm (Jantzie 1976) and very little is known of its toxicity to aquatic organisms.

Copper has been well studied and is a component of saline groundwater. It served as a reference toxicant for the vanadium studies and provided a basis for evaluating certain aspects of the toxicological screening system applied to saline groundwater.

2. RESUME OF CURRENT STATE OF KNOWLEDGE

The detailed inorganic chemical composition of saline groundwater has been described for a limited number of indiviudual depressurization wells (McMahon et al. 1977). More recently, the heavy metal composition of 40 wells has been measured by Syncrude Canada Ltd. (Telephone communication October 1976 from R. Wilson, Co-ordinator, Aquatic Toxicology, Aquatic Fauna Technical Research Committee, AOSERP). The major components of saline groundwater recorded in the latter study are shown below (Table 1), while details of the composition of the saline groundwater employed in this study are provided in Section 4.1. The preliminary results of these and other current studies performed under contract to AOSERP (Lake and Rogers 1979) would suggest that the chemical composition of saline groundwater is highly variable. Major factors influencing this variability would appear to include location and depth of well, season, and climatic conditions.

Toxicity testing of saline groundwater has also been relatively limited. Initial tests indicate that this effluent is moderately toxic to various aquatic organisms although lethal concentrations differ considerably from species to species within both fish and invertebrate groups (McMahon et al. 1977). In addition, the lethal concentrations of different waste water samples were significantly different. Virtually no information has been published on the sublethal toxicity of saline groundwater although McMahon did suggest that short-term exposure to waste water concentrations $\geq$20% or longer term exposure to 5 to 10% effluent affected the swimming performance of juvenile rainbown trout (*Salmo gairdneri*).

With one exception, no information on the toxicity of vanadium to freshwater invertebrates or fish was located in a computerized search of the toxicological literature. Tarzwell and Henderson (1956, cited by McKee and Wolf 1963) reported that vanadium pentoxide was moderately toxic to fathead minnows (96 h TLm 13 and 30 mg/L as vanadium in soft and hard water, respectively),

Table 1. Concentrations (µg/L) of heavy metals in saline groundwater after various periods of storage at the Freshwater Institute. Analyses were performed at the FWI with the exception of those noted as Chemex analyses which were performed by Chemex Labs (Alberta) Ltd. through AOSERP project 2.5. The mean concentrations of heavy mteals from 40 mine depressurization wells were provided by R. Wilson (personal communication). The filtered samples were filtered through 0.22µ pore size Millipore filters prior to analysis. All values refer to the composite sample collected 18-19 October 1976 and are included for purposes of comparison.

Metal		Sample Storage Treatment Time (Days)									Mean of 40 Wells±1 s.d.
		0[a]	6[a]	6	8	11	14	17	27[a]	27	
Manganese	N.F.[b]	240	210	250	230	20	10	160	18	10	202±65
	F.[c]		180	270	240	<10	<10	<10	18	<10	
Lead	N.F.	<2	<2	<9	<9	<9	<9	<9	<2	<9	68±16
	F.		<2	<9	<9	<9	<9	<9	<2	<9	
Copper	N.F.	3	58	35	22	3	2	27	4	8	24±9
	F.		12	18	17	2	3	4	6	8	
Nickel	N.F.	<4	<2	<4	<4	<4	<4	<4	<2	<4	123±31
	F.		<2	<4	<4	<4	<4	<4	<2	<4	
Arsenic	N.F.	<1	<1	19	9.1	11	12	13	1.5	13	-
	F.		<1	9	8.6	13	11	11	1.5	13	
Cadmium	N.F.	<1	<1	<2	<2	<2	<2	<2	<1	<2	26±3
	F.		<1	<2	<2	<2	<2	<2	<1	<2	
Mercury	N.F.	2.4	<.1	1.9	0.4	0.2	<0.2	<0.2	4.3	<0.2	0.7±0.3
	F.		72	1.2	0.3	0.2	0.2	<0.2	70	<0.2	
Selenium	N.F.	<0.5	<0.5	2.5	2.1	2.1	2.0	2.0	<0.5	-	2±0
	F.		<0.5	2.1	2.1	2.1	1.9	2.1	<0.5	-	
Zinc	N.F.	28	48	16	17	4	<4	13	2	<10	40±15
	F.		19	31	14	23	11	5	1	<10	

Continued...

Table 1. Concluded.

Metal		Sample Storage Treatment Time (Days)									Mean of 40 Wells±1 s.d.
		0[a]	6[a]	6	8	11	14	17	27[a]	27	
Vanadium	N.F.	<1	<1	<10	<10	<10	<10	<10	<1	<10	7±4
	F.		<1	<10	<10	<10	<10	<10	<1	<10	
Silver	N.F.	<0.5	-	<1	<1	-	<1	<1	<5	-	14±9
	F.		-	<1	<1	-	<1	<1	<5	-	
Chromium	N.F.	<3	<3	<1	<1	<1	<1	<1	<3	<1	10±4
	F.		<3	<1	<1	<1	<1	<1	<3	<1	
Cobalt	N.F.	<2	<2	<3	<3	<3	<3	<3	<2	<3	112±25
	F.		<2	<3	<3	<3	<3	<3	<2	<3	

[a]Chemex Labs (Alberta) Ltd. analyses

[b]Sample not filtered prior to analysis

[c]Sample filtered prior to analysis

while vanadium in the form of vanadyl sulphate was approximately twice as toxic (96 h TLm 4.8 and 55 mg/L in soft and hard water, respectively). No sublethal acute or chronic studies of vanadium toxicity to aquatic have been published, although substantial literature is available on the responses of mammals to this toxicant (National Academy of Sciences 1974).

In view of the scarcity of detailed toxicological information on saline groundwater and vanadium, a broad-based toxicological program employing acute lethal and acute sublethal tests with fish and aquatic invertebrates was conducted in 1976-77 as subprojects AF 3.2.1 under contract to AOSERP. The results of these experiments are contained in this final report.

3. STUDY AREA

Although the entire research program was conducted at the Freshwater Institute in Winnipeg, Manitoba, samples of saline groundwater were obtained from the AOSERP study area (Figure 1). Saline groundwater was collected from five depressurization wells in the Syncrude Canada Ltd. lease near Mildred Lake. The well locations are illustrated in Figure 2 and their exact locations were designated as 2900E/1000S, 5300E/1400S, 4800E/14600S, 4900E/15700S, and 5300E/11400S for Wells 1, 2, 3, 4, and 5, respectively. Sample collection was performed by Alberta Environment personnel under a separate AOSERP project (Lake and Rogers 1979).

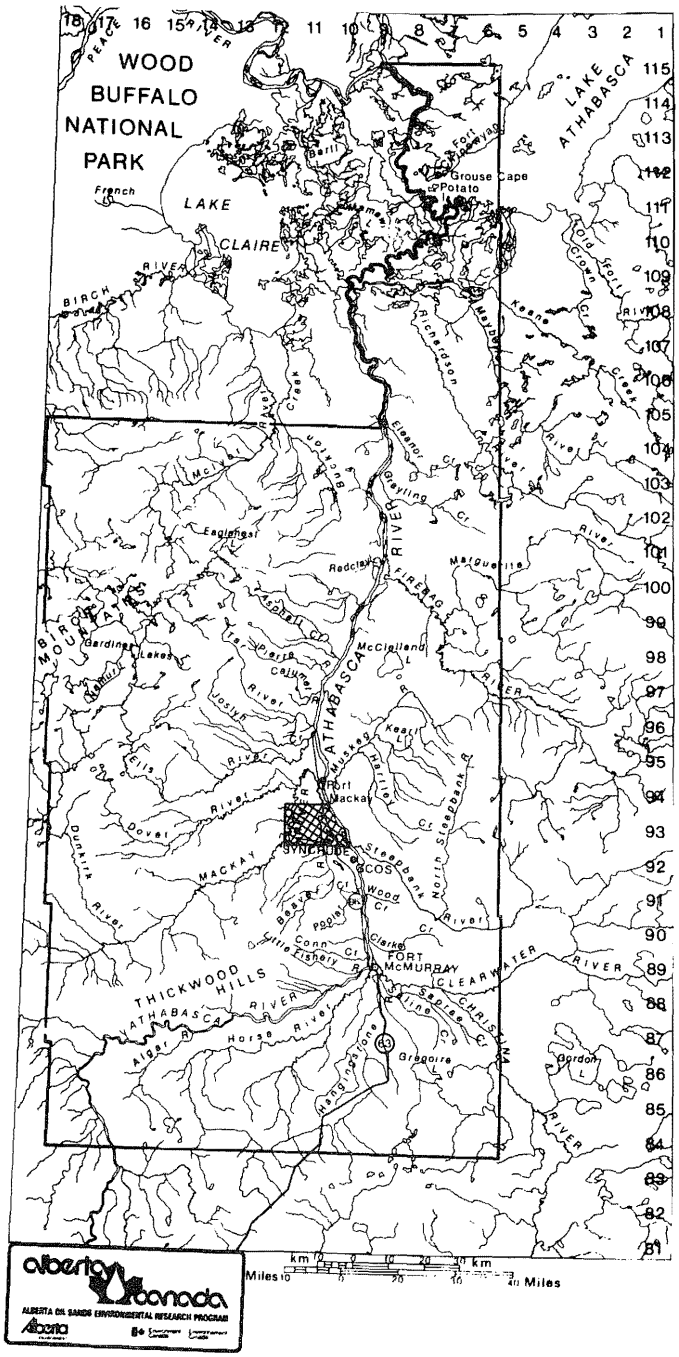

Figure 1. AOSERP study area. The cross-hatched area denotes the site of the Syncrude Lease No. 17.

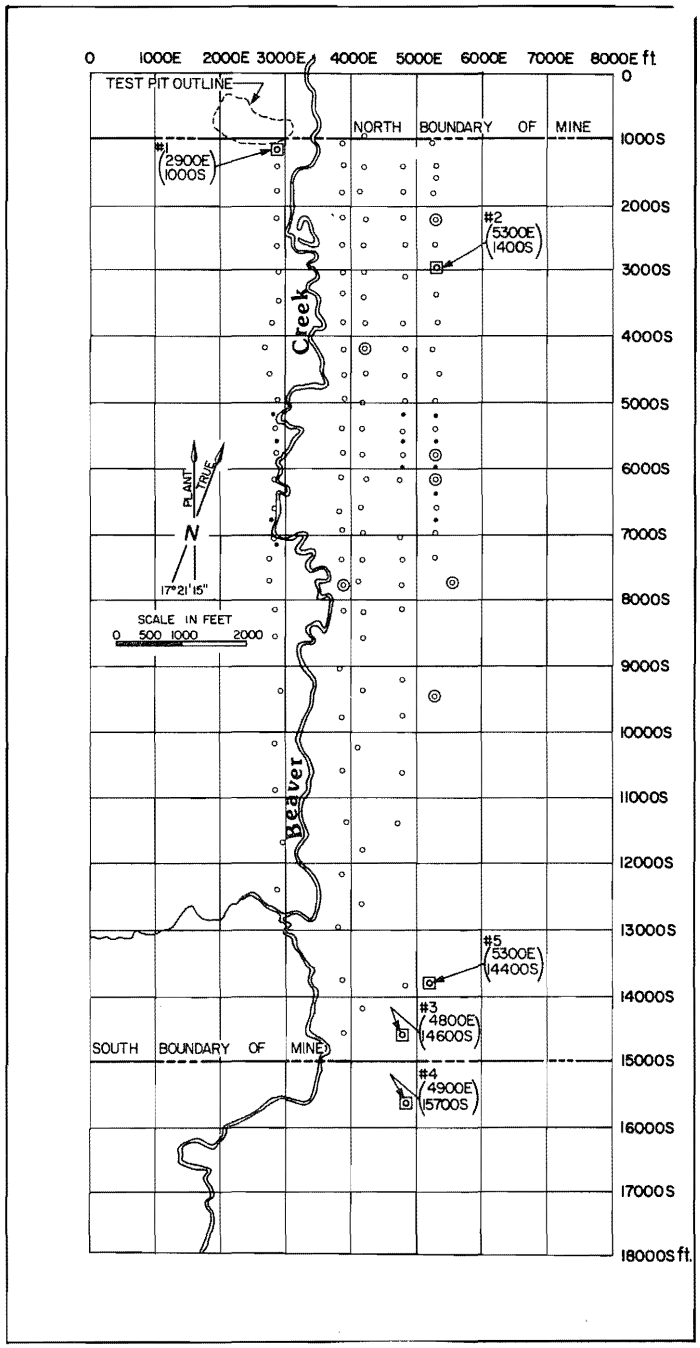

Figure 2. Location of depressurization wells on Syncrude Canada Ltd. Lease site 17 used as a source saline groundwater. The wells labelled numbers 1 to 15 supplied all groundwater used in this present study.

4. SALINE GROUNDWATER TOXICITY STUDIES

4.1 CHEMICAL CHARACTERISTICS OF SALINE GROUNDWATER

4.1.1 Introduction

Toxicity testing of whole effluents is complicated by several factors not normally encountered in studies of pure chemicals. Whole effluents are generally chemically complex which allows for the possibility of interaction of toxic components. In addition, the chemical composition of whole effluents may change during storage as a result of aeration, oxidation, decomposition, precipitation, or settling of various components. Finally, whole effluents are subject to changes in chemical composition on a daily or seasonal basis as a result of changes in activities related to production of the effluent. All the foregoing factors are expected to have profound effects on the results of any toxicity tests. In an attempt to facilitate the interpretation of toxicity tests and to provide further information on the chemical composition and factors influencing the effects of storage, the saline groundwater effluents used to generate toxicological data in this study were subjected to detailed chemical analyses. Portions of this work were performed by Chemex Labs (Alberta) Ltd. through a separate contract, while the remainder was conducted by the analytical chemictry program of the Toxicology section of the Freshwater Institute.

4.1.2 Methods

Composite samples (20 450 L) of saline groundwater from five wells on the Syncrude lease adjacent to Beaver Creek were collected by AOSERP personnel on 18-19 October and on 6-7 July 1976. The five wells were designated as Sites 1, 2, 3, 4, and 5 (Figure 2). The effluent was transported to the Freshwater Institute, Winnipeg, Manitoba in a steam-cleaned stainless steel tank truck and stored in six 3400 L fiberglass containers at 4^{o}C until used. The

storage containers were cleaned thoroughly prior to use and were housed in an enclosed shed to prevent contamination of the stored effluent. During storage, the effluent was continuously recirculated to reduce settling of suspended materials. Samples of the effluent from each well and the composite were removed during the collection period for chemical analysis by Chemex Ltd. Upon arrival at the FWI, suitably preserved samples of the composite effluent were sent to W. Lake, Alberta Environment, for complete chemical analysis by Chemex Labs (Alberta) Ltd. In addition, samples of the stored effluent were analyzed for heavy metal concentrations by atomic absorption (Varion AA5 or Perkin-Elmer 403 absorption spectrophotometers) at the FWI. Heavy metal analysis of the whole effluent and of effluent filtered through a cellulose-acetate (0.22 µm pore-size) Millipore filter was performed at intervals throughout the toxicity testing period for the 18-19 October composite.

A 45 L sample of groundwater was collected on 13 December 1976 from Wells 4 and 5 and transported to the FWI to complete the invertebrate toxicity tests. The chemical composition of this sample at the time of collection was determined, but no further characterization during the bioassay period was performed.

4.1.3 Results and Discussion

4.1.3.1 Chemical composition. The chemical characteristics of the saline groundwater from each well and the composite at the time of collection and of the composite after transport and storage at the Freshwater Institute are presented in Tables 2, 3 and 4 for the 8 July, 18 October, and 13 December 1976 collection periods, respectively. A composite sample of groundwater from Wells 4 and 5 collected on 13 December was not analyzed and the proportion of effluent from each well used in the composite is unknown.

Table 2. Chemical composition of saline groundwater from Wells 1-5 and composite sampled 8 July 1976 and of composite after 7 days of transport and storage at the Freshwater Institute. All concentrations are presented as mg/L except where other units are indicated.

Parameter	Wells 1	2	3	4	5	Composite (8 July)	Composite (15 July)
Calcium	318	211	160	107	237	136	14
Magnesium	96	207	220	253	158	233	218
Sodium	4588	7054	5982	6339	4583	5982	5600
Potassium	24.7	37.6	37.6	37.6	28.2	36.5	57.5
Chloride	6667	10000	8415	9024	5532	8272	7821
Sulphate	24	<10	26	17	17	29	<10
Total Alkalinity	2410	2877	2733	2702	2477	2728	2644
pH (units)	7.4	7.4	7.5	7.5	7.3	7.5	7.9
Carbonate							
Bicarbonate	2937	3507	3381	3293	3019	3325	3223
Total Hardness	1191	1377	1305	1309	1214	1300	934
Fluoride	0.92	0.72	0.72	0.72	0.72	0.75	1.25
Silica							
Conductance umhos/cm	20000	29000	25500	27000	20500	26000	24500
Threshold Odor No.							10
Color T_1 units	98	98	98	98	98	98	99
Color T_2 units	97	97	97	97	97	97	98

Continued...

Table 2. Continued.

Parameter	Wells 1	2	3	4	5	Composite (8 July)	Composite (15 July)
Color T_3 units	96	96	96	96	96	96	97
Tannin & Lignin	0.4	0.5	0.3	0.4	0.3	0.3	0.1
Total Residue	13106	18138	15840	15978	12810	16142	15796
Total Residue Fixed	12604	17602	15386	16446	12388	15680	
Surfactants	0.9	1.5	1.36	1.32	1.02	1.58	1.71
Total Organic Carbon	286	294	291	297	297	301	21.0
Total Inorganic Carbon	464	545	523	517	453	546	303
Total Carbon	750	839	814	814	750	847	21.0
Nitrate & Nitrite Nitrogen	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.033
Ammonia Nitrogen	7.76	11.85	11.06	11.48	9.36	10.55	10.47
Total Kjeldahl Nitrogen	7.22	10.53	10	10.53	8.86	10.3	10.81
Total Phosphorus	0.44	0.46	0.34	0.51	0.58	0.21	0.20
Total Dissolved Solids	13164	19245	16504	17400	12042	16326	15297
Phenol							0.002
Oil and Grease							4.6
Sulphide							<0.02
Cyanide	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chemical Oxygen Demand	74.5	84.6	72.7	72.7	81.0	81.9	105.6
Cadmium							0.02
Chromium $^{+6}$	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002

Continued...

Table 2. Concluded.

Parameter	Wells 1	2	3	4	5	Composite (8 July)	Composite (15 July)
Copper							0.006
Iron	0.4	0.2	1.8	3.0	3.3	0.7	0.6
Lead							<0.003
Manganese							0.143
Zinc							<0.001
Mercury	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Arsenic	<0.0002	<0.0002	<0.005	<0.0002	<0.009	<0.0002	
Nickel							<0.001
Cobalt							<0.002

Table 3. Chemical composition of saline groundwater from Wells 1 to 4 and composite samples 18-19 October 1976, and of composite after six (25 October sample) and 27 (6 November sample) days of storage at the Freshwater Institute. The "filtered" samples were filtered through cellulose acid Millipore filters (pore size 0.22 µm) prior to preservation. All concentrations are presented as mg/L except where other units are indicated.

Parameter	Well Number 1	2	3	4	Composite (19 Oct.)	Composite (25 Oct.) Unfiltered	Filtered	Composite (16 Nov.) Unfiltered	Filtered
Calcium	70.0	16.0	28.0	106.0	85.0	66.0	85.0	3.4	3.5
Magnesium	100.0	72.0	135.0	150.0	120.0	120.0	120.0	112.0	112.0
Sodium	5000.0	4500.0	6250.0	6250.0	5500.0	5500.0	5500.0	5750.0	5750.0
Potassium	42.0	42.0	55.0	55.0	48.0	49.0	49.0	38.5	38.5
Chloride	6950.0	5425.0	8500.0	8800.0	7650.0	7700.0	7700.0	7175.0	7175.0
Sulphate	1.9	0.5	0.5	0.5	0.5	0.5	0.5	31.3	30.2
Total Alkalinity	2230.0	3096.0	2700.0	2630.0	2620.0	2560.0	2560.0	2160.0	2160.0
pH (units)	7.6	7.7	7.7	7.7	7.8	8.0	8.0	9.1	9.1
Carbonate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	396.0	396.0
Bicarbonate	2718.4	3774.0	3291.3	3206.0	3193.8	3120.6	3120.6	1828.5	1828.5
Total Hardness	586.5	336.4	625.7	882.2	706.3	658.8	706.3	469.6	469.8
Fluoride	1.05	0.81	0.74	0.81	0.81	0.66	0.64	0.59	0.61
Silica	3.9	2.0	2.7	2.9	2.7	2.8	2.8	3.1	3.1
Conductance (umhos/cm)	23000	17200	25800	30500	26000	27000	27000	24000	24000
Threshold Odor No.	8	32	8	8	16	8	8	16	4
Color (units)	<5	<5	<5	<5	<5	<5	<5	<5	<5
Tannin & Lignin	0.80	<0.2	0.35	0.5	0.5	<0.10	<0.10	<0.10	<0.10

Continued...

Table 3. Continued.

Parameter	Well Number 1	2	3	4	Composite (19 Oct.)	Composite (25 Oct.) Unfiltered	Composite (25 Oct.) Filtered	Composite (16 Nov.) Unfiltered	Composite (16 Nov.) Filtered
Total Filt. Residue	13595	11590	14645	14645	16440	14535	14670	14500	
Fixed	13595	11590	14645	14645	16440	14535	14670	14500	
Total Non-Filt. Residue	12.4	9.6	19.2	13.2	20.4	14.8	0.4	1.2	0.4
Total Non-Filt. Residue Fixed	3.6	2.0	10.8	5.2	11.6	110	<0.4	<0.4	<0.4
Surfactants	<0.05	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Humic Acids	<1	<1	<1	<1	<1	<1	<1	<1	<1
Total Organic Carbon	75	125	50	50	50	50	50	5	20
Total Inorganic Carbon	475	725	450	450	250	425	425	530	470
Total Diss. Organic Carbon	50	75	50	50	37.5	50	50	5	20
Nitrate & Nitrite Nitrogen	<0.01	<0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01
Ammonia Nitrogen	6.90	6.70	10.1	10.0	7.8	8.0	8.1	9.3	9.2
Total Kjeldahl Nitrogen	8.50	8.80	12.0	11.5	10.5	9.05	8.95	9.85	9.80
Total Phosphorus	0.70	0.11	0.35	0.34	0.15	0.03	0.04	0.05	0.04
Ortho-Phosphorus	0.11	0.08	0.05	0.04	0.04	0.04	0.04	0.05	0.04
Phenol	0.012	0.001	0.001	0.004	0.019	0.005	0.001	0.001	0.001
Oil and Grease	0.13	0.6	0.8	0.8	0.3	<0.1	<0.1	0.7	0.8
Sulfide	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Cyanide	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chemical Oxygen Demand	100	56	338	79	84	98	86	194	190

Continued...

Table 3. Concluded.

Parameter	Well Number 1	2	3	4	Composite (19 Oct.)	Composite (1 Nov.) Unfiltered	Composite (1 Nov.) Filtered	Composite (19 Nov.) Unfiltered	Composite (19 Nov.) Filtered
Cadmium	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hexavalent Chromium	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
Copper	0.001	0.001	0.011	0.002	0.003	0.058	0.012	0.004	0.006
Iron	0.45	1.10	0.85	0.80	2.75	0.70	0.30	0.14	0.14
Lead	<0.002	<0.002	<0.006	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Manganese	0.17	0.14	0.18	0.20	0.24	0.21	0.18	0.018	0.018
Silver	<0.005	<0.005	<0.005	<0.005	<0.005			<0.005	<0.005
Zinc	0.016	0.011	0.019	0.018	0.028	0.048	0.019	0.002	0.001
Vanadium	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Selenium	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Mercury	0.0001	<0.0001	0.0003	0.0011	0.0024	0.072	<0.0001	0.0043	0.070
Arsenic	<0.001	<0.001	0.007	<0.001	<0.001	<0.001	<0.001	0.0015	0.0015
Nickel	0.004	<0.002	0.004	<0.002	0.004	<0.002	<0.002	<0.002	<0.002
Aluminum	0.06	0.17	0.17	0.09	0.23	0.11	0.09	0.08	0.08
Cobalt	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Boron	2.10	2.20	3.21	1.99	2.93	2.82	2.08	2.32	1.93
Ion Balance	0.96	0.95	0.97	0.97	0.95	0.95	0.95	1.06	1.06

< symbol indicates a concentration less than stated value

Table 4. Chemical composition of saline groundwater from Wells 4 and 5, sampled 13 December 1976. All concentrations are presented as mg/L except where other units are indicated.

Parameter	Well Number 4	5
Calcium	41.5	24.5
Magnesium	146.0	119.0
Sodium	6150.0	5750.0
Potassium	41.5	41.0
Chloride	8750.0	8100.0
Sulphate	70.5	61.0
Total Alkalinity	2660.0	2800.0
pH (units)	7.8	7.8
Carbonate	0.0	0.0
Bicarbonate	3242.5	3413.2
Total Hardness	704.7	551.1
Fluoride	0.52	0.50
Silica	2.9	2.8
Conductance (umhos/cm)	26800	25600
Threshold Odor No. (units)	16	32.0
Color (units)	<5	5
Tannin & Lignin	1.60	1.0
Total Filt. Residue	16105.0	15392
Total Filt. Residue Fixed	15917	14912
Total Non-Filt. Residue	9.2	14.0
Total Non-Filt. Residue Fixed	6.0	8.8
Surfactants	<0.02	<0.02
Humic Acids	<1.0	<1.0
Total Organic Carbon	50.0	5.0
Total Inorganic Carbon	530.0	470.0
Total Dis. Organic Carbon	50.0	<5.0
Nitrate & Nitrite Nitrogen	0.02	0.02

Continued...

Table 4. Concluded.

Parameter	Well Number 4	Well Number 5
Ammonia Nitrogen	11.0	11.0
Total Kjeldahl Nitrogen	12.18	11.6
Total Phosphorous	0.10	0.09
Ortho-Phosphorous	0.07	0.09
Phenol	<0.001	<0.001
Oil and Grease	1.1	2.9
Sulphide	<0.05	<0.05
Chemical Oxygen Demand	74.0	208.0
Cadmium	0.001	0.001
Hexavalent Chromium	<0.003	<0.003
Copper	<0.006	<0.006
Iron	0.39	0.77
Lead	0.004	0.004
Manganese	0.14	0.14
Silver		
Zinc	0.017	0.006
Vanadium	<0.001	<0.001
Selenium	0.0009	0.0007
Mercury	0.0014	0.0024
Arsenic	<0.001	<0.001
Nickel	0.003	0.004
Aluminum	0.09	0.11
Cobalt	<0.002	<0.002
Boron	1.42	1.45
Ion Balance	0.94	0.92

Several discrepancies in the concentrations of various components in the "composite" sample and the individual well effluents comprising the composite were observed in the July and October groundwater analyses. In the July sample (Table 2), the concentration of sulphate, surfactants, total organic carbon, and total inorganic carbon in the composite were all higher than that observed in any of the five wells sampled, whereas tannin and lignin and total phosphorus were lower than expected. In the October sample (Table 3), pH, total filterable residue, total non-filterable residue, phenol, iron, manganese, zinc, and aluminum were higher in the composite, while total inorganic carbon and total dissolved organic carbon were lower than would be expected from the respective values measured in the individual well effluents. These discrepancies could be attributed to inaccuracies in chemical analysis, contamination of samples during collection, or ineffective preservation and storage procedures. Since the volume of effluent from each well used to produce the "composite" is unknown, it was not possible to calculate the expected values of each parameter for comparison with the measured concentrations.

4.1.3.2 Variation in chemical composition during storage. Attempts were made to monitor changes in the chemical composition of saline groundwater during transport and storage at the Freshwater Institute by returning suitably preserved samples to Alberta Environment periodically after receipt of the effluent in Winnipeg. In addition, samples of the October composite were analyzed at FWI frequently. These October samples were filtered through 0.22µm pore Millipore filters to separate the contributions of precipitated components from those still in solution.

The data in Tables 2 and 3 indicate that major changes did occur in the chemical composition of the composite effluent during storage. The magnitude of these changes can be observed in Tables 1 and 5 where the relative concentration of each parameter, after various storage times, is expressed as a percentage of the

Table 5. Relative concentration of selected components of saline groundwater after various periods of storage as a percentage of the respective concentrations measured at the time of collection. Values for pH represent the arithmatic difference. Only parameters which varied in concentration by more than 15% from time of collection in at least one sample are included in this table.

	Relative Concentration (% of Concentration at Time of Collection)				
	July Composite	October Composite			
Storage Time	7 d	6 d		27 d	
		(Filtered)	(Unfiltered)	(Filtered)	(Unfiltered)
Parameter					
Calcium	10.3	100.0	77.6	4.1	4.0
Potassium	157.5	102.0	102.0	80.2	80.2
Sulphate	<34.5	100.0	100.0	6040.0	6260.0
Total Alkalinity	96.9	97.7	97.7	82.4	82.4
pH (units)	+0.4	+0.2	+0.2	+1.3	+1.3
Carbonate	n.d.	100.0	100.0	∞[a]	∞[a]
Bicarbonate	96.9	97.7	97.7	58.6	58.6
Total Hardness	71.8	100.0	93.3	57.3	57.3
Fluoride	166.7	79.0	81.5	75.3	72.8
Tannin & Lignin	<33.3	<20.0	<20.0	<20.0	<20.0
Total Organic Carbon	7.0	100.0	100.0	40.0	10.0
Total Inorganic Carbon	55.5	170.0	170.0	188.0	212.0
Ammonia Nitrogen	99.2	103.8	102.6	117.9	119.2
Total Phosphorous	95.2	26.7	20.0	26.7	33.3

Continued...

Table 5. Concluded.

	Relative Concentration (% of Concentration at Time of Collection)				
	July Composite	October Composite			
Storage Time	7 d	6 d		27 d	
		(Filtered)	(Unfiltered)	(Filtered)	(Unfiltered)
Parameter					
Phenol	-	5.3	26.3	<5.3	<5.3
Oil & Grease	-	<33.3	<33.3	266.7	233.3
Chemical Oxygen Demand	128.9	102.4	116.7	226.7	231.0

[a]Carbonate ion concentration increased from 0.0 mg/L at time of collection to 369.0 mg/L after 27 d of storage.

concentration at the time of collection. Only parameters which varied by more than 15% (with the exception of pH) over the entire storage period are included.

Major changes (21 to 80%) in the concentrations of fluoride, tannin and lignin, and total inorganic carbon were observed within one week in both the July and October composites. In addition, variations exceeding 20% were observed within 7 d in calcium, potassium, sulphate, total hardness, total organic carbon, total phosphorus, and chemical oxygen demand in either the July or the October composite. This observation suggests that the time course of chemical change during storage is highly variable and may be dependent upon complex interactions related to the actual concentrations of the components. Direct comparison of the effects of transport and storage upon the July and October effluents is further complicated by the effects of increased temperature in the July sample during the 4 d collection and transport period.

Storage of the October composite for 27 d resulted in extremely large changes in chemical composition. Of the 17 parameters, excluding pH, presented in Table 1, eight (calcium, sulphate, carbonate, tannin and lignin, total organic carbon, phenol, oil and grease, and chemical oxygen demand) changed by more than 75%; two (bicarbonate and total hardness) increased by 25 to 50%; and four (potassium, fluoride, total alkalinity, and ammonia nitrogen) changed by 20 to 25%. A pH rise of 1.3 units occurring over the 27 d storage period may account for changes in calcium carbonate, bicarbonate, hardness, and total inorganic carbon. The 231% rise in chemical oxygen demand, coupled with the decrease in the organic fractions, suggests that oxidation of the organic components results in the release of smaller organic molecules which are more readily oxidized than the parent compounds. In any case, it is clear that, even when saline groundwater is collected, transported, and stored at temperatures of $4^{o}C$ or less, major changes in chemical composition can be expected.

With the exception of tannin and lignin, phenol, ammonia, pH, and possibly hardness, few of the parameters listed in Table 5 would be expected to change radically the toxicity of the effluent. Many heavy metals, however, are known toxicants to aquatic organisms. To determine if changes in heavy metal concentrations are occuring during effluent storage, samples of the October composite effluent were analyzed by the analytical chemistry section of the Freshwater Institute. These data along with the relevant Chemex analyses are presented in Table 1.

Of the 13 heavy metals analyzed, seven (lead, nickel, cadmium, vanadium, silver, chromium, and cobalt) were below detectable limits of analysis. Of the remainder, two (arsenic and selenium) did not change in concentration over the 30 d storage period. Of the four that did change (manganese, copper, mercury, and zinc), decreases of 92.0, 91.4, 92.0, and 75.0%, respectively, were observed in unfiltered effluent in the interval from day six to day 11 of storage. Only minor changes in concentration occurred over the remaining 19 d of storage. Only minor differences in the concentration of heavy metals in filtered and unfiltered effluent were observed with the exception of the 5 November samples. These latter samples were obtained from the last few gallons of effluent in storage tank No. 2 and contained large amounts of precipitate which resulted in abnormally high values of manganese, copper, and zinc in the unfiltered effluent.

These observations clearly indicate that certain heavy metal components of saline groundwater do precipitate during storage of effluent. The mechanism of the precipitation is unknown and may be a result of the aeration with an associated rise in pH producing an insoluble carbonate salt. McMahon et al. (1977) observed large quantities of precipitate in a saline groundwater sample from a well on the Syncrude lease. The precipitate contained considerable quantities (from 10 to 4000 mg/kg) of various heavy metals including, in order of decreasing concentration, barium, baron, zinc, iron, strontium, manganese, copper, lead, nickel, vanadium, and cobalt.

Considering the observations of McMahon (1977) and the apparent difference between the concentrations of the heavy metal components of the October composite at the time of collection (19 October) and the respective mean concentrations of heavy metals (Table 1) in a sample of 40 mine depressurization wells on the Syncrude lease site (R. Wilson, personal communication; see Page 3), it is not unreasonable to suggest that a substantial proportion of the heavy metals was removed from solution during the two-day collection period for the October composite groundwater sample prior to transport to the Freshwater Institute. A more detailed analysis of this latter possibility is complicated by the apparent discrepancies in chemical analyses performed by Chemex and the Analytical Chemistry Department of the Freshwater Institute as illustrated by a comparison of the analyses of 25 October and 16 November (Table 1).

4.1.3.3 Seasonal variability in chemical composition. Since samples of saline groundwater obtained at three different dates were employed in toxicity tests, seasonal variability in the chemical composition represented a potential complication in the interpretation of toxicity data. Table 6 presents the relative change (percent) of selected constituents of groundwater from the July to October and July to December sampling periods of each individual well and the composite sample. A value of 100% indicates no change in concentration from the July sample whereas a value >100 or <100 represents a proportional concentration increase or decrease, respectively. Heavy metals were not analyzed in the July samples and are, therefore, not included in Table 6.

Of the 18 parameters presented in the composite samples from July and October, only seven (sodium, chloride, total alkalinity, bicarbonate, conductance, Kjeldahl nitrogen, and chemical oxygen demand) changed by less than 20%, while 21 to 40% changes in calcium, potassium, ammonia nitrogen, and total phosphorous, 41 to 60% changes in magnesium, hardness, total organic carbon, and total

Table 6. Relative concentration of selected components of saline groundwater on 18-19 October and 13 December 1976 as a percentage of the respective concentrations on 6-7 July 1976. Values for pH represent the difference in pH at each time. All analyses were performed on samples preserved at the time of collection.

Parameter	Relative Concentration (% of 6-7 July)						
Well No. / Sample Date	1 October 18-19	2 October 18-19	3 October 18-19	4 October 18-19	4 December 13	5 December 13	Composite October 18-19
Calcium	22.0	7.6	17.5	99.1	38.8	10.3	62.5
Magnesium	104.0	34.8	61.4	59.3	57.7	75.3	51.5
Sodium	109.0	63.8	104.5	98.6	97.0	125.5	91.9
Potassium	170.0	111.7	146.3	146.3	110.4	145.4	131.5
Chloride	104.2	54.3	101.0	97.5	97.0	146.4	92.5
Sulphate	7.9	>5.0	1.9	2.9	414.7	358.8	1.7
Total Alkalinity	92.5	107.6	98.9	97.3	107.4	113.0	96.0
pH	+0.2	+0.3	+0.2	+0.2	+0.3	+0.5	+0.3
Bicarbonate	92.6	107.6	97.3	97.4	98.5	113.1	94.4
Total Hardness	49.2	24.4	47.9	67.4	53.8	45.4	54.3
Conductance	115.0	59.3	101.2	113.0	99.3	124.9	100.0
Tannin and Lignin	200.0	<40.0	116.7	125.0	400.0	333.3	166.7
Total Organic Carbon	26.2	42.5	17.2	16.8	16.8	1.7	45.8
Total Inorganic Carbon	102.4	133.0	86.0	87.0	102.5	103.8	45.8
Ammonia Nitrogen	88.9	56.5	91.3	87.1	95.8	117.5	73.9
Kjeldahl Nitrogen	117.7	83.6	120.0	109.2	115.7	130.9	101.9

Continued...

Table 6. Concluded.

Parameter	Well No. / Sample Date	Relative Concentration (% of 6-7 July)						
		1 October 18-19	2 October 18-19	3 October 18-19	4 October 18-19	4 December 13	5 December 13	Composite October 18-19
Total Phosphorous		159.1	23.9	102.9	66.7	19.6	15.5	71.4
Chemical Oxygen Demand		134.2	66.2	464.9	108.2	101.8	256.8	102.6

inorganic carbon, a 67% change in tannin and lignin, and a 98% change in sulphate were observed. Although the values for tannin and lignin, sulphate, total organic carbon, and total inorganic carbon may not be completely dependable (possible analtyical errors or contamination), it is clear that the composite sample collected in October was substantially different in chemical composition from that collected in July.

In terms of seasonal variability in composition of individual well effluents (Table 6), it is clear that the seasonal changes in composition from well to well are not uniform in magnitude or direction (for example, total inorganic carbon and sodium in Wells 1 to 4, July to October). In addition, there is no apparent trend in the composition of the effluents, i.e., some constituents increase in concentration while others decrease. It would appear, however, that Wells 3 and 4, which are 305 m apart (Figure 1), exhibit relatively similar seasonal composition changes compared to those of Wells 1 and 2 which are more than 3000 m from the location of Wells 3 and 4. Wells 1 and 2, which are approximately 945 m apart and separated by Beaver Creek, are vastly different in terms of seasonal changes in chemical composition (Tables 2, 3, 4, and 6).

These observations indicate that the groundwater pumped from the wells used as sources of effluent in this study are derived, not from a common subetrranean pool, but from discrete pockets of water trapped in various geological strata. In addition, it is unlikely that the seasonal changes observed are simply a product of changes in the rate of percolation of surface waters into the water table as a result of variation in rainfall, but represent actual changes in the characteristics of the saline groundwater itself. The mechanisms whereby these changes occur are unknown but could relate to changes in the strata drained or seepage from new pockets of groundwater.

4.2 ACUTE LETHALITY BIOASSAYS - RAINBOW TROUT

(Principle Researchers: R. Danell, D. Hodgins, and G. Watts.)

4.2.1 Introduction

The initial phase of a broad-based toxicological assessment of a potentially harmful substance is usually a measurement of the acute lethal response of a test organism to a range of concentrations of the material. In the case of saline groundwater from the AOSERP study area, the acute lethality parameters have not been thoroughly investigated and, in view of the considerations presented in Section 4.1 of this report, may be expected to be extremely complex. McMahon et al. (1977) studies the acute lethality of groundwater from three wells on Syncrude Canada Ltd., Lease 17, to eight species of freshwater fish, and found that the toxicity of effluent from different wells did differ significantly. Since individual well effluents do vary in toxicity, a composite effluent from several depressurization sites may more closely reflect the "average" effluent. The following experiments were conducted on composites of the effluents of five wells identified in Section 4.1 of this report to provide information on the acute lethal responses of trout and a point of reference for additional acute sublethal toxicity tests with rainbow trout.

4.2.2 Methods

A total of five, 10 d bioassays of saline groundwater were completed using rainbow trout (*S. gairdneri*) (Idaho strain). The high density and suspended solids interfered with normal operation of the proportional diluter systems, preventing the successful completion of any flow-through bioassay and only static or daily replacement tests were conducted.

Two static bioassays were performed on the "8 July 1976 composite" (Section 4.1), one using dechlorinated Winnipeg city water and the other using Athabasca River water as diluent. Five trout (mean weight 1.8 g) were exposed to 10 concentrations of saline groundwater in 20 L polyethylene vessels in a controlled temperature water bath at 15°C. All fish were acclimated to the test temperature for at least 10 d prior to use. Routine chemical analyses were performed on the test solutions at 1 to 2 d intervals (Table 55). A 12 h light : 12 h dark photoperiod was used in all these experiments.

One daily replacement and two static bioassays (10 d duration) were performed on the "19 October 1976 composite" (Section 4.1), using dechlorinated Winnipeg city water as diluent. Five trout (mean weight 12.4 g) were placed in 20 L polyethylene containers containing various dilutions of groundwater at 15°C. Each test combination was duplicated and the mortality data pooled for each duplicate prior to statistical analysis. For the replacement bioassay, appropriate dilutions of saline groundwater were prepared daily and allowed to equilibrate to 15°C overnight. The next day, approximately 18 L of effluent were siphoned from each test vessel and replaced with fresh temperature-equilibrated groundwater to provide a 90% daily replacement of test material. Saline groundwater concentrations used in the replacement bioassay were 20, 30, 40, 50, 60, 80, and 100% of full strength and the test was initiated 8 d after effluent had been collected (test period 27 October to 6 November 1976). The static bioassays were performed in the period of 27 October to 6 November, and 16 November to 25 November 1976, which represents a storage time of 8 and 27 d, respectively, for the effluent. All vessels were vigorously aerated and routine chemical analyses were performed daily on each test vessel.

Probit analysis was used to obtain estimates of the LC50 and median survival times from the mortality data by the graphical methods of Litchfield (1949), Litchfield and Wilcoxon (1949), and,

when sufficient partial mortality was observed, from the BMD03S, Biological Assay, Probit Analysis Program, Health Sciences Computing Facility, University of California, Los Angeles.

4.2.3 Results

4.2.3.1 Bioassay test conditions. Table 55 presents the results of the routine chemical analyses performed during the course of each bioassay. Water temperature was maintained within the range of 14 to 16°C in all experiments, and dissolved oxygen was maintained in excess of 90% of air-saturation. Conductivity and un-ionized ammonia concentration increased proportionally with increasing saline groundwater concentration. Un-ionized ammonia concentrations in control vessels using Winnipeg city water diluent were generally less than 100 µg/L after a 10 d period in the static bioassay, while Athabasca River water control vessels had concentrations over 350 µg/L. The pH of all saline groundwater concentrations greater than 10% was approximately 8.6 to 9.0 and did not change in a uniform matter with time over the 10 d bioassay periods. This may be a reflection of the relatively high buffering capacity of the effluent. The pH of control vessels increased by 0.3 to 0.5 units over the test period in the static bioassays, while the 10% saline groundwater increased or decreased by up to 0.3 units in different static bioassays. No significant time-related changes in the conductivity, pH, temperature, dissolved oxygen concentrations, or un-ionized ammonia concentrations were observed in any of the daily replacement bioassay vessels.

4.2.3.2 Comparison of Athabasca River and Winnipeg city water diluents. The cumulative mortalities of rainbow trout in saline groundwater (July composite) with dechlorinated Winnipeg city water or Athabasca River water as diluent are presented in Tables 56 and 57, respectively. The median survival times (MST) and 95% confidence intervals calculated from these data are presented in Table 7.

Table 7. A comparison of the median survival times (MST) of rainbow trout in saline groundwater (SGW). The various test conditions employed in each bioassay are presented in the table. The 95% confidence intervals (95% C.I.) is presented for each MST estimate and wherever sufficient data permitted the slope function (S) was calculated.

	July Composite (Static Bioassay)				October Composite (Winnipeg Water Diluent)								
	Winnipeg Water Diluent		Athabasca R. Diluent		27 Oct. to 6 Nov.						16 Nov. to 25 Nov.		
					Static Bioassay			Replacement Bioassay			Static Bioassay		
SGW (%)	MST (h)	(95% C.I.)	MST (h)	(95% C.I.)	MST (h)	(95% C.I.)	S	MST (h)	(95% C.I.)	S	MST (h)	(95% C.I.)	S
100	1.4	(1.2- 1.6)	1.5	(1.4- 1.7)	1.6	(1.3- 1.9)	1.4	1.5	(0.9- 2.6)	2.5	0.75	(0.71-79)	1.1
90	1.2	(1.1- 1.4)	2.0	(1.9- 2.1)	1.5	(1.2- 1.8)	1.4	-	-	-	0.80	(0.58- 1.10)	1.4
80	2.0	(1.8- 2.2)	2.0	(1.9- 2.1)	2.1	(0.7- 6.1)	6.1	3.0	(1.3- 6.8)	3.7	0.88	(0.79- 0.98)	1.1
70	2.1	(1.8- 2.5)	2.8	(2.2- 3.6)	3.0	(1.2- 7.7)	4.6	-	-	-	1.0	(0.89- 1.1)	1.1
60	2.3	(1.8- 2.9)	1.7	(1.4- 2.0)	1.5	(1.2- 2.0)	1.6	12.0	(2.1- 34.2)	5.8	0.96	(0.85- 1.1)	1.1
50	12.0	(6.3- 22.8)	10.0	(5.8- 17.4)	1.7	(0.7- 4.3)	4.5	91.0	(75.2-110.1)	1.4	0.96	(0.85- 1.1)	1.1
40	55.0	(48.2- 62.7)	78.0	(30.0-203.0)	67.5	(30.3-150.5)	3.7	190.0	(177.9-202.9)	1.1	1.8	(1.7 - 1.9)	1.1
30	115.0	(97.5-135.7)		>240	115.0	(100.0-132.0)	1.3		>240		2.2	(1.9 - 2.5)	1.1
20		>240		>240	204.0	(117.9-352.9)	-		>240			>240	
10		>240		>240		>240			>240			>240	
24 h LC50 (% SGW)		28		35		19			33%			20 -30%	

No significant differences in MST using the two diluents were observed at groundwater concentrations ≥40%. At 30% groundwater, 40% mortality occurred with Athabasca River diluent while 100% mortality was observed with Winnipeg city water diluent. The significance of these differences is questionable since the respective estimates of the 10 d LC50 were 28 and 35%. These LC50 estimates were obtained from extrapolation of the log MST versus log % groundwater relationship.

4.2.3.3 Acute lethality of the "October composite" on saline groundwater. The cumulative mortalities of rainbow trout in the bioassays of the "October composite" of saline groundwater are presented in Tables 58, 59, and 69. The MST's of trout in the static bioassay performed after 8 d of effluent storage (from time of collection) are similar to those observed in the "July composite" for groundwater concentrations of 60 to 100% (Table 7). In the 27 October to 6 November bioassay, however, 60% mortality occurred at 20% groundwater, whereas no mortality occurred in the July tests at this concentration. This difference resulted in a 10 d LC50 estimate of 19% in the former bioassay compared to 28 to 35% in the July tests.

The MST's of trout in the "October composite" effluent after 8 d of storage were substantially different in the daily replacement bioassay compared to the static bioassay. Trout survived much longer in the replacement experiment, especially at groundwater concentrations ≤60%, and this is reflected in the 10 d LC50 of 33% for the replacement bioassay. Inspection of the daily chemical analyses (Table 55) does not provide an explanation of this difference since all parameters measured were similar in both experiments. It is possible, however, that some toxicants were decreasing in the saline groundwater stock used in the replacement bioassay (Section 4.1).

The MST's of trout in the static bioassay performed 16 to 25 November (28 d after effluent collection) differed from all previous results. All mortality occurred within the first 8 h of the test and the MST's ranged from 0.75 to 2.2 h over an effluent concentration of 30 to 100% (Table 7). The 12 h and 10 d LC50's were both 20 to 30%. This result was unexpected since chemical analysis of the effluent suggested that toxicity should be reduced. No major changes in routine chemical analyses were observed during the bioassay (Table 55). It is possible, however, that a toxic product from the breakdown of an organic component of the effluent may have been responsible for the high mortality during the first few hours of this bioassay.

Double logarithmic plots of exposure time versus LC50 for the 27 October to 6 November static and replacement bioassays are presented in Figures 3 and 4, respectively. Both relationships exhibit a slow rate of change of LC50 during the first 2 to 4 d of exposure, followed by an acceleration to the end of the 10 d period. Both phases were approximately linear and did not exhibit any tendency to become asymptotic to the time axis. This suggests that a 10 d bioassay period is insufficient to estimate the incipient lethal level of saline groundwater to rainbow trout.

4.2.4 Discussion

Few acute lethality bioassays of saline groundwater have been performed using rainbow trout. McMahon et al. (1977) studied the lethal responses of several species of fish to saline groundwater and reported a 7 d LC50 value of 36.8% for rainbow trout under conditions similar to those employed in the present study. The results of on-site and laboratory-based bioassays of groundwater (Lake and Rogers 1979) have not been published to date. The present studies produced 10 d LC50 estimates of 19 to 33% which are similar to those of McMahon. However, close comparison of the time mortality relationships observed in the two studies highlights several major differences. McMahon interpreted a convergence of

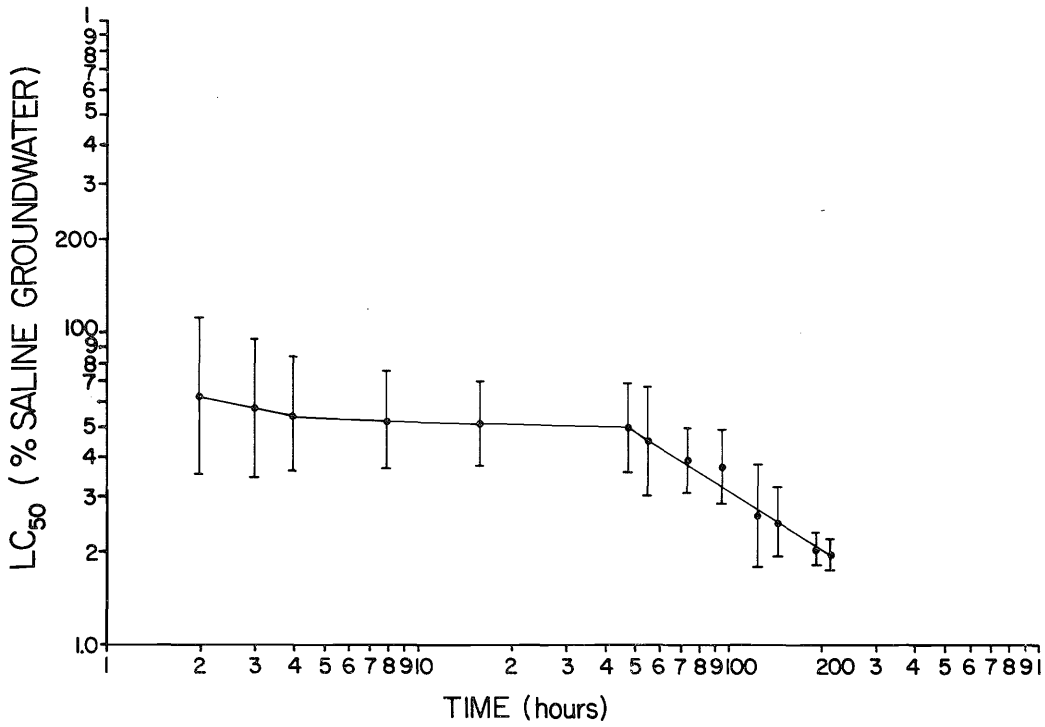

Figure 3. Toxicity of saline groundwater to juvenile rainbow trout (*Salmo gairdneri*) in a static bioassay. The vertical bars denote the 95% confidence interval of the LC50 estimate.

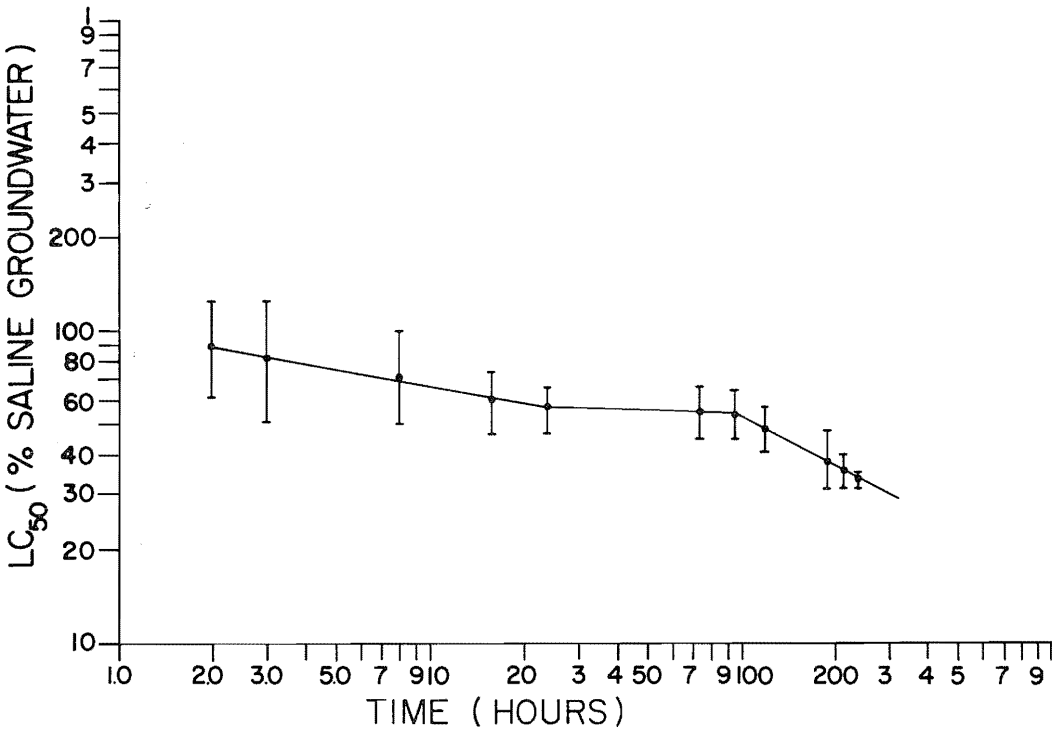

Figure 4. Toxicity of saline groundwater to juvenile rainbow trout (*S. gairdneri*) in a daily replacement bioassay. The vertical bars denote the 95% confidence interval of the LC50 estimate.

the toxicity curves of two different concentrations of groundwater to represent evidence of two modes of toxic action. The MST relationships in the present experiments do not indicate more than one mode of action, however. In addition, the time-LC50 relationship observed by McMahon did become asymptotic to the time axis, whereas the mortality accelerated over the course of the 10 d bioassay period in the present tests.

The data are insufficient to resolve these differences. On the basis of frequent chemical analysis of the saline groundwater during storage, it could be expected that the toxicity would decrease with time, contrary to the observed pattern of mortality. In general, however, it would appear that the 10 d LC50 of saline groundwater to rainbow trout lies between 20 and 40%, and that the time-mortality relationship is highly variable depending somewhat upon storage time, bioassay procedure, and one or more unidentified factors. The incipient lethal concentration is less than 20% and a long-term (at least 30 d) bioassay procedure may be required to define this parameter adequately.

4.3 HISTOPATHOLOGICAL EFFECTS OF SALINE GROUNDWATER IN FISH

(Principal Researchers: R. Evans and D. Klaprat.)

4.3.1 Introduction

As an element in broad-based toxicological testing systems, histopathological examination sometimes ranks as one of the most sensitive procedures for observing a toxic effect. Histological observations are also useful in providing information as to the target organs and the mode of actions of a toxicant. This may be of major importance when a whole effluent, possibly containing a variety of toxicants with differing modes of action, is examined in a series of increasingly sensitive toxicity tests. A histopathological examination of liver, gill, and kidney from rainbow trout and whitefish exposed to sublethal levels of saline groundwater was performed to describe more fully the possible toxic effects of this effluent.

4.3.2 Methods

Rainbow trout (*S. gairdneri*) and whitefish (*Coregonus clupeaformis*) surviving from the acute lethal bioassays of saline groundwater (Sections 4.2 and 4.5, respectively) were used in this study. Immediately following the bioassay, the fish were weighed, measured, and the abdominal cavity opened by mid-ventral incision. The gills were exposed by removing the opercula and the entire fish preserved in Bouins fluid. Normally, a maximum of three fish was obtained from each saline groundwater concentration.

After fixation for at least 24 h, the mid-region of the posterior kidney, the first gill arch, and the entire liver were dissected and washed in 70% alcohol for 1 to 3 d. The tissues were embedded in paraffin in the normal manner and sectioned at 10 μm. The sections were stained in Harris Hematoxylin and eosin (Jones 1967). Approximately 50 to 100 sections were examined from each tissue from each fish.

To facilitate discussion of results, the following histopathological terms (Robbins 1974; Florey 1970) have been used:

1. Cloudy swelling is a universal regression alteration reflecting any mild injury to the normal biochemical or metabolic state of cells. Cells are enlarged and the cytoplasm may be filled with granules. Cellular death may result.
2. Hydropic degeneration is a more pronounced form of cloudy swelling in which vacuoles appear in the cytoplasm. Both forms of cellular edema are associated with disturbance of active transport processes of the cell and are encountered most frequently in liver, kidney, and heart muscle cells. Cellular death may result from this condition.

3. Hyaline degeneration refers to a regressive cellular change in which the cytoplasm takes on a glassy, eosinophilic appearance and generally becomes necrotic. This histopathological condition indicates more serious tissue damage than cloudy swelling.
4. Hyperplasia refers to a localized increase in the number of cells in a tissue, such as the gills causing a thickening of the epithelium.
5. Hematoma is a swelling or edema in which the intercellular spaces contain blood cells and plasma.

4.3.3 Results

4.3.3.1 Liver histopathology. No definite histopathological changes were observed in the livers of rainbow trout exposed to saline groundwater concentrations at 6.25 to 50%. Fifty-four fish from a total of five bioassays were examined and, of these, 15 animals exhibited a slight increase in the number of hemopoietic sites. This increase, however, was not correlated to the saline groundwater concentration. At 20% and 50% groundwater, enlarged macrophages were observed in the blood sinuses but these did not appear in fish exposed to 30% effluent.

4.3.3.2 Kidney histopathology. A summary of the degenerative changes (cloudy swelling, hydropic degeneration, hyaline degeneration, and enlarged macrophages) observed in rainbow trout surviving in each of five bioassays is presented in Table 8. The concentrations of groundwater given for the flow-through bioassay of 14-24 July 1976 represent maximum concentrations, since dilutor malfunction resulted in a higher dilution (by a factor of 1.5 to 2.0) during most of the 10 d test period. Sections of kidney from control fish and those exhibiting cloudy swelling, hydropic degeneration, hyaline degeneration, and enlarged macrophages are presented in Figure 5 (Sections 1 to 5, respectively). It would appear from

Table 8. Histological effects of saline groundwater (SGW) on kidneys of rainbow trout (*Salmo gairdneri*) after 10 d of exposure.

Bioassay Test Conditions	Diluent	No. of Fish	Relative Degree[a] of Cloudy Swelling	Hydropic Degeneration	Hyaline Degeneration	Enlarged Macrophages
10 d Static (July Composite)						
Control	W[b]	3	0	0	0	0
10% SGW	W	3	3	1	1	1
20% SGW	W	3	3	2	2	2
Control	A[c]	3	0	0	0	0
10% SGW	A	3	3	2	2	1
20% SGW	A	3	3	3	3	2
10 d Flow-through (July Composite)						
Control	W	3	0	0	0	0
6.25% SGW	W	3	3	1	1	1
12.5% SGW	W	3	3	2	1	1
15.8% SGW	W	3	3	2	1	1
21.1% SGW	W	3	3	3	1	2
25.0% SGW	W	3	3	3	2	2
28.2% SGW	W	3	3	3	3	2
37.5% SGW	W	3	3	3	3	2
50.0% SGW	W	3	3	3	3	3

Continued...

Table 8. Concluded.

Bioassay Test Conditions	Diluent	No. of Fish	Relative Degree[a] of Cloudy Swelling	Hydropic Degeneration	Hyaline Degeneration	Enlarged Macrophages
10 d Replacement (October Composite)						
Control	W	6	0	0	0	0
20%	W	6	6	3	3	2
30%	W	6	3	3	3	3
10 d Static (October Composite)						
Control	W	3	0	0	0	0
10%	W	3	3	2	2	1
20%	W	3	3	3	3	3

[a]Ranking Code: 0 - no aberration
1 - small amount
2 - moderate amount
3 - large amount

[b]W refers to dechlorinated Winnipeg city water diluent.

[c]A refers to Athabasca River water dilutent.

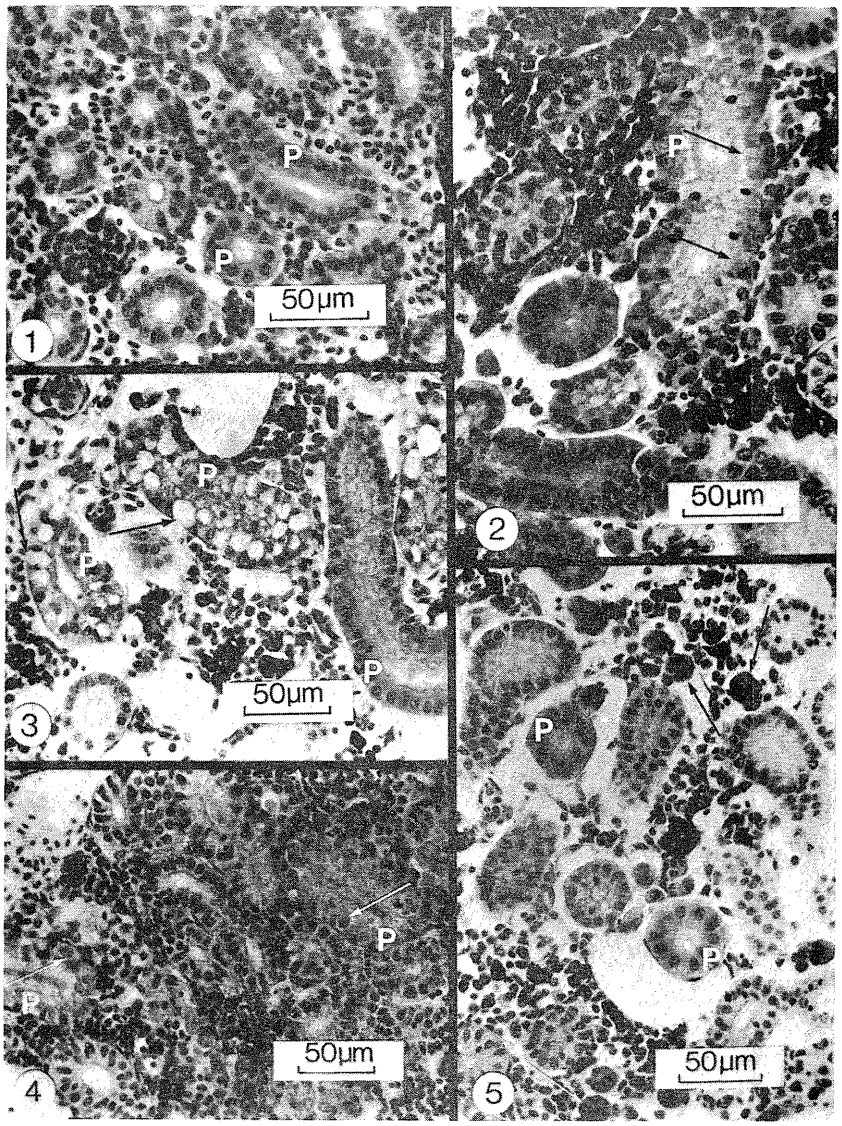

Figure 5. Longitudinal section of the posterior kidney of rainbow trout (*Salmo gairdneri*) showing:
1. normal morphology of proximal tubule;
2. cloudy swelling in proximal tubule;
3. hydropic degeneration in proximal tubule;
4. hyaline degeneration in proximal tubule; and
5. enlarged macrophages in the intertubular spaces. Sections 2 to 5 are from trout exposed to saline groundwater for 10 d.

the data in Table 8 that the incidence and severity of degeneration increases with increasing concentration of groundwater. In general, with increasing concentration of effluent, cloudy swelling is first observed, followed by hydropic generation, and finally be hyaline associated with the appearance of enlarged macrophytes. The use of Winnipeg city of Athabasca River water had no apparent effect on the histopathological effects of saline groundwater (Table 8).

4.3.3.3 Gill histopathology. Saline groundwater at concentrations ranging from 6.25 to 30% induced a general thickening of the secondary gill lamellae (Figure 6, Sections 6 to 9) of rainbow trout. This histological response was observed at all groundwater concentrations tested but did not occur in every trout, and no apparent dose-response relationship was indicated. The hyperplasia varied in intensity from mild (Section 7) to severe (micrograph not shown) in which over 60% of the lamellae were affected. Hyperplasia of the distal end of the secondary lamellae (Section 8) and development of distal hematomas (Section 9) were also observed frequently in fish exposed to all concentrations of saline groundwater tested in the different bioassays. Whitefish gills exhibited interlamellar hyperplasia at groundwater concentrations of 12.5 and 25.0% but not 6.25%. Distal hyperplasia or hematoma was not observed in whitefish.

4.3.4 Discussion

Exposure of rainbow trout to sublethal concentrations of saline groundwater induces definite histopathological changes in both the kidneys and gills and lesser changes in the liver. Mild injury to kidney cells was manifested by cloudy swelling in every fish exposed to the saline groundwater. The degree of hydropic degeneration present was clearly shown to be related to the saline groundwater concentration to which fish were exposed. Both these forms of cellular edema are related to a disturbance of active transport (Florey 1970). Serious cell damage in the form of hyaline degeneration was also prominent and related to saline concentration.

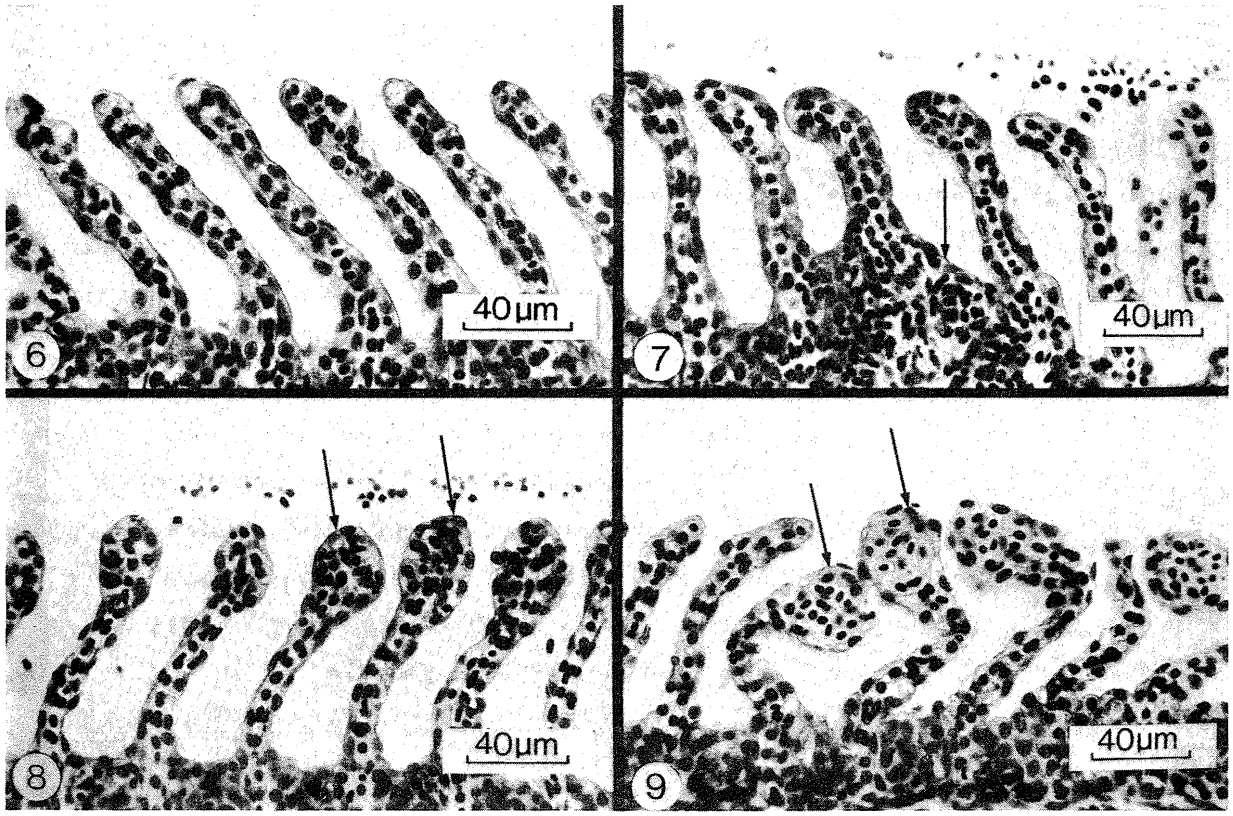

Figure 6. Longitudinal section of the secondary gill lamellae of rainbow trout (*Salmo gairdneri*) showing: 6. normal morphology; 7. minor interlamellar hyperplasia; 8. distal clubbing of lamellae; 9. distal hematomas in lamellae. Sections 7 to 9 are from trout exposed to saline groundwater for 10 d.

The increase in phagocytic marcophages reflects a response to the degeneration and death of the kidney cells, and not a change in kidney structure. The macrophages form a portion of the normal response to cellular infection or injury, and would not have a major physiological effect on the fish.

The slight increase in thickness of the secondary lamellar epithelium in fish exposed to saline groundwater would be expected to result in less efficient oxygen-carbon dioxide exchange due to an increased diffusion distance. Hyperplasia or fusion of lamellae occurred with variable severity in the majority of exposed fish. Clubbing and hematomas appeared randomly in about one third of the groundwater-exposed samples. However, there was no indication that they were more prevalent in the higher concentrations. These aberrations no doubt have some effect in decreasing the efficiency of respiration. A breakdown in the pillar cell system may be responsible for the hematomas (Smart 1976).

Liver tissues showed no definite lesions due to the saline groundwater exposure. The expanded hemopoietic areas observed in roughly 33% of the fish exposed to effluents were perhaps a response for increased leucocyte production. Saline groundwater toxicity may have initiated this response to bolster an inflammatory response at another site (e.g., kidney) within the fish. It is known that mononuclear blood cells (e.g., lymphocytes) hypertrophy in the first day or two after the onset of inflammation and become transformed into phagocytic cells, the hematogenous macrophages which supplement the macrophages already present in the tissue. Stimulated by a noxious material, many of these are mobilized at the site as free macrophages (Bloom and Fawcett 1968).

The degenerative histological changes observed in this study may explain the apparent increase in the rate of mortality observed in the latter stages of the 10 d acute lethality bioassays (Section 4.2). The accumulation of cell damage in the gill and kidney would result in a decreased capacity to maintain critical physiological processes such as ionic-osmotic regulation, waste excretion, and blood-gas transport. Experiments relating the time sequence of histopathological changes

during exposure to groundwater are necessary to substantiate this possibility.

4.4 SUBLETHAL CARDIOVASCULAR/RESPIRATORY RESPONSES OF RAINBOW TROUT

(Principal Researchers: V. Blouw and H. Majewski)

4.4.1 Introduction

Physiological responses of test organisms have long been an element in pharmacological screening systems and can provide comparative information on minimum toxicant concentrations eliciting a response. Such studies can also provide some insight into the modes of toxic action in relation to toxicant concentration. In addition, application of physiological monitoring procedures to field programs has been proposed (Spoor et al. 1971; Sparks et al. 1972). In fish, the cardiovascular/respiratory (CVR) responses to a wide variety of toxicants have been examined and several sublethal bioassay procedures developed (Schaumburg et al. 1967; Morgen and Kuhn, 1974). In the present study, the CVR responses of rainbow trout to saline groundwater were investigated in an attempt to provide insight into the mode of toxic action of this effluent, and provide the basis for development of an acute sublethal bioassay procedure for possible application to future field studies in the AOSERP study area.

4.4.2 Methods

Twenty-four rainbow trout of either sex, weight of 543.63± 147.38 g, and length of 34.40±2.84 cm, were acclimated in sodium thiosulphate dechlorinated (1.0 to 5.0 µg/L) water at 10°C for at least one month prior to use. The fish were anesthetized in water containing 0.3 g/L of 2-phenoxyethanol. A PE 60 catheter (Clay-Adams) was implanted to obtain buccal amplitudes and respiratory rate. Electrocardiogram electrodes were inserted (Bahr 1973) and the fish were placced into 10 L restraining chambers modified from Majewski et al. (1977) (Figure 29).

A flow of 500 ml/min of ultra-violet dechlorinated Winnipeg city water was supplied to each chamber and the fish allowed to recover for 24 h. After recovery, city water delivery was discontinued and diluted effluent was delivered by pump to a head-tank and from there to the restraining chambers at a flow rate of 500 mL/min/chamber. The outflow from each chamber was run to waste until the conductance (Radiometer CDM-2C) was identical to that in the head-tank (99% replacement time 1.2 h). At this time, the outflow from the chambers was collected and recirculated to the header tank. Fresh toxicant was added to the header system at the rate of 500 mL/min to provide a 25%/min replacement of recirculated effluent.

Heart rate, metabolic rate, ventilation rate, and buccal pressure were monitored hourly for 3 h prior to exposure to toxicant and of a log-based time scale after the toxicant attained the desired concentration in the outflow from each chamber. The electrocardiogram was obtained with a bioelectric amplifier (Hewlett-Packard model 8811A) and the ventilatory parameters with a pressure transducer (Hewlett-Packard model 1280C-02) and a pressure amplifier (Hewlett-Packard model 8805-C). A Hewlett-Packard physiography (model 7754A) was used to record the foregoing parameters. Oxygen uptake was estimated as the difference in oxygen partial pressure in water entering and leaving the restraining chamber with a Radiometer BMS 3 MK 2 acid-base analyser. All experiments were conducted at 10^{o}C with a 16:8, light:dark photoperiod. Oxygen levels in water or effluent delivered to the restraining chambers were maintained at >95% saturation.

4.4.3 Results

The effects of 10 to 100% saline groundwater on the heart rate, metabolic rate, ventilation rate, and buccal amplitude are presented in Tables 9 to 12. The fish dies in 100% groundwater within 8 h of exposure and in 30% effluent three of the four animals died within 12 h of exposure. An increase in all parameters was observed 2 to 4 h prior to death, followed by a decrease in all parameters in the observation period immediately preceding death. These effects have little or no bearing on acute sublethal responses and the experiments

Table 9. Effect of saline groundwater on heart rate (beats/min) in adult rainbow trout (*Salmo gairdneri*). Results are presented as mean ± 1 standard deviation for N=4.

Time (h)	SGC					
	100%	30%	25%	20%	10%	Control
-3	55 ±7.83	38.5 ±3.70	49.75 ±3.86	43.25 ±10.24	- -	34 ±9.06
-2	55.5 ±9.26	38.75 ±3.50	42.5 ±9.71	43.25 ±10.78	45.75 ±7.27	33 ±8.49
-1	55.5 ±11.12	38.5 ±3.70	43.75 ±13.23	49 ±13.49	43.5 ±7.42	34.25 ±7.14
0	43.2 ±23.50	37.75 ±4.92	44.5 ±5.69	45.75 ±8.77	45.5 ±6.40	33.25 ±6.70
0.25	48.22 ±18.39	42.25 ±2.87	49.25 ±3.20	46.25 ±13.87	47 ±6.88	33 ±6.98
0.5	52 ±6.06	41.75 ±3.20	56.75 ±14.59	46.25 ±12.69	46.75 ±3.95	32 ±7.70
1	51.75 ±6.40	43.25 ±5.91	43.25 ±8.58	45.25 ±12.28	42.5 ±5.97	33 ±4.90
2	55.25 ±4.35	45.75 ±3.40	52.5 ±5.45	41.5 ±13.89	43.5 ±7.59	30.5 ±6.81
4	45 ±17.63	46 ±4.32	54 ±4.83	43.25 ±8.46	44.25 ±8.92	31.5 ±4.73
8		28 ±18.02	55.75 ±4.35	43.75 ±14.31	42.75 ±7.97	29 ±6.63
12			54.5 ±6.81	47 ±11.52	44.75 ±8.46	32 ±6.48
21			54 ±4.62	45 ±8.04	41 ±9.06	28.75 ±7.09
24				45.25 ±9.43	41.25 ±9.18	30 ±6.73

Table 10. Effect of saline groundwater on metabolic rate ($mgO_2/h/kg$) in adult rainbow trout (*Salmo gairdneri*). Results are presented as mean ± 1 standard deviation for N=4.

Time (h)	SGW Concentration					
	100%	30%	25%	20%	10%	Control
-3		89.9 ±19.9	127.8 ±24.5	103.5 ±43.8		70 ±18.5
-2	113.7 ±45.2	71.6 ±6.7	128.7 ±61.5	90.8 ±29.2	87.2 ±13.1	104.5 ±57.5
-1	68 ±29.7	78.3 ±13.7	97.9 ±38.5	84.2 ±8.6	98.5 ±16.9	104 ±10.2
0		81.1 ±12.0	96.9 ±25.1	103.7 ±29.1		108 ±27.3
0.25	174.6 ±73.5					
0.5		123.2 ±12.0	84 ±51.6	96.2 ±8.2		92.6 ±34.3
+1		111.8 ±33.8	138.7 ±36.8	85.7 ±27.6	153.3 ±53.7	91 ±21
2	203.2 ±12.4	147.1 ±12.5	117 ±27.4	73.6 ±22.5	136.5 ±33.1	89 ±9.5
4	146.9 ±123.3	138.3 ±23.2	121.4 ±31.9	73 ±35.8	120.7 ±21.6	112 ±28.9
8		45.1 ±44.4	121.8 ±3.0	121.2 ±35.0	86.2 ±18.7	62.8 ±2.6
12			119.6 ±45.5	98.5 ±31.1	109.5 ±21.4	76.5 ±30
21			114.2 ±21.2	93 ±31.3	103.9 ±51.5	74.3 ±19.5
24			79.7 ±44.6	106.3 ±23.3	67.3 ±9.6	118.3 ±10.7

Table 11. Effect of saline groundwater on ventilation rate (buccal cycles/min) in adult rainbow trout (*Salmo gairdneri*). Results are presented as mean ± 1 s.d. for N=4.

Time (h)	SGW Concentration					
	100%	30%	25%	20%	10%	Control
-3	66.25 ±14.89	52.5 ±5.8	65.5 ±9.18	67 ±3.83	- -	63.5 ±7.51
-2	71.5 ±16.11	50.5 ±3.32	64.5 ±14.18	70.25 ±6.29	66.75 ±9.11	64.75 ±7.27
-1	62.5 ±13.60	51 ±2.83	60.5 ±15.78	73.25 ±3.95	71.25 ±7.59	62.5 ±5.26
0	67.5 ±16.05	53 ±7.39	55.75 ±11.59	72.75 ±6.99	69 ±6.83	61.25 ±7.23
0.25	74 ±12.68	60 ±10.23	61.5 ±11.47	69.5 ±4.43	66 ±8.52	60 ±8.64
0.5	55 ±37.41	52.25 ±7.68	65.25 ±12.69	66.5 ±6.45	65.75 ±8.75	64 ±5.48
1	80.75 ±10.28	57.75 ±6.5	68.75 ±8.34	65.25 ±7.23	68.5 ±16.05	62.75 ±6.02
2	78.5 ±8.35	64 ±4.08	61.25 ±8.10	67.25 ±8.73	62.75 ±15.52	61.25 ±5.44
4	74 ±4.24	60.25 ±10.25	66.25 ±13.89	65.5 ±13.08	65.25 ±15.97	64.25 ±4.5
8		59 0	59.5 ±13	66.25 ±8.54	64.25 ±16.78	60.6 ±4.04
12			61 ±11.17	63.5 ±5.20	69.5 ±9.98	63 ±12.99
21			58.25 ±9.0	56.25 ±11.64	65 ±11.17	58.75 ±7.46
24				56.25 ±4.57	65 ±10.17	61.5 ±13.30

Table 12. Effect of saline groundwater on buccal pressure (mm Hg) in adult rainbow trout (*Salmo gairdneri*). Results are presented as mean ± 1 s.d. for N=4.

Time (h)	SGW Concentration					
	100%	30%	25%	20%	10%	Control
-3	1.55 ±.723	1.18 ±.57	1.50 ±.70	0.88 ±.38	- -	0.40 ±.08
-2	1.75 ±.714	0.93 ±.21	2.2 ±1.57	0.73 ±.34	0.8 ±.48	0.56 ±.32
-1	1.36 ±.50	1.03 ±.47	1.60 ±1.60	1.03 ±.62	1.20 ±.81	0.33 ±.13
0	1.60 ±.79	1.10 ±.16	1.07 ±.38	0.65 ±.19	1.10 ±.70	0.35 ±.13
0.25	1.88 ±.99	1.78 ±.26	1.50 ±.87	0.58 ±.29	0.80 ±.50	0.38 ±.13
0.5	2.33 ±1.25	1.18 ±.30	2.0 ±1.53	0.55 ±.06	0.83 ±.43	0.35 ±0.10
1	2.95 ±1.59	1.75 ±.82	2.3 ±1.27	0.60 ±.14	1.3 ±1.1	0.35 ±.13
2	2.83 ±1.17	2.3 ±1.01	1.25 ±.30	0.78 ±.35	0.95 ±.55	0.43 ±.21
4	2.4 ±.28	2.08 ±.59	1.125 ±.40	0.95 ±.41	0.9 ±.51	0.45 ±.26
8		1.8 ±0	1.88 ±1.09	0.90 ±.20	0.9 ±.55	0.30 ±.17
12			2.23 ±.61	0.83 ±.21	1.35 ±.68	0.38 ±.10
21			2.25 ±.66	0.98 ±.48	1.35 ±.64	0.35 ±.19
24			1.43 ±0.52	0.85 ±.10	1.15 ±42	0.43 ±.21

at 100 and 30% groundwater concentrations were discontinued.

No consistent response was observed in any of the four parameters measured in fish exposed to 10, 20, and 30% saline groundwater (Tables 9 to 12). Normalization of the data reduced the variability over time substantially but no toxicant-induced effect was detected within the 24 h exposure period. Substantial variability was observed in the parameters among the different test conditions (control, 10, 20, and 25% effluent) although this was greatly reduced in normalized data. These experiments indicate that the cardiovascular/respiratory systems of rainbow trout are not affected by sublethal concentrations of groundwater during a 24 h exposure period.

4.4.4 Discussion

In conducting physiological toxicity testing with restrained fish, the experimental conditions may induce a stress factor sufficient to mask any effects induced by the toxicant. Although this possibility cannot be dismissed completely, the heart rates, metabolic rates, and ventilatory parameters observed in the present study are within the range of values reported for lightly restrained, unstressed rainbow trout (Stevens and Randall 1967; Hughes and Roberts 1970 Wood 1974; Lunn et al. 1975; Majewski et al. 1977).

The lack of reaction of fish exposed to saline groundwater, with the exception of reactions observed just prior to death at high effluent concentrations, may have resulted from the relatively short period of exposure to the effluent. The results of the acute lethality bioassays (Section 4.2) and histopathological examinations (Section 4.3) suggest that the toxic effects of saline groundwater may accelerate after 4 to 6 d of exposure. This could reflect an accumulation of physiological and histological damage producing a progressively increasing stress on the fish. Under these circumstances, the relatively small physiological changes induced within the first 24 h of exposure to the effluent would be masked by the relatively high individual variability occurring in the test animals. Further, it it probable that the relatively minor changes in cardiovascular respiratory responses of trout at 30 and 100% effluent are

not the cause of death but reflect the toxic action of the effluent upon some other target organ or physiological system.

In view of the foregoing considerations, it is not possible to state that sublethal concentrations of saline groundwater do not produce adverse effects on the cardiovascular/respiratory systems of trout. The results of 10 d acute lethality bioassays, histopathological examinations, and short-term physiological bioassays would indicate that longer term (4 to 6 d) exposure to saline groundwater is required in future experiments to assess adequately the physiological responses of fish to sublethal concentrations of this effluent.

4.5 AVOIDANCE-PREFERENCE REACTIONS OF WHITEFISH (*Coregonus clupeaformis*) AND *Gammarus lacustris*

(Principal Researchers: D. Hodgins and H. Maciorowski)

4.5.1 Introduction

The avoidance or preference reactions of aquatic organisms to industrial effluents will affect the actual impacts of the effluent on aquatic organisms. An avoidance reaction to industrial effluents may result in the alteration of patterns of dispersion and migration formation of abiotic zones in lakes and river and of fish and aquatic invertebrates. Alternatively, a preference reaction may result in prolonged exposure to an industrial effluent which may produce lethal or sublethal toxic effects.

Saline groundwater from the Athabasca Oil Sands area is toxic to fish and to aquatic invertebrates (McMahon et al. 1977). The impact of the saline groundwater on the fauna of the Athabasca River may depend upon whether the saline groundwater attracts or repels aquatic organisms. Therefore laboratory tests were performed to determine the avoidance-preference reactions of lake whitefish (*C. clupeaformis*) and the aquatic invertebrate, *Gammarus lacustris* (Sars), to saline groundwater.

4.5.2 Material and Methods

The following studies were performed on the saline groundwater composite collected 19 October 1976. The chemical composition of this effluent has been described previously (Section 4.1). Ultra-violet dechlorinated Winnipeg city water was employed as diluent for the groundwater and for the control and is characterized in Table 61.

Lake whitefish (*C. clupeaformis*) were obtained from the Manitoba provincial hatchery at Anama Bay and raised in a natural pothole lake at Erickson, Manitoba. The seven month old whitefish were transported to the Freshwater Institute where they were acclimated to ultra-violet dechlorinated (Armstrong and Scott 1974) Winnipeg city water at 10°C for two weeks prior to the first test. The mean weight and fork length of the whitefish were 12.3 g and 113 mm, respectively.

Gammarus lacustris were collected from a freshwater pothole lake near Erickson, Manitoba, and transported to the Freshwater Institute where they were acclimated to sodium thiosulphate dechlorinated (Armstrong and Scott 1974) Winnipeg city water at 14°C for 10 d prior to the first test.

The lethal toxicity of the saline groundwater to whitefish and *G. lacustris* was determined in static bioassays. Whitefish were exposed to 6.25, 12.5, 25, 50, and 100% saline groundwater for 10 d prior to avoidance-preference tests. Duplicate 20 L plastic-lined test vessels, each containing five whitefish, with a loading density of 0.1 L/g/day were employed. *Gammarus lacustris* were exposed for 96 h to 3, 13, 26, 51, and 80% saline groundwater before the avoidance-preference tests and to 3, 13, 51, 80, and 100% saline groundwater after the avoidance-preference tests in duplicate 2 L glass test vessels for each concentration. Ten *G. lacustris* were placed in each test vessel. Dissolved oxygen, pH, temperature, and conductivity were measured daily for the whitefish toxicity test and for both *G. lacustris* toxicity tests (Table 62). Mortality was recorded on a geometric time series and LC50s were calculated by the logit method (Ashton 1972).

A rectangular counter-current trough (Scherer and Nowak 1973) was used to assess the avoidance-preference reactions of whitefish and *G. lacustris* to saline groundwater. The trough was reduced in size to 25.5 x 3.75 cm for use with *G. lacustris*. Saline groundwater and water were controlled by rotameters and adjustable needle valves. The flow rate was 7.8 L/min with a resultant velocity of 0.55 cm/sec in tests with whitefish, and 110 ml/min with a resultant velocity of 0.33 cm/sec in tests with *G. lacustris*. In tests with whitefish , the inner baffles were placed 30 cm from the centre of the trough and airstones were placed between the outer and inner baffles; this ensured that the saline groundwater did not flow beneath the water.

The avoidance-preference reactions of whitefish were tested at 10±1°C to 0.1, 1, 10, 25, and 35% saline groundwater. The avoidance-preference reactions to *G. lacustris* were tested at 13±1°C to 0.5, 1.5, 1, 5, 15, and 25% saline groundwater. Tests at higher concentrations of saline groundwater were not possible since the density prevented the formation of a water-toxicant interface. Ten whitefish or five *G. lacustris* were tested separately at each concentration and each test organism was used in one trial only. Each whitefish was habituated to the test trough for 10 min and each *G. lacustris* for 5 min. The movements of test organisms were monitored for 10 min while water was introduced into both ends, and for an additional 10 min while saline groundwater was introduced into one end. The saline groundwater was introduced into the end of the trough for which *G. lacustris* had shown a preference during the water-water trial, whereas the introduction of the saline groundwater was alternated between the ends of the trough in trials with whitefish. The conductance of the saline groundwater and water was measured at the end of each trial (Table 63).

The arcsine transformation (Snedecor and Cochran 1967) was applied to the percent time data before further analysis. A t-test determined if there was a statistically significant preference for one end during the water-water trials, and one-way analysis of variance determined if there was a statistically signficant difference in

the results of water-water trials at different test concentrations. Paired t-tests determined if there was a statistically significant change in test organism behaviour from the water-water trial to the saline groundwater trial in percent time spent in the half of the trough in which the saline groundwater was introduced.

4.5.3 Results

The 4 d and 10 d LC50 estimates for whitefish in saline groundwater were 62 and 37.5%, respectively. No whitefish mortality was observed in groundwater concentrations less than 30%. In the *G. lacustris* acute lethality tests, mortality was observed only at 80% groundwater. The mortalities recorded at this concentration after 96 h exposures were 15% and 30%, respectively, for tests performed before and after the behavioural experiments, providing a rough 96 h LC50 estimate of 90 to 100% saline groundwater.

The possibility of test organisms preferring one segment of the avoidance-preference chamber was examined by monitoring their movements when uncontaminated water was present in both sections. No statistically signficant preference occurred for one end of the trough with whitefish ($t = 0.67$, $df = 49$, $p>0.05$) or *G. lacustris* ($t = 1.38$, $df = 24$, $p>0.05$), nor was there a statistically significant difference in the results of water-water trials for different concentrations of saline groundwater with whitefish ($F = 0.75$, $df = 4.45$, $p>0.05$) and *G. lacustris* ($F = 1.71$, $df = 4.20$, $p>0.05$).

Whitefish exhibited a significant avoidance to 1.0% saline groundwater but neither preferred nor avoided higher or lower concentrations (Table 13, Figure 7). In view of the highly variable response of whitefish to 1.0% groundwater (Figure 7), the reliability of the statistically significant avoidance observed is questionable. At groundwater concentrations of 10% and higher, considerable difficulty was encountered in maintaining a sharp effluent:water interface and this may have adversely affected the behavioural tests at these concentrations.

Gammarus lacustris avoided all groundwater concentrations tested (Figure 7) but the avoidance reaction was statistically significant

Table 13. The avoidance-preference responses of whitefish (*Coregonus clupeaformis*) and *Gammarus lacustris* to various concentrations of saline groundwater.

% SGW	% Time Spent in SGW: Water-Water Trial	% Time Spent in SGW: Toxicant Water Trial	Calculated Paired t-test	Significance
Coregonus clupeaformis				p=0.05; t=2.262
0.1%	54.47	48.82	1.19	no significant difference
1.0%	53.33	36.98	2.55	increased avoidance
10.0%	52.43	49.08	0.66	no significant difference
20.0%	49.09	52.97	0.87	no significant difference
35.0%	48.02	52.42	0.67	no significant difference
				p=0.05; t=2.78
Gammarus lacustris				p=0.01; t=4.60
0.5%	55.40	36.53	2.40	no significant difference
1.0%	69.17	25.57	4.09	increased avoidance
5.0%	60.97	23.00	5.07	increased avoidance
15.0%	55.87	33.39	6.79	increased avoidance
25.0%	63.50	13.31	7.46	increased avoidance

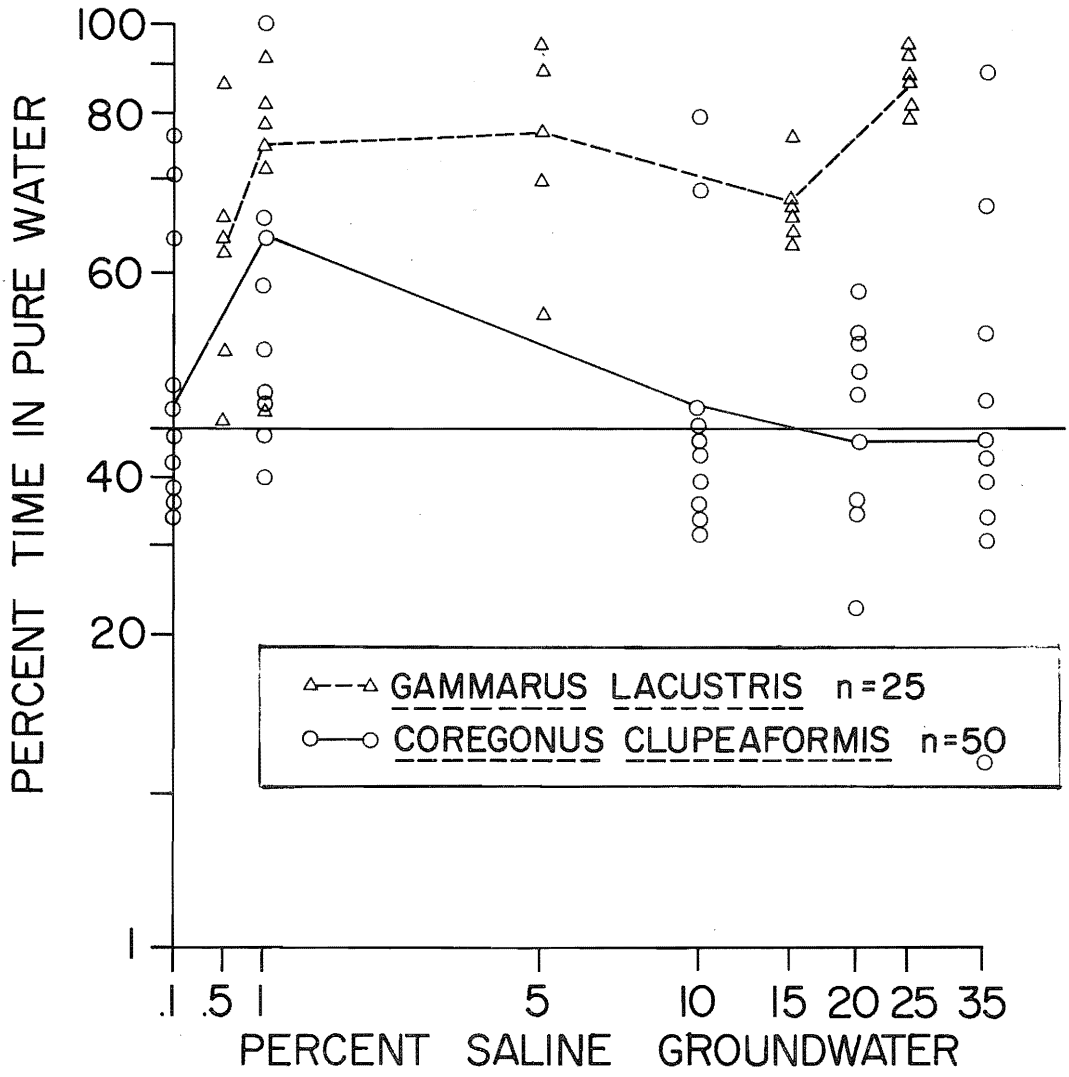

Figure 7. Avoidance-preference responses of whitefish (*Coregonus clupeaformis*) and *Gammarus lacustris* exposed to saline groundwater.

($p<0.05$) at concentrations from 1.0 to 25.0%. The degree of avoidance in this species was not correlated to the concentration of groundwater, although a lesser degree of avoidance to concentration <0.5% may occur. Under no circumstances did whitefish or *G. lacustris* exhibit a preference reaction for saline groundwater.

4.5.4 Discussion

The toxicity of saline groundwater to whitefish (*C. clupeaformis*) was substantially lower than that observed by McMahon et al. (1977) with juvenile mountain whitefish (*Prosopium williamsoni*). The respective 4 d LC50 values were 62% and 42.8% and the comparable 10 d LC50 values were 37.5% and 22.3%. In view of the probable differences in chemical composition of the effluents, test conditions, and species used in these two studies, this lack of similarity is not surprising. The toxicity of groundwater to *G. lacustris* was extremely low (96 h LC50>80%). This observation is in agreement with those of McMahon et al. (1977) who observed that the tolerance to short-term exposure to groundwater was greater for four benthic invertebrates than for nine species of fish.

Whitefish did not exhibit either preference or avoidance reactions to saline groundwater concentrations of 0.1, 10.0, 20.0, or 30.0%. The avoidance response of whitefish at 1.0% groundwater was statistically significant at the 95% confidence level but not at the 99% confidence level. Although the avoidance-preference reaction of this species may differ under natural conditions from those in the laboratory, the present data would indicate that whitefish would not avoid potentially harmful concentrations of saline groundwater released into the Athabasca River or its tributaries.

Gammarus lacustris activity avoided all effluent concentrations 1% or above. This reaction may be due to the presence of high concentrations of sodium chloride or possibly other major ions since *G. pulex*, a closely related species, actively avoids sea water concentrations as low as 1.0% (Bettison and Davenport 1967). However, both species are known to be tolerant of saline conditions and can be acclimated to 70% sea water (Sutcliffe and Shaw 1967). Although other

sublethal toxic effects of saline groundwater to *G. lacustris* have not been investigated, the present laboratory data would indicate that this species would actively avoid effluent concentrations less than 1 to 2% of those causing death in a 96 h exposure period. Clearly the potential for active avoidance-preference reactions of a number of endemic fish and invertebrate species is required prior to assessing the impact of saline groundwater upon the behaviour of aquatic organisms in the AOSERP study area.

4.6 INVERTEBRATE ACUTE TOXICITY BIOASSAYS

(Principal Researchers: M. Friesen, S. Leonard, and B. Townsend)

4.6.1 Introduction

Invertebrates form an essential part of the aquatic ecosystem and, as a group, exhibit a wide range in their abilities to tolerate various toxicants. Although fish have been widely used in toxicological assessment of the impact of pollutants on the aquatic environment, it is clear that additional attention must be given to invertebrate toxicology. As part of a broad toxicological study, five species, *Daphnia magna, Artemia salina, Orconectes virilis, Chironomus tentans,* and *Hexagenia rigida,* representing four invertebrate orders, were examined for their responses to acute exposure to saline groundwater.

4.6.2 Methods

4.6.2.1 *Daphnia magna*. Toxicity testing with daphnids was performed on two saline groundwater composites, one collected on 7 to 8 July 1976 and the other on 13 December 1976. The chemical characteristics of these effluents have been described previously (Section 4.1). Slightly different procedures were employed in the *Daphnia* toxicity studies with these two composites; therefore, methods and results are described separately.

"July Composite Studies": Two sets of experiments were performed with this effluent using 4 d old daphnids from a stock culture maintained at 15°C. On 14 July 1976, a flow-through bioassay was performed in which 30 daphnids were exposed to nine concentrations of groundwater from 6.25% to 100% using ultra-violet dechlorinated Winnipeg city water as diluent. The daphnids were maintained in partially submerged glass chambers (47 x 55 mm) enclosed at one end with #170 nylon screen to allow circulation of toxicant. The chambers were placed in 11 L vessels supplied with effluent dilutions from a modified Mount and Brungs proportional dilutor. A pair of static bioassays were performed using either Athabasca River water or dechlorinated Winnipeg city water as diluent. These tests were initiated 20 July 1976 under the same conditions as previously described with the exception that the proportional dilutor was disconnected. All tests were performed at 15°C. The criteria for death were complete immobility of appendages and cessation of heart beat and gut contractions. Animals were not fed during exposure to toxicant.

"December Composite Studies": Static bioassays were initiated on 15 January 1977 using a reconstituted medium (Table 64) as diluent. Considerable difficulty in maintaining daphnid cultures in dechlorinated city water necessitated this change in diluent. Acid cleaned 100 mL beakers containing 80 mL of diluted effluent and five 4 d old daphnids were used as exposure vessels. Each test concentration was duplicated and the mortalities for each test condition pooled. All other test conditions were similar to those employed in the July studies. The routine chemical analyses performed on each test vessel are presented in Table 65.

4.6.2.2 *Artemia salina*. These experiments were performed to determine the effect of saline groundwater on the hatching success of brine shrimp. The groundwater composite collected in December 1976 was employed in this study. *Artemia* cysts were collected from Chaplin Lake, Saskatchewan. The diluent was prepared by dissolving 250 g of raw sodium sulphate in 6 L of distilled water. Triplicate 20 mL

samples of each groundwater dilution were placed in polyethylene insect-rearing vials and approximately 1800 dry, oviparous *Artemia* cysts (measured by volume) were added. Immediately after hatching was observed in the control vial, the contents of two of each triplicate were fixed and stained in a solution of formalin and Rose Bengal to facilitate counting of hatched and unhatched cysts. Twenty nauplii were transferred from each remaining vial to fresh toxicant of the same concentration to observe cumulative mortality of meta-nauplii. The remaining cysts and nauplii were preserved and stained after 96 h of exposure and the percent hatch determined. All tests were performed at room temperature. The conductivity, pH, temperature, and ammonia concentrations recorded during this experiment are presented in Table 66.

4.6.2.3 *Orconectes virilis*. These tests were performed using the "July composite" of saline groundwater. Crayfish were collected from the Rat River, Manitoba, in early June 1976 and maintained in aerated, sodium thiosulphate-dechlorinated water at 15°C. On 14 July 1976, 15 crayfish were transferred in 20 L vessels in a flow-through proportional dilutor system described in Section 4.2, using dechlorinated Winnipeg city water as diluent. Two static bioassays were performed, using Athabasca River or Winnipeg city water as diluents and using five crayfish per test concentration in 20 L vessels. All tests were performed at 15°C. Lack of movement of the gills and antennae and failure to produce a tail flexion-extension response were used as criteria of death. Moulting animals were excluded from mortality records since they normally die from predation by non-moulting crayfish.

4.6.2.4 *Chironomus tentans*. The standard 96 h bioassays were run on the July composite of saline groundwater using third instar 16 d old larvae cultured at 20°C. Thirty larvae were used per concentration in both static and flow-through tests and were maintained individually in a floating bioassay rack. The rack was constructed

by fixing 30 9.5 mm diameter, 76 mm glass tubes, sealed at the submerged end with 500 µm nitrex screen, in a board fitted with styrofoam floats. At each mortality check the rack was lifted from the solution and the larvae were considered dead when they did not respond to a stream of solution expelled from a Pasteur pipet.

The emergence success study was run using fourth instar 22 d old larvae cultured at 20°C. Four polyethylene containers, each containing 20 fourth instar larvae, were used per test concentration. These containers isolated the chironomid larvae within the dilutor system but contained silica sand substrate in which the larvae could construct tubes. Unemerged larvae were examined for mobility at the end of the experiment.

The hatching first instar bioassay was conducted in glass petri dishes using two egg masses (approximately 3000 eggs) per test concentration. Eggs were obtained within 12 h of deposition and incubated in the test solutions at 20°C. At this temperature, hatching normally occurs within 40 to 60 h. The middle of each egg mass was examined daily for hatching.

4.6.2.5 *Hexagenia rigida*. The July composite of saline groundwater was employed in the mayfly studies. The chemical characteristics of effluent are presented in Section 4.1.

Eggs were obtained from females collected on the shore of the Red River at the South Perimeter Bridge in Winnipeg, Manitoba. Bioassays were conducted on two groups of eggs: eggs with little or no embryological development (early-stage eggs) which were not expected to hatch during the exposure period, and eggs more advanced in development (late-stage eggs) which were expected to hatch during the exposure period.

Eggs for the early-stage egg bioassay were obtained from 10 females collected the day the bioassay was begun (14 July 1976). The eggs were dissected into Winnipeg or Athabasca water and thoroughly mixed. An aliquot (minimum of 240 eggs/aliquot) was then transferred to each of three dishes for each concentration (control, 20, 40, 60, 80, and 100%) of groundwater using Winnipeg ulta-violet (UV)

dechlorinated water or Athabasca River water as diluents. Eggs were incubated at 21.4°C where start of hatch was expected to occur after 15 d. After 10.5 d the developing eggs were photographed (Polaroid Land Instrument Camera, model ED-10), solutions were decanted, and conductivity and pH readings were taken (Table 67), and fresh Winnipeg thiosulfate-dechlorinated water added. Eggs were monitored daily for start of hatch. Counts of hatched and unhatched eggs were made 2 and 6 d after first hatch in all concentrations, with an additional count after 10 d in 60% saline groundwater. A maximum of 500 eggs were counted per petri dish. The same eggs were not necessarily examined at each count. Only nymphs which had successfully freed themselves from their egg cases were considered as having hatched. Counts were made using a binocular microscope (Wilde model M5). Counts were discontinued when little further hatching was expected to occur.

Eggs for the late-stage egg bioassay were obtained from 10 females which had been collected on 29 June 1976. At this time eggs were dissected into Winnipeg thiosulfate-dechlorinated water, thoroughly mixed, and aliquots (270 to 1695 eggs/aliquot) transferred to petri dishes. Eggs were incubated to 16°C (at which temperature hatching occurs after about 30 d). Sixteen days later the incubation water was decanted and solutions of groundwater at various concentrations (control, 20, 40, 50, 80, and 100%) at 15°C were added. Duplicate samples for each concentration, using both diluents, were run. Eggs were then incubated at 21.4°C for 10 d after which time the water was decanted, conductivity and pH readings taken (Table 68), and fresh Winnipeg thiosulfate-dechlorinated water added. Counts of hatched and unhatched eggs up to a maximum of 500 eggs were made at this time, 4 d later, in all concentrations, as well as 8 d later in 80% and 100% concentrations. The total number of hatched and unhatched eggs per petri dish was counted 8 d after the fresh water was added in control 20%, 40%, and 60% groundwater and after 13 and 18 d in 80% and 100% groundwater.

4.6.3 Results

4.6.3.1 *Daphnia magna*. The July composite of saline groundwater was extremely toxic to *Daphnia* at all concentrations tested in the flow-through bioassay (Table 14). The MST at concentrations ≥15.8% was less than 30 min while the MST's at 6.25 and 12.5% were 1.7 and 1.0 h, respectively. In static tests the toxicity was slightly lower, but essentially complete mortality was observed within 4 h at all concentrations of groundwater tested (Table 15). No significant difference in toxicity was apparent when Athabasca River or Winnipeg city water was employed as diluent (Table 15).

Since the 24 h LC50 of groundwater to *Daphnia* was apparently less than 6.25%, a second test was performed on the "December composite" effluent using a lower range of test concentrations (Table 16). It is clear that this effluent was considerably less toxic to *Daphnia* than the July composite with a 96 h LC50 >20% and <50%. No meaningful mortality occurred within 96 h at concentrations less than 10%. These results may relate to the effect of the culture medium on toxicity, or changes in the toxicity of the effluent. No data are available to explain these effects and this potential variability must be considered in future experiments.

4.6.3.2 *Artemia salina*. No major effects of saline groundwater concentrations ranging from 0.01 to 100% were observed on the hatching efficiency of oviparous cysts or survival of meta-nauplii of *A. salina*. Hatching was initiated after 26 h incubation in all effluent concentrations, although the percent hatched at this time in full strength groundwater may be reduced (Table 17). After 96 h the percentage hatch was similar in all concentrations of effluent. The high variability of hatching success of these brine shrimp cysts (Table 17) is not unusual and in these experiments the lack of correlation between effluent concentration and hatching success was interpreted as a lack of direct toxic action. None of the newly hatched nauplii

Table 14. Cumulative mortality of *Daphnia magna* to saline groundwater (July composite) in a flow-through bioassay. Sample size, N=30.

Time (h)	SGW Concentration									
	Control	6.25%	12.5%	15.8%	21.1%	25%	28.2%	37.5%	50%	100%
0	0	0	0	0	0	0	0	0	0	0
0.25	0	0	0	0	100.00	80.00	96.67	93.33	100.00	100.00
0.5	0	16.67	16.67	100.00		100.00	96.67	96.67		
1	0	20.00	16.67				100.00	100.00		
2	0	60.00	86.67							
4	0	83.35	100.00							
8	0	83.35								
12	0	83.35								
16	0	83.35								
48	0	90.00								
72	0	90.00								
96	0	93.33								
120	0	100.00								
Terminate										

Table 15. Cumulative mortality percent of *Daphnia magna* in saline groundwater (July composite) using Winnipeg city and Athabasca River water as diluents under static conditions. Sample size, N=30.

Time (h)	SGW Concentration											
	Winnipeg Water Diluent						Athabasca					
	Control	20%	40%	60%	80%	100%	Control	20%	40%	60%	80%	100%
0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0	0	0	33.0	40.0	0	0	0	0	0	0	0
0.5	0	0	40.0	100.0	100.0	100.0	0	0	30.0	100.0	100.0	100.0
1	0	23.3	100.0				0	30.0	100.0			
2	0	80.0					0	60.0				
4	0	100.0					0	83.3				
8	0						0	90.0				
12	0						0	96.6				
48	0						0	96.6				
72	0						0	100.0				
96	0						0					
120	0						0					
MST (h)		1.38	0.52					1.54	0.58			
95% confidence		1.18	0.49					1.12	0.53			
Interval of MST		1.62	0.56					2.13	0.64			

Table 16. Cumulative mortality percent of *Daphnia magna* in saline groundwater (December composite) under static conditions. Sample size, N=20.

Time (h)	SGW Concentration									
	Control	.01%	.05%	.10%	.50%	1.0%	5.0%	10.0%	50.0%	100.0%
0	0	0	0	0	0	0	0	0	0	0
0.25	0	0	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0	0	100
1	0	0	0	0	0	0	0	0	0	
2	0	0	0	0	0	0	0	0	0	
4	0	0	0	0	0	0	0	0	40	
8	0	0	0	0	0	0	0	0	100	
12	0	0	5	0	0	0	0	0		
24	0	0	5	0	0	0	0	5		
48	0	0	5	0	0	0	0	5		
72	0	5	10	0	0	15	5	10		
96	0	5	10	0	0	20	5	20		

Table 17. Hatching efficiency of *Artemia salina* oviparous cysts in saline groundwater.

SGW (%)	Percent Hatch 26 h Incubation	96 h Incubation
Control	11.42	23.23
0.01	12.12	13.51
0.05	13.18	14.04
0.10	10.15	16.30
0.50	6.54	10.45
1.00	5.12	9.52
5.00	9.78	17.56
10.00	9.86	25.61
50.00	3.28	14.28
100.00	1.66	11.12

died when exposed to groundwater concentrations from 0.01 to 100% of full strength, again indicating that this species is extremely tolerant of this effluent.

4.6.3.3 *Orconectes virilis*. The results of the flow-through bioassay are presented in Table 18. It is evident that the July composite of groundwater was not acutely toxic at concentrations less than 100% over an 11 d exposure period. At 100% saline groundwater, mortality was 33% at 10 d exposure and 53% after 11 d exposure. Although this may reflect a toxic action during the latter part of the test period, other factors such as starvation may also be involved.

The toxicity of groundwater to crayfish was not modified by the use of Winnipeg city or Athabasca River water in the static bioassays (Table 19). In these 11 d bioassays, groundwater was also demonstrated to be non-toxic in terms of the LC50 criterion.

4.6.3.4 *Chironomus tentans*. The 48 h and 96 h LC50 of saline groundwater to third instar *Chironomus* larvae were 69 and 62% effluent, respectively, using Athabasca River diluent and 68.5 and 63% effluent, respectively, using dechlorinated Winnipeg city diluent (Table 20).

Population variance as indicated by the slope functions was similar in all test conditions. Since third instar larvae normally burrow into the substrate, the results in Table 20 may be somewhat biased. The flow-through bioassays failed to provide reliable toxicity data since the proportional dilutor systems could not function with the dense groundwater effluents.

The emergence data (Table 21) clearly demonstrate that fourth instar larvae cannot survive in 60% groundwater. At this concentration larvae did not penetrate the substrate provided and either died or failed to pupate. In 40% effluent, growth was retarded as demonstrated by the high proportion of larvae which did not emerge during the bioassay period but did remain alive.

Table 18. Cumulative mortality percent of *Orconectes virilis* in saline groundwater in a flow-through bioassay. Sample size, = 15.

Time (h)	SGW Concentration									
	Control	6.25%	12.5%	15.8%	21.1%	25%	28.2%	37.5%	50%	100%
0	0	0	0	0	0	0	0	0	0	0
0.25	0	0	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	6.7	0
72	0	0	0	0	0	0	0	0	6.7	0
96	0	0	0	0	0	0	0	6.7	6.7	0
120	0	0	0	6.7	0	0	13.3	6.7	6.7	13.3
144	0	0	0	6.7	0	0	20.0	6.7	6.7	13.3
168	0	0	13.3	6.7	0	0	20.0	6.7	6.7	26.6
192	0	0	13.3	6.7	0	0	20.0	6.7	6.7	33.3
216	0	0	13.3	13.3	0	0	20.0	6.7	6.7	33.3
240	0	0	13.3	13.3	0	0	20.0	6.7	6.7	33.3
264	0	0	20.0	13.3	0	0	20.0	6.7	6.7	53.3

Note: All of the corpses were encrusted with an orange precipitate.

Table 19. Cumulative mortality percent of *Orconectes virillis* in static bioassays of saline groundwater with Athabasca River and Winnipeg city water as diluent. Sample size = 5.

Time (h)	SGW Concentration											
	Winnipeg Diluent						Athabasca Diluent					
	Control	20%	40%	60%	80%	100%	Control	20%	40%	60%	80%	100%
0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0	0	0	0	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	20
72	0	0	0	0	0	0	0	0	0	0	0	20
96	0	0	0	0	20	0	0	0	20	20	0	20
120	0	0	0	0	20	0	0	0	20	40	0	20
144	0	0	0	0	20	20	0	0	20	40	0	20
168	0	0	0	0	20	20	0	0	40	40	20	20
192	0	0	0	0	20	20	0	0	40	40	20	20
216	0	0	0	0	40	40	0	0	40	40	20	20
240	0	0	0	0	40	40	0	0	40	40	20	20
264	0	0	0	0	40	60	0	20	40	40	20	20

Table 20. Cumulative mortality percent of third instar larvae of *Chironomus tentans* in saline groundwater at 15°C using Athabasca River and Winnipeg city water as diluents. Thirty larvae were exposed at each effluent concentration static bioassay conditions.

SGW (%)	Exposure Time			
	48(h)		96(h)	
	Athabasca R. Diluent	Winnipeg Diluent	Athabasca R. Diluent	Winnipeg Diluent
Control	0	0	0	6.7
20%	0	0	0	13.3
40%	0	3.3	6.7	6.7
60%	3.3	16.7	20.0	33.3
80%	100	100	100	100
100%	100	100	100	100
LC50 (% Effluent)	69.0	68.5	62.0	63.0
(95% Confidence)	-	(65.3)	(57.7)	(58.0)
(Interval)	-	(72.9)	(66.7)	(68.4)
Slope function	-	1.056	1.075	1.085

Table 2]. Effect of saline groundwater on emergence success of fourth instar larvae of *Chironomus tentans*. Total possible emergence (%) was calculated at the termination of the bioassays from the sum of the numbers emerged and the number of viable larvae remaining in each test vessel.

SGW (%)	Actual Emergence (%)	Total Possible Emergence (%)
Control	52.5	56.3
5	38.8	41.3
10	47.5	52.5
20	55.0	72.5
40	20.0	58.8
60	0.0	0.0

The hatching of *Chironomus* eggs was delayed by 1 d in 10% effluent and by 2 d in 20 and 40% effluent. Control animals and those exposed to 5% groundwater hatched within 48 h with an approximate success of hatching of 90%. At 20%, effluent larvae survived and successfully dissociated from the egg mass while, at 40%, the larvae hatched but most died prior to leaving the egg mass.

4.6.3.5 *Hexagenia rigida*, Early-Stage Eggs. After 10.5 d of exposure to saline groundwater, the most advanced embryological development (presence of eye-spots) occurred in the control and 20% groundwater (Figures 8 and 9). Development was retarded at 40 and 60% effluent (embryonic form visible but eye-spots not present), and totally inhibited at 80 and 100% effluent (Figures 8 and 9). No differences related to the use of Winnipeg city (Figure 8) or Athabasca River (Figure 9) waters as diluents were observed in the development of early-stage mayfly eggs.

Hatching of early-stage eggs began after 15 d of incubation in control vessels. The initiation of hatching was delayed at groundwater concentrations of 20, 40, and 60% by 1, 2, and 4 d, respectively, and was similar in Winnipeg city water (Figure 10, Table 69) and Athabasca River (Figure 11, Table 70) diluents. The success of hatch was similar in controls and 20% groundwater, slightly reduced at 40%, and less than 50% at 60% effluent. At 60% groundwater, a large proportion of the nymphs died while freeing themselves from the egg case and actual survival to the nymph stage was therefore less than 50%. No hatching occurred in eggs exposed to 80 and 100% effluent concentrations.

4.6.3.6 *Hexaginia rigida*, Late-Stage Eggs. After 10 d exposure to groundwater, hatching of late-stage eggs occurred in control tests and effluent dilutions of 20 to 60% (time = 0, Figures 12 and 13). Percent hatch at this time was similar in controls and 20% effluent, reduced by about 25% at 40% effluent, and by more than 80% at 60% effluent in both Winnipeg city water (Figure 12, Table 71)

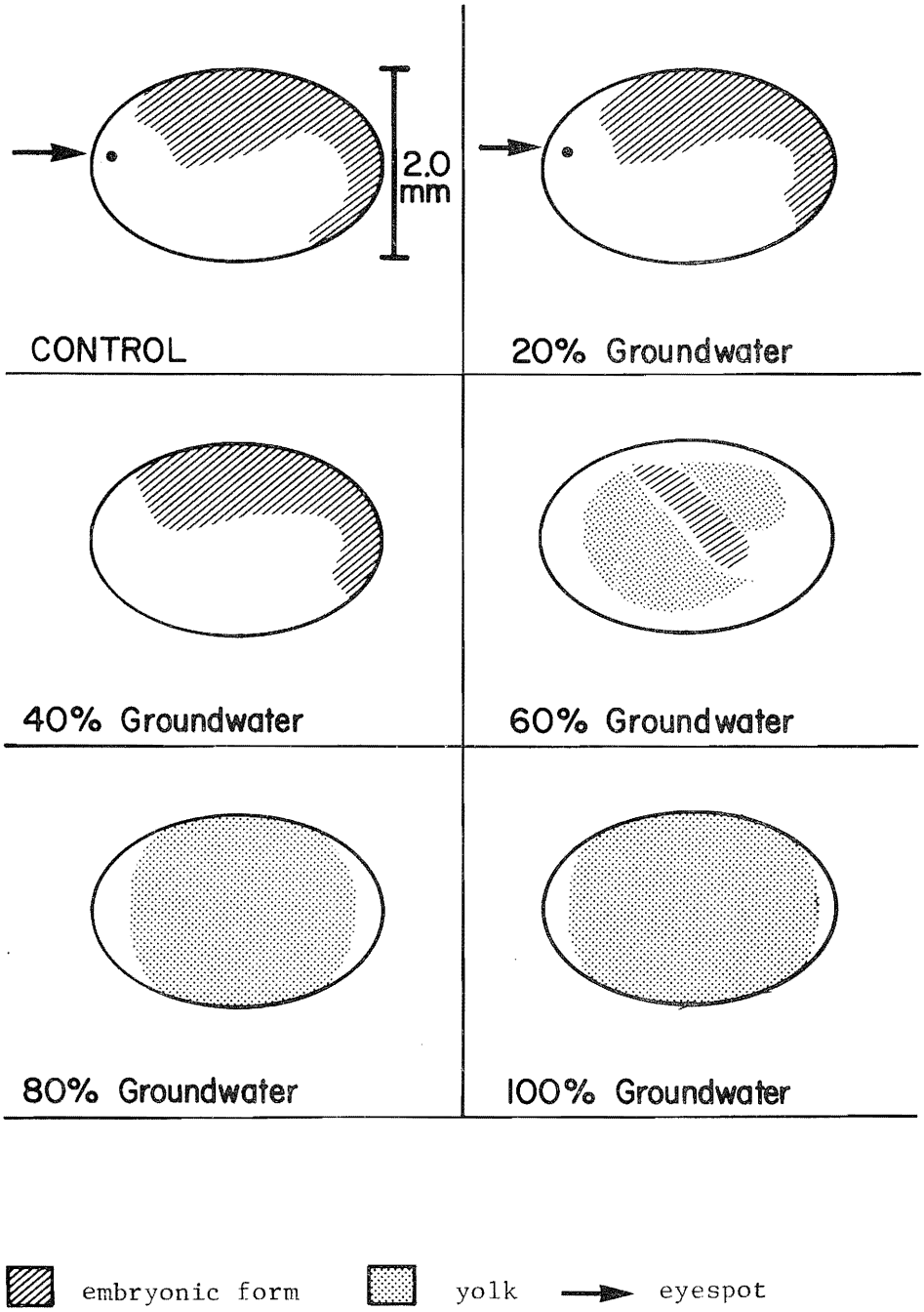

Figure 8. Embryological development of early-stage eggs of *Hexagenia rigida* for 10.5 d in various concentrations of saline groundwater in Winnipeg city water diluent (drawn from photographs).

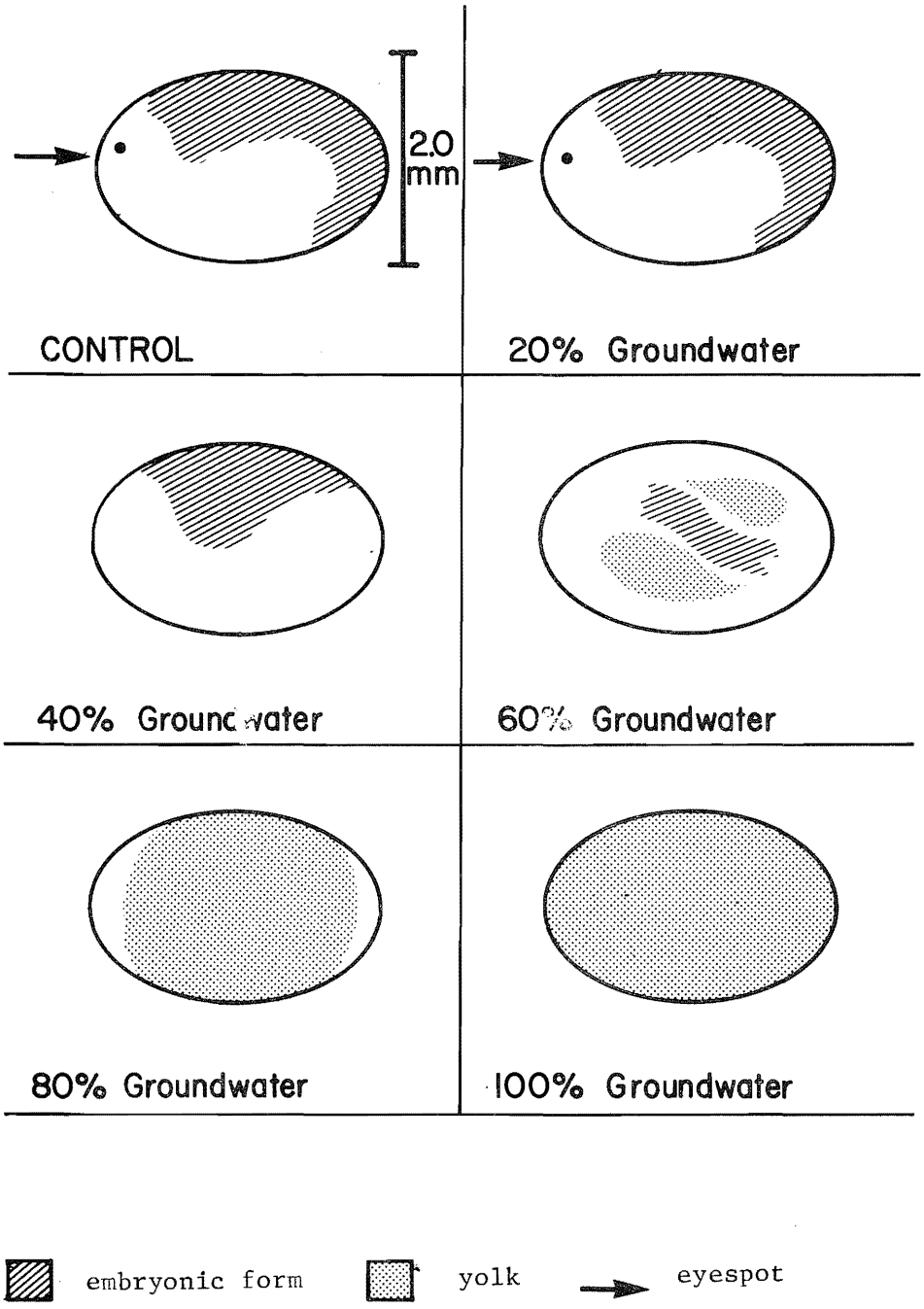

Figure 9. Embryological development of early-stage eggs of *Hexagenia rigida* after incubation for 10.5 d in various concentrations of saline groundwater in Athabasca River water diluent (drawn from photographs).

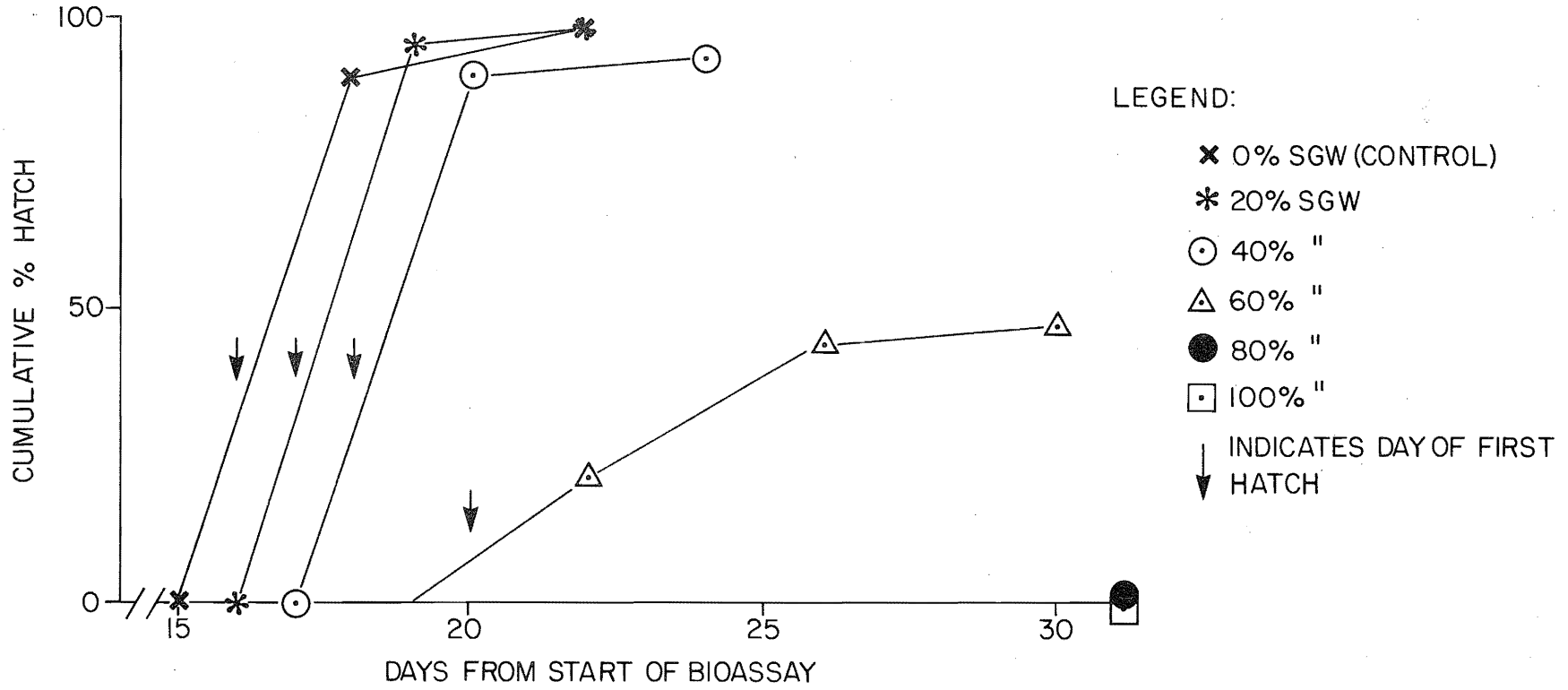

Figure 10. Cumulative percent hatch of early-stage eggs of *Hexagenia rigida* in dechlorinated Winnipeg city water after incubation for 10.5 d in saline groundwater (SGW). The period of saline groundwater exposure occurred during the first 11 d of the bioassay.

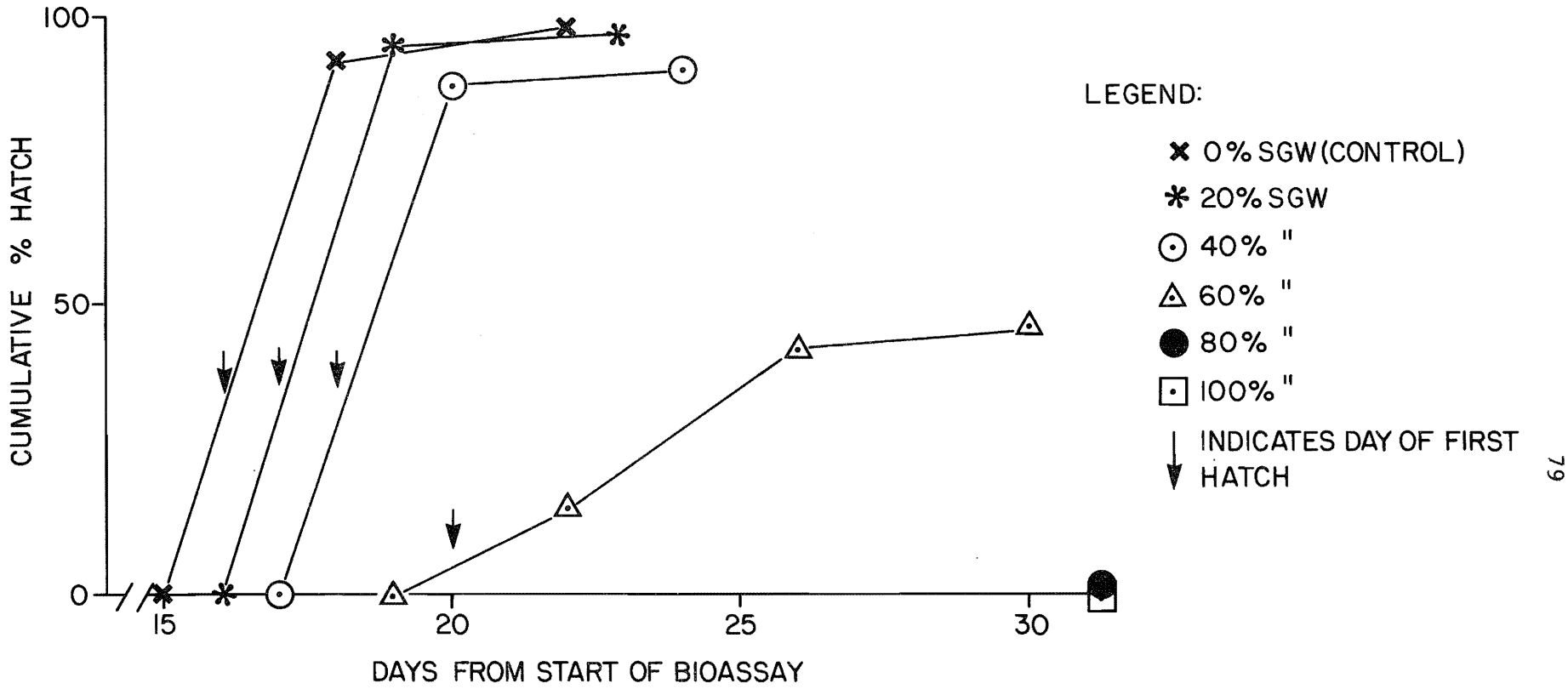

Figure 11. Cumulative percent hatch of early-stage eggs of *Hexagenia rigida* in Athabasca River water after incubation for 10.5 d in saline groundwater (SGW). The period of saline groundwater exposure occurred during the first 11 d of the bioassay.

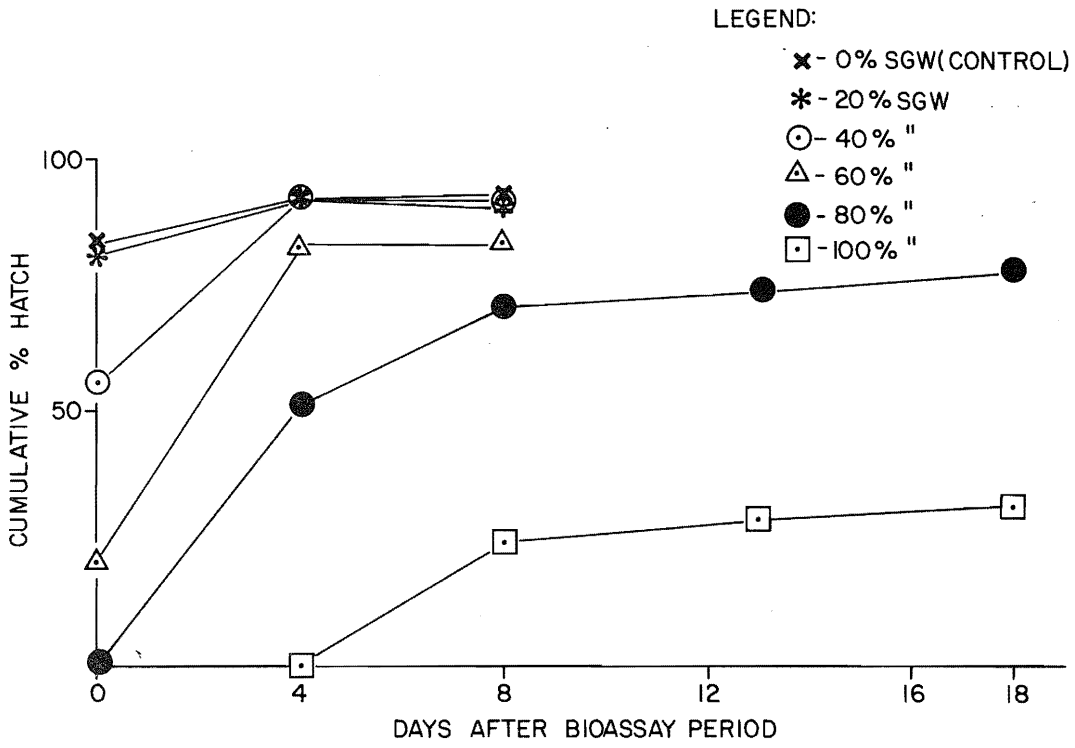

Figure 12. Cumulative percent hatch of late-stage eggs of *Hexagenia rigida* in dechlorinated Winnipeg city water after incubation for 10 d in saline groundwater (SGW). The abscissa is the time after transfer from groundwater to pure diluent.

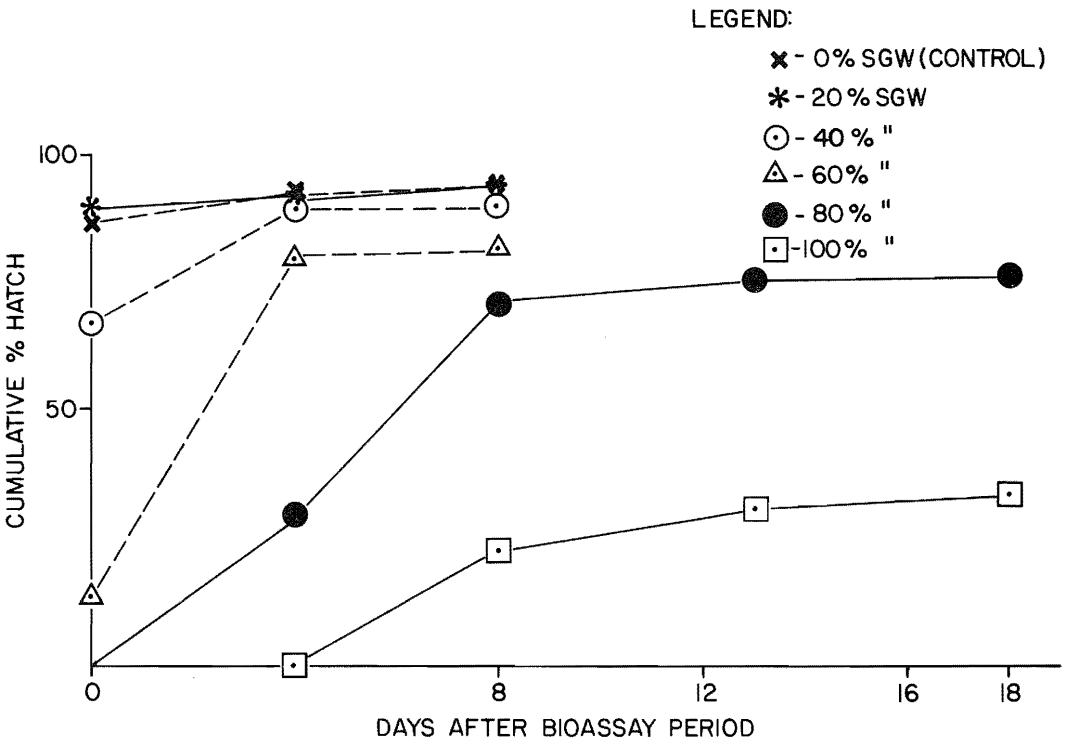

Figure 13. Cumulative percent hatch of late-stage eggs of *Hexagenia rigida* in Athabasca River water after incubation for 10 d in saline groundwater (SGW). The abscissa is the time after transfer from groundwater to pure diluent.

and Athabasca River (Figure 13, Table 72) diluents. No hatching had occurred in 80 or 100% effluent concentrations. At this time the saline groundwater was replaced with diluent and hatching monitored for a further 18 d. The results demonstrate that hatching was increased after removal of groundwater. The increase in cumulative percent hatch was progressively reduced in eggs which had been exposed to higher concentrations of groundwater and delayed at concentrations $\geq$80%. No difference in response was observed relating to the two diluents employed.

4.6.4 Discussion

Several approaches using different test conditions, species, and developmental stages were employed in these studies to assess the toxicity of saline groundwater. Such diversity makes detailed comparison of specific tolerances to the effluent somewhat difficult. In general, however, the present results indicate that the five species exhibited increasing tolerance to saline groundwater in the following order: *Daphnia<<Chironomus<Hexagenia<<Orconectes<Artemia*. *Daphnia* are known to be extremely sensitive to many aquatic toxicants and it is not surprising that they represent the most sensitive test organism in this study. *Artemia* are exceptionally tolerant to high salinity water and the complete tolerance of groundwater by this species lends support to the observations of McMahon et al. (1977) which suggested that the toxicity of saline groundwater may relate in part to its salinity. Relatively little is known of the tolerances of the remaining species to toxicants. McMahon, using larval mayflies *Heptagenia marginalis* and *Paraleptophlebia bicornuta*, observed 7 d LC50's of approximately 50 and 30% groundwater, respectively, at 5°C. He also demonstrated that the toxicity to *Heptagenia* increased as the incubation temperature was elevated to 15°C. McMahon's studies were conducted using larvae collected from natural populations and the results may not be strictly comparable to those from the eggs of Red River populations used in the present study.

In addition, both the *Hexagenia* and *Chironomus* results indicate that the earlier developmental stages are least tolerant of saline groundwater.

Although most of the invertebrate species were tested using only the July composite effluent, the *Daphnia* experiments on the July and December composites would suggest that the toxicity of different groundwater samples may vary by an order of magnitude. In view of the complex changes occurring in this effluent on a seasonal basis and during storage, the invertebrate toxicity data must be interpreted with caution. On a comparative basis, however, it would appear that, with the exception of *Daphnia magna*, the invertebrate species tested exhibited a tolerance to saline groundwater which is as great or greater than that observed in rainbow trout or whitefish.

5. ACUTE TOXICITY OF VANADIUM AND COPPER

5.1 EFFECTS OF pH ON VANADIUM AND COPPER TOXICITY TO RAINBOW TROUT

(Principal Researchers: W.R. Lillie and G. Watts)

5.1.1 Introduction

The acidification of water by industrial processes (Likens 1974; Reuss 1975) can produce direct deleterious effect on aquatic organisms as well as modify the toxicity of other chemicals. Reviews of these effects have been provided by Duodorff and Katz (1950), McKee and Wolf (1963), European Inland Fisheries Advisory Committee (1969), and Gregory (1974).

Vanadium emissions to air are predominantly from oil-fired industrial furnaces, with a smaller amount coming from catalytic cracking of fuel oil (Tullar and Suffet 1975). Tullar and Suffet also point out that this vanadium may be in close association with SO_2 and that it may catalyse the reaction to form H_2SO_4. Since Athabasca bitumen contains considerable amounts of vanadium (Camp 1974) which may be released during mining and/or upgrading processes, and sulphur which could be released to the environment, it was decided to conduct preliminary acute bioassays to assess the pH-dependence of vanadium toxicity. Copper, a relatively widespread heavy metal toxicant for which considerable toxicological information has been published, was tested for comparison with vanadium.

5.1.2 Methods

The diluent used was chlorinated Winnipeg city water (hardness 90 mg/L as $CaCO_3$, pH 7.3). Other chemical parameters are shown in Tables 61, 73, and 74. Dechlorination was performed by first passing the water through an ultra-violet dechlorination unit; then through activated charcoal.

The bioassay system described by Harrison et al. (1975) was used with two modifications. A dilutor which produced 10 concentractions (at a 75% dilution rate) instead of five concentrations

in duplicate was used. An additional modification was made by passing the incoming dechlorinated water through a 20 L header tank. A pH titrator (Radiometer TTT-2) controlled a pump which delivered acid (H_2SO_4) or base (NaOH) into this tank to produce water of pHs 6, 7, 8, or 9. Aeration partially removed CO_2 liberated from acid addition. This water was then delivered to the dilutor system.

Hatchery-raised rainbow trout (*S. gairdneri*) fingerlings were used as the test organisms. Idaho stock were used for the copper bioassays and the 14 d vanadium bioassays. Nesquali stock trout were used for the acute vanadium bioassays. All fish were acclimated at least 3 wks to 15°C and a 12-12 h photoperiod before experimental use. Fish size for each experiment is shown in Tables 73 and 74.

A stock solution of 20 g V/L was prepared with vanadium pentoxide. To assist in dissolving V_2O_5 and to keep the solutions at the proper pH, NaOH was used at the rate of 0.71 g NaOH/g V_2O_5 for pH 9, and 0.5 g NaOH/g V_2O_5 for pHs 6, 7, and 8. After the pH 6 V_2O_5 stock was dissolved, 0.2 mL concentrated H_2SO_4/g V_2O_5 was added to lower the pH of the stock. All solutions were completely dissolved after 2 d of mixing. The pH 6 stock, however, turned orange after the acid addition. Stock solutions of copper (386-965 mg Cu/L) were prepared from regeant grade anhydrous copper sulphate.

Twenty litre plastic-lined test vessels containing 10 fish per vessel were employed in these bioassays. The fish were allowed to acclimate to the test pH for 60 h at a flow rate of 100 mL/min before addition of toxicant. Toxicant was then added directly to the test vessels to produce desired concentrations and the flow-through dilutor system activated. Dissolved oxygen and temperature were monitored daily and pH thrice daily in all bioassays. Analyses for the test chemical (V or Cu) were performed every second day and analysis for NH_3, PO_4, CO_2, Cl, SO_4, Na, K, Ca, Mg, and Fe were performed weekly. These data are recorded in Tables 73 and 74.

Mortalities were monitored three times daily in the 96 h acute bioassays and daily in the 14 d bioassay. Lengths and weights were recorded with time of death. At the termination of all bioassays,

the surviving fish were removed, weighed, and measured, and three animals from each concentration were preserved for histological examination.

The LC50 values were determined using the log-probit BMDO3S computer method. Median survival times were determined graphically using the method described by Litchfield (1949).

5.1.3 Results

5.1.3.1 Vanadium. The MST of trout in vanadium pentoxide are presented in Table 22. Fish survival time increased with increasing pH from pH 7 to 9. The relationship between median survival time and vanadium concentration was approximately linear (Figure 14) over the concentration range tested for all values of pH, although the data for pH 9 exhibit a slight degree of curvature.

The LC50 values and their 95% confidence intervals are presented in Table 23 for pH 6 to 9. For any exposure time, the LC50 estimates could be ranked in relation to pH such that the LC50 at pH 6 > pH 9 > pH 8 > pH 7 in increasing order of toxicity. The slope function was between 3.7 and 10.6 for all LC50 estimates at pH 7, 8, and 9 but was less than 1.5 for both estimates at pH 6. Since the slope function is an index of population variability, it is probable that the toxic response of the population was more uniform at pH 6 than at pH 7-9. A double logarithmic plot of exposure time versus LC50 (Figure 15) indicated that, although the toxicity of vanadium differed at the different hydrogen ion concentrations, the time-toxicity relationships were essentially parallel. In addition, with the possible exception of pH 9, no indication of an asymptote to the time axis was observed (Figure 15) within the 96 h bioassay period and therefore an incipient lethal level (ILL) for vanadium could not be defined. A 14 d bioassay (Table 24, Figure 16) also failed to produce an ILL for vanadium and indicates that, if such an ILL exists, it is less than 20 mg/L. Histological examination indicated that many of the fish used in the 14 d bioassay had nephro-calcinosis and this may account for the lack of a well-defined cessation of mortality

Table 22. Effect of pH on median survival time (MST) of rainbow trout *(Salmo gairdneri)* in V_2O_5.

Vanadium Concentration (mg/L)	MST (h)			
	pH 6	pH 7	pH 8	pH 9
40		30.2	31	46
30	52	30.2	44	47
22.5		48	52	62
16.8		53	82	92
12.6	2	80	82	
9.5		96		

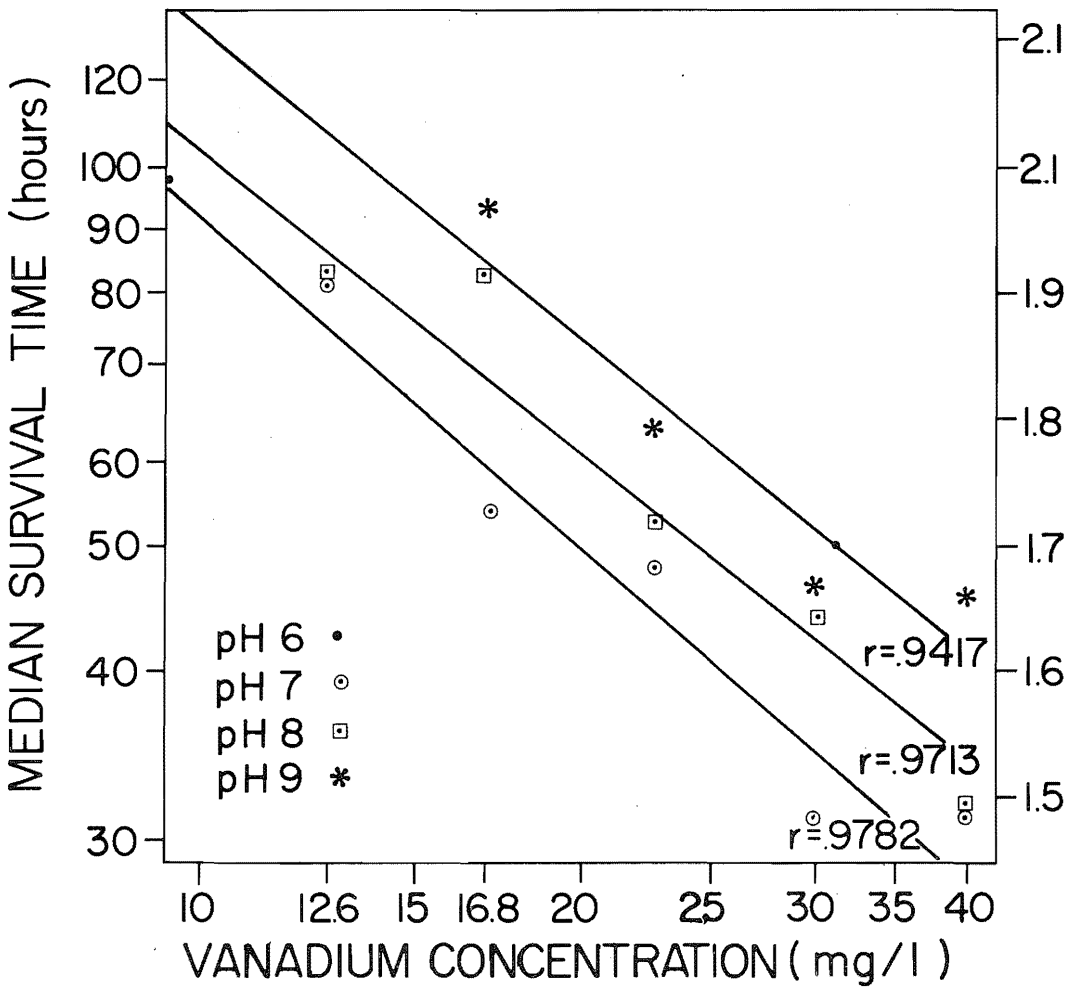

Figure 14. Effect of pH on the median survival time of juvenile rainbow trout *(Salmo gairdneri)* exposed to vanadium in a 96 h continuous flow bioassay.

Table 23. The LC50 (mg V/L) of V_2O_5 to rainbow trout *(Salmo gairdneri)* in a 96 h bioassay at pH 6 to 9.

h		pH 6	pH 7	pH 8	pH 9
31	LC50		34.89		
	95% Conf. Interval		18.27-62.62		
	Slope Function		3.735		
40	LC50			34.99	
	95% Conf. Interval			20.39-60.05	
	Slope Function			10.594	
48	LC50		20.43	24.68	31.83
	95% Conf. Interval		13.99-29.83	16.90-36.06	17.57-57.68
	Slope Function		6.384	7.541	3.874
72	LC50	36.91	13.70	16.73	18.09
	95% Conf. Interval	10.63-128.19	9.47-19.83	11.17-24.05	12.56-26.08
	Slope Function	1.421	5.757	5.983	6.294
96	LC50	21.75	8.15	11.43	15.73
	95% Conf. Interval	7.67-61.65	5.12-12.99	8.30-15.74	11.34-21.81
	Slope Function	1.199	5.191	6.659	7.049

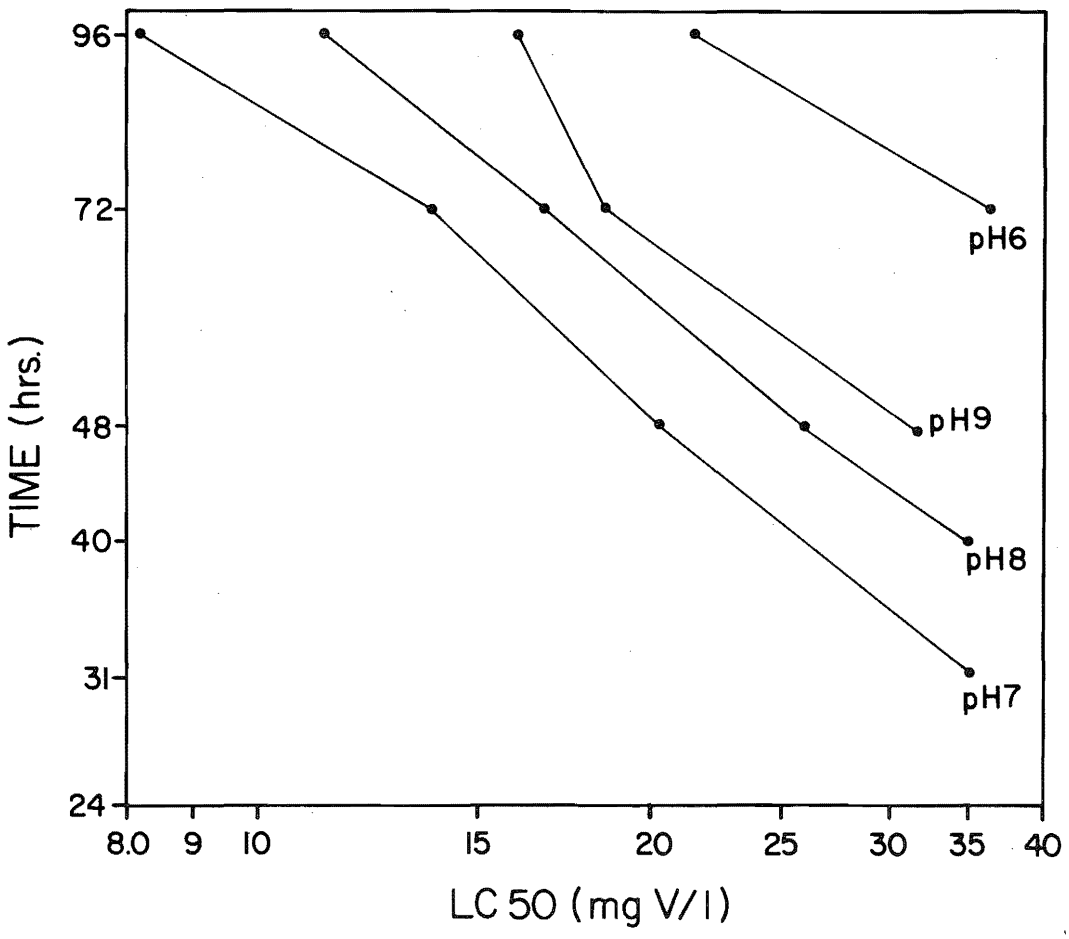

Figure 15. Effect of pH on the LC50 of vanadium to rainbow trout *(Salmo gairdneri)* in a 96 h continuous flow bioassay.

Table 24. The LC50 of V_2O_5 to rainbow trout *(Salmo gairdneri)* in a 96 h bioassay at pH 8.

Time (H)	LC50 (mgV/L)	95% Confidence Interval	Slope Function
96	6.43	3.92-10.52	4.898
120	4.34	3.04-6.19	5.985
144	3.21	2.07-4.98	4.854
168	2.81	1.96-4.04	5.329
192	2.49	1.78-3.48	8.485
216	2.41	1.78-3.28	9.373
240 + 264	2.28	1.62-3.20	8.383
288 + 312	2.13	1.44-3.14	6.221
336	1.95	1.22-3.12	5.108

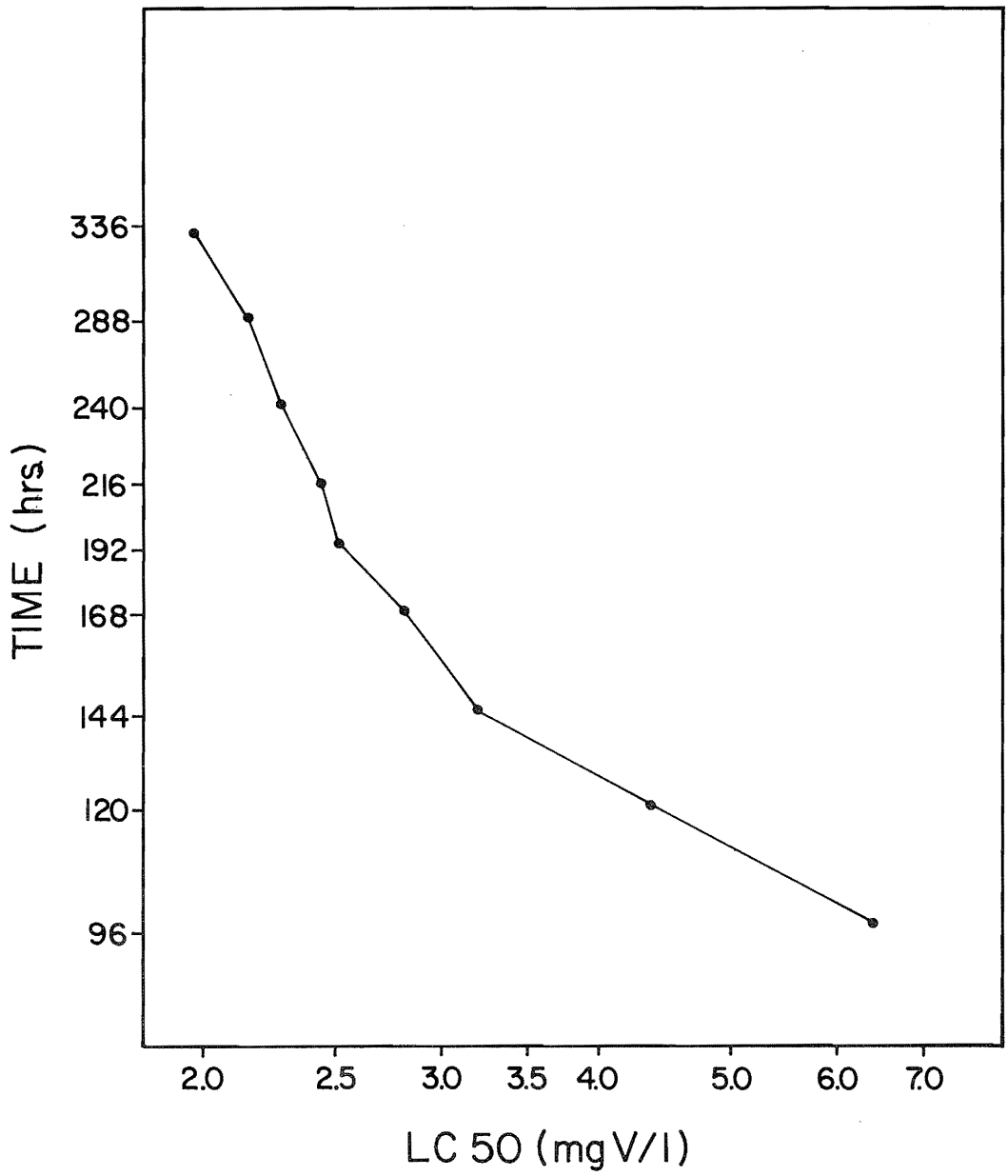

Figure 16. Acute toxicity of vanadium to rainbow trout *(Salmo gairdneri)* in a 96 h continuous flow bioassay.

within the experimental period.

5.1.3.2 Copper. The MST's of trout in copper sulphate are presented in Table 25. These data failed to demonstrate a consistent relationship between survival and toxicant concentration over the pH range of 6 to 9. This may be the result of mortality occurring in the first 24 h in which insufficient checks were made to provide reliable MST estimates.

The LC50's, 95% confidence limits, and slope functions for the copper bioassays at pH 6 to 9, are presented in Table 26. In a manner similar to that observed in the vanadium bioassays, the slope functions observed at pH 6 were consistently less than those at higher pH. The time-LC50 relationships (Figure 17) were approximately parallel at pH 7, 8, and 9. At pH 6, however, toxicity appeared to increase after prolonged exposure. Thus, at 24 h exposure the LC50/pH could be ranked in decreasing order of toxicity, pH $7<6\leq8<9$, while at 48 to 96 h the order was pH $6<7<8<9$.

A comparison of the toxicity of vanadium and of copper in relation to pH is presented in Figure 18. It is apparent that the slopes of all the curves are approximately equal in the pH range of 7 to 9, and that copper is approximately two orders of magnitude more toxic than vanadium. The parallelism in the toxicity-pH relationships indicates that the modification of toxicity by pH is similar for both metals and may suggest a similar target organ for their toxic action.

5.1.4 Discussion

5.1.4.1 Vanadium. In exploratory tests, Tarzwell and Henderson (1956, cited by McKee and Wolf 1963) found the 96 h TLm of the fathead minnow to be 4.8 mg V/L in soft water and 30 mg V/L in hard water using vanadyl sulphate, and 13 mg/L in soft water and 55 mg/L in hard water using vanadium pentoxide. Similar tests with the bluegill sunfish gave 96 h TLm values of 6 mg V/L in soft water and 55 mg V/L in hard water with vanadyl sulphate. Sprague (in prep.)

Table 25. The effect of pH on median survival times (MST) of rainbow trout *(Salmo gairdneri)* in copper sulphate.

Copper Concentration (mg/L)	MST (h)			
	pH 6	pH 7	pH 8	pH 9
2.000			14.7	10.9
1.500			13.0	10.9
1.125			10.0	13.0
0.844			10.0	14.5
0.800	13.6	9.2		
0.633			12.4	16.5
0.600	20.2	11.[illegible]		
0.475			12.4	26.5
0.450	20.2	12.4		
0.356			21.0	29.0
0.338	21.3	14.5		
0.267			21.4	37.0
0.253	34.0	20.2		
0.200				
0.190		24.0		

Table 26. The LC50 (mg Cu/L) of copper sulphate to rainbow trout *(Salmo gairdneri)* at pH 6 to 9.

h		pH 6	pH 7	pH 8	pH 9
24	LC50	0.258	0.182	0.256	0.432
	95% Conf. Interval	0.158-0.421	0.133-0.249	0.193-0.338	0.293-0.637
	Slope Function	3.919	6.735	13.128	6.203
48	LC50	0.143	0.155	0.213	0.278
	95% Conf. Interval	0.071-0.291	0.105-0.227	0.165-0.275	0.199-0.389
	Slope Function	2.998	5.523	14.308	8.512
72	LC50	0.114	0.141	0.191	0.270
	95% Conf. Interval	0.061-0.214	0.084-0.238	0.118-0.308	0.191-0.382
	Slope Function	3.388	4.088	7.667	8.232
96	LC50	0.111	0.137	0.191	0.263
	95% Conf. Interval	0.067-0.210	0.083-0.225	0.118-0.308	0.192-0.360
	Slope Function	3.719	4.265	7.667	9.064

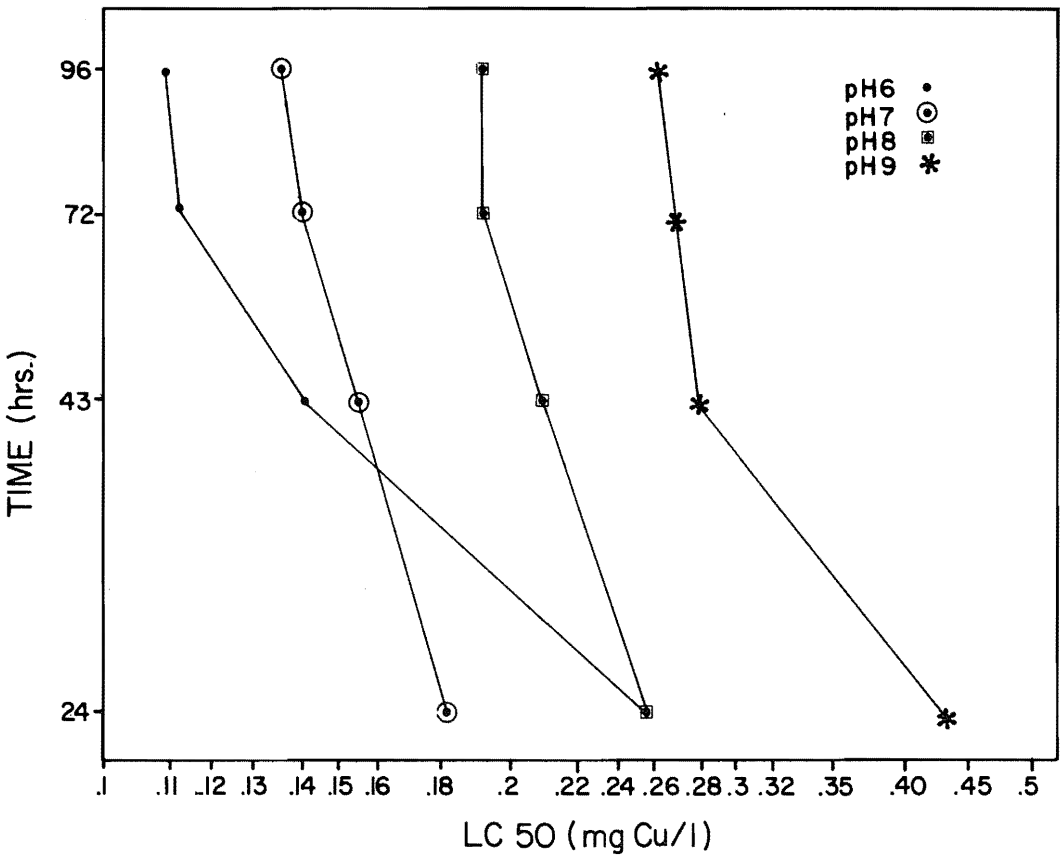

Figure 17. Effect of pH on the LC50 of copper to rainbow trout (*Salmo gairdneri*) in a 96 h continuous flow bioassay.

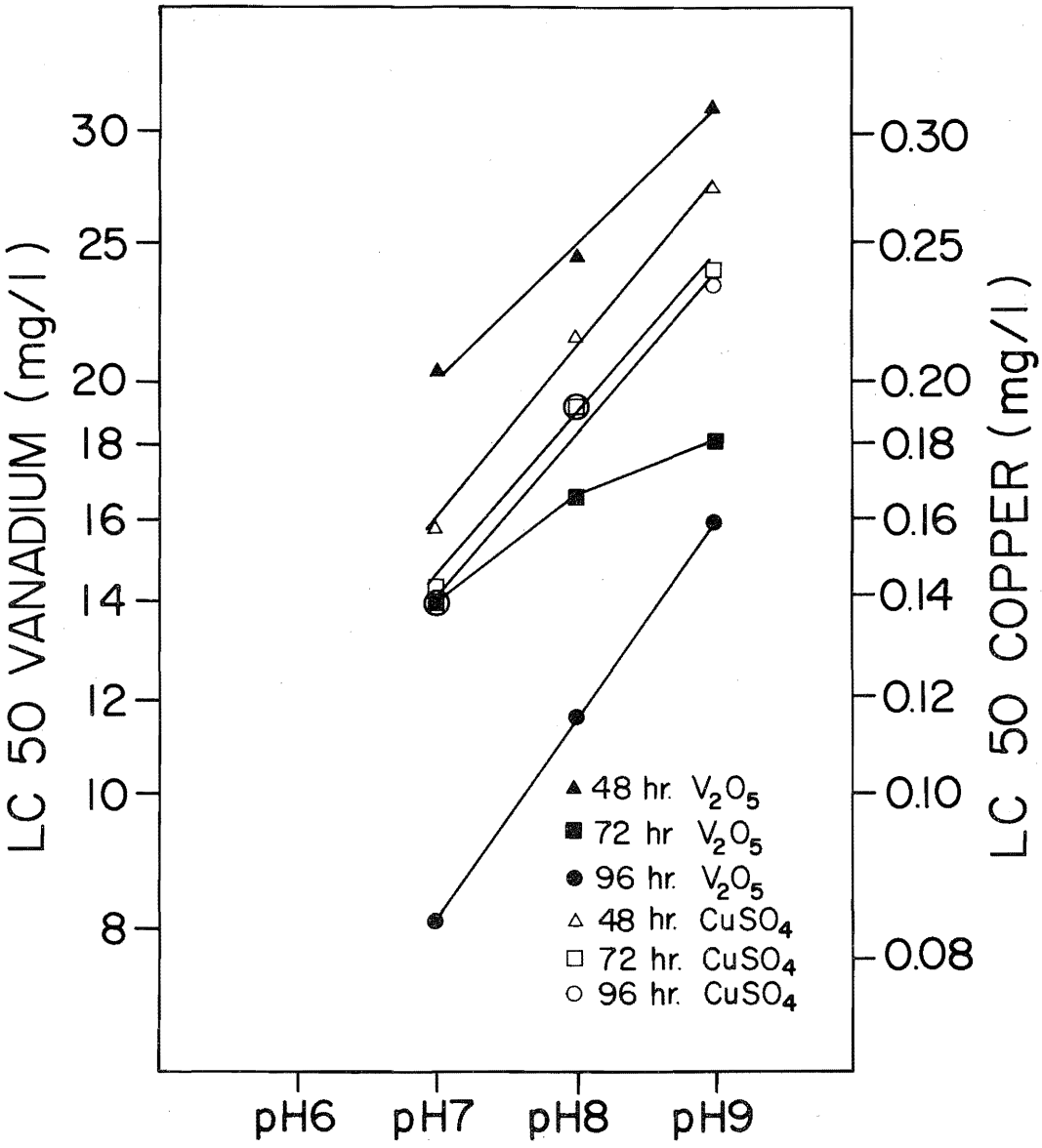

Figure 18. A comparison of the effect of pH on the acute lethality of copper and vanadium to rainbow trout *(Salmo gairdneri)*.

found the 4 week LC50 for flagfish to be 1.8 mg/L and found that an ILL was not reached. They also found 22.4 mg/L to be lethal to zebrafish in 32 h. The data in this report agree with the above findings.

Sprague (in prep.) has discussed vanadium chemistry in relation to pH. It would appear that HVO_4^{2-} is the dominant ionic form at pH 9 and that $H_2VO_4^-$ is the dominant form at pH 7. At pH 8, each species may exist in approximately equal amounts. The shift between these two forms may be reflected in the higher toxicity at pH 7, indicating that $H_2VO_4^-$ is the more toxic of the two forms. This is a simplification since water hardness, alkalinity, or chemical chelating or complexing may be involved as well.

Lowering the pH of the stock solution (20 000 mg/L V) to 6.0 produces a change in colour from clear to orange in the solution. Sprague (in prep.) states that complicated orange-coloured polymers may be formed at high concentrations below pH 6.5. After dilution to 40 mg V/L, the solution turned yellow, a condition which persisted in the test tanks throughout the experiment. This observation may indicate that a different ionic species, or a polymer that did not break down quickly, from that at higher pH's was formed and may explain the relatively low toxicity at pH 6.

Chemical factors affecting copper toxicity are discussed extensively in the literature (Shaw and Brown 1974; Brown et al. 1974; Pagonkopf et al. 1974; Stiff 1971a, 1971b; Sylva 1976; Sellen and Martell 1964). In general, more of the total copper exists as Cu^{++} in waters with low pH, hardness, and alkalinity. The data in this report do not correlate with the theoretical concentration of Cu^{++} in relation to pH, which would indicate that more factors are involved. Interpretation is complicated by the fact that changing pH can change hardness and alkalinity. The effects of the latter were not examined in these experiments. It may be beneficial to repeat these experiments with more extensive monitoring of hardness, alkalinity, CO_2, and free Cu^{++}.

Vanadium and copper toxicity decreases in the same proportion with increasing pH. This similarity strongly suggests that a

common parameter is affecting their toxicities. This parameter may be a common pH effect on chemical dissociation, ionic form, chelation, water hardness, or alkalinity. The effect may also be directly on the fish physiology; gill permeability, ionic regulation, or blood chemistry could be changed by differing hydrogen ion concentrations. Further experimentation will be required to isolate toxicity factors.

5.2 HISTOPATHOLOGICAL EFFECTS OF VANADIUM AND COPPER ON RAINBOW TROUT

(Principal Investigators: R. Evans and D. Klaprat)

5.2.1 Introduction

As outlined previously, histopathological examination of organisms exposed to toxicants provides insight into modes of toxic action, suggests target organs, and assists in the interpretation of physiological effects observed in associated studies. Sublethal histological bioassays of toxicants also hold promise but have not adequately been developed for most aquatic pollutants. The following studies were undertaken with the primary purpose of providing support for the various acute lethality, physiological, and biochemical studies of vanadium and copper toxicity and, secondarily, to examine the feasibility of developing a histopathological bioassay for heavy metals.

5.2.2 Methods

The rainbow trout (*S. gairdneri*) employed in this study were obtained from the survivors of the acute lethality bioassays of vanadium and copper described in Section 5.1 of this report. All histological procedures and histopathological terminology employed have been previously described in Section 4.3.2.

5.2.3 Results

5.2.3.1 Histopathological Effects of Vanadium

5.2.3.1.1 Kidney Histopathology. Exposure of juvenile rainbow trout to vanadium concentrations which were not lethal within 96 h at pH 6 to 9 induced histopathological effects similar to those observed in saline groundwater-exposed fish. Cloudy swelling, hydropic degeneration (Figure 19, Section 11), and hyaline degeneration (Figure 19, Section 12) were all present in at least some of the test animals. A further abnormality in which the proximal and distal tubules were dilated (Figure 19, Sections 12, 13) was also observed in vanadium-exposed trout. The incidence and degree of each of these histopathological effects in relation to pH and vanadium concentration after 96 h exposure is presented in Table 27. It is clear that at pH 6 to 8, the histopathological effects of vanadium on trout kidney were similar, and in general induced a similar degree of damage at 5.3 to 16.8 mg V/L. At 3.0 and 4.0 mg V/L, histopathological effects were reduced to frequent incidences of cloudy swelling and occasional observations of hyaline degeneration and hydropic degeneration. The histopathological effects observed at pH 9 appear to be more severe than those occurring at less alkaline conditions, although additional studies are required to confirm this observation.

The effects of longer term (15 d) exposure to vanadium on kidney histology are shown in Table 28. All abnormalities observed in the 96 h exposure studies were present in fish exposed for 15 d which suggests that tissue repair does not occur under these test conditions. In addition, it is clear that histological damage continues to develop during longer exposures to vanadium since virtually all the fish exhibited severe lesions at 3.75 mg V/L after 15 d exposure compared to the relatively minor damage observed at 4.0 mg V/L after 4 d of exposure (Table 27). The lowest vanadium concentration tested in the 15 d experiments (0.375 mg/L) was sufficient to induce mild histopathological aberrations, which suggests that much longer term exposures may be required to define the concentration of

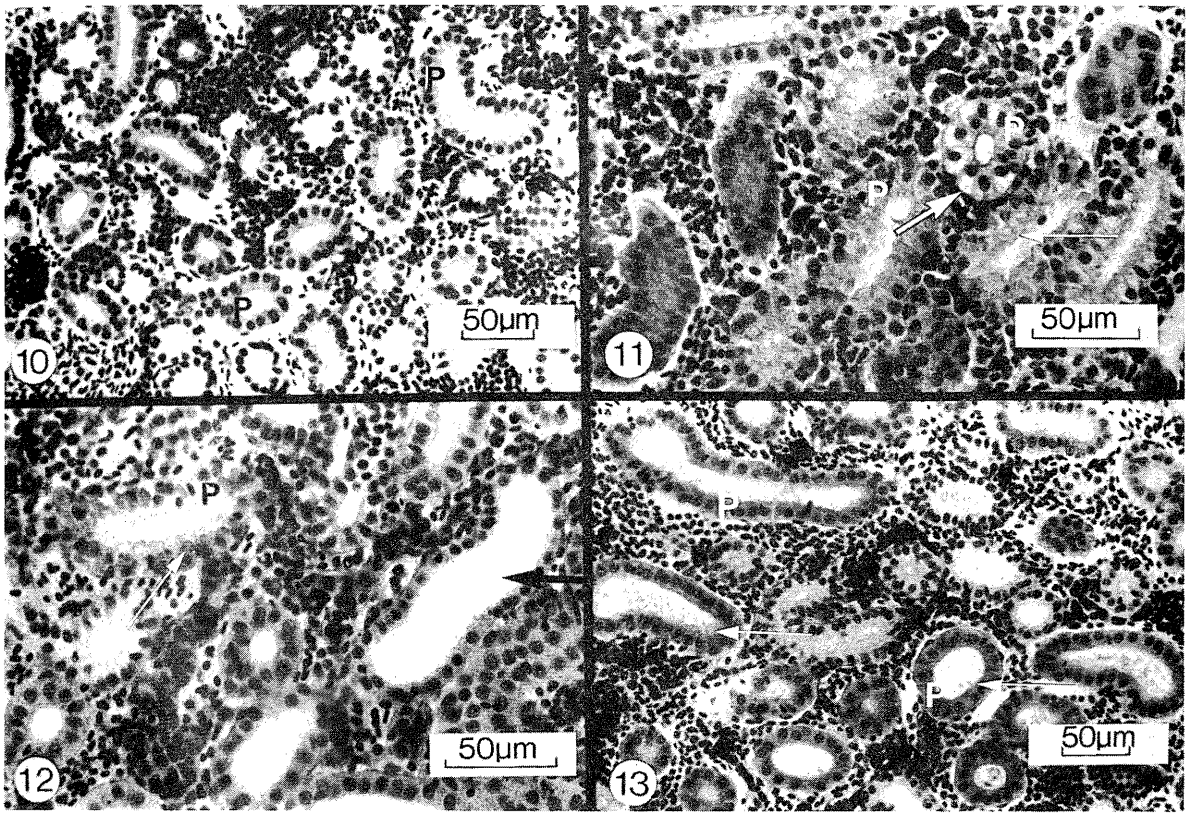

Figure 19. Longitudinal section of the posterior kidney of rainbow trout *(Salmo gairdneri)* showing: 10. normal morphology of proximal tubule: 11. cloudy swelling (small arrow) and hydropic degeneration (large arrow); 12. hyaline degeneration (small arrow) and tubule dilation (large arrow); and 13. proximal tubule dilation (arrows). Sections 11 to 13 are from trout exposed to vanadium (7-17 mg/L) for 96 h.

Table 27. Histopathological effects of vanadium on kidney of rainbow trout (*Salmo gairdneri*) after 96 h of exposure.

	Number of Fish	Relative Degree[a] of Hyaline Degeneration	Hydropic Degeneration	Tubule Dilation	Cloudy Swelling
pH 6.0					
22.5 ppm	3	2	1	2	3
16.8 ppm	3	1	0	1	3
12.6 ppm	3	1	0	1	3
9.5 ppm	2	1	1	2	3
7.1 ppm	2	0	0	1	3
5.3 ppm	2	0	0	0	3
4.0 ppm	2	1	0	2	2
3.0 ppm	2	0	0	1	1
Control	2	0	0	0	0
pH 7.0					
12.6 ppm	2	2	0	2	3
9.5 ppm	3	1	2	1	2
7.2 ppm	3	1	1	1	3
5.3 ppm	3	1	0	1	2
4.0 ppm	2	1	1	0	2
3.0 ppm	2	1	0	0	2
Control	2	0	0	0	0
pH 8.0 Replicate 1					
16.8 ppm	1	1	1	1	3
12.6 ppm	3	1	0	1	3
9.5 ppm	3	3	3	2	3
7.1 ppm	3	2	0	3	3
5.3 ppm	2	1	2	2	2
4.0 ppm	2	0	0	0	2
3.0 ppm	2	0	0	0	1
Control	2	0	0	0	0
pH 8.0 Replicate 2					
16.8 ppm	3	1	1	3	3
12.6 ppm	3	2	1	3	3
9.5 ppm	3	1	0	1	3
7.1 ppm	2	2	0	1	3
5.3 ppm	2	1	0	0	3
4.0 ppm	2	1	0	0	2
3.0 ppm	2	1	1	1	1
Control	2	0	0	0	0

Continued...

Table 27. Concluded.

	Number of Fish	Relative Degree[a] of Hyaline Degeneration	Hydropic Degeneration	Tubule Dilation	Cloudy Swelling
pH 9.0					
16.8 ppm	3	3	3	1	3
12.6 ppm	3	3	3	1	3
9.5 ppm	3	2	1	1	3
7.1 ppm	3	2	0	2	2
5.3 ppm	2	2	0	1	2
4.0 ppm	2	2	2	1	3
3.0 ppm	2	2	0	0	2
Control	2	0	0	0	0

[a]Ranking code used in this table:

0 - no aberration observed

1 - small degree of aberration

2 - moderate degree of aberration

3 - large degree of aberration

Table 28. Histopathological effects of vanadium on kidney of rainbow trout (*Salmo gairdneri*) after a 15 d exposure period at pH 8.0.

Vanadium (mg/L)	Number of Fish	Relative Degree of[a]			
		Cloudy Swelling	Hyaline Degeneration	Hydropic Degeneration	Tubule Dilation
5.000	1	3	3	1	2
3.750	3	3	3	3	2
2.870	3	2	0	1	2
2.110	3	2	1	1	2
1.580	3	1	0	2	0
1.190	3	2	0	1	0
0.890	3	1	0	0	0
0.670	3	2	0	0	0
0.500	3	0	0	0	0
0.375	3	1	0	1	0
Control	2	0	0	0	0

[a] Ranking code as in Table 27.

vanadium which produces no observable effect on kidney histology.

5.2.3.1.2 Gill Histopathology. Gill morphology was normal (Figure 20, Section 14) in control fish in the pH range of 6 to 9 (Table 29). Hyperplasia (Figure 20, Sections 15 to 16), however, occurred in virtually all fish exposed to vanadium concentration of 3.0 to 22.5 mg/L regardless of pH (Table 29). Hematomas (Figure 20, Section 15 and 17) appeared randomly among the fish with no evident pattern relating to vanadium concentration and/or pH. Epithelial thickening of the secondary lamellae (Figure 20, Section 17) was observed in 65 of the 66 vanadium-exposed fish examined in this study.

Although the degree of severity of the three histological abnormalities was apparently independant of pH or vanadium concentration, it is clear that much lower toxicant concentrations and longer exposure times must be tested to determine the minimum vanadium concentration required to produce these histopathological changes.

5.2.3.1.3 Liver Histopathology. A total of 66 vanadium-exposed and 30 control fish were examined for histopathological lesions of the liver. No abnormalities were observed in any of the treated fish, indicating that the liver is not a target organ of this toxicant.

5.2.3.2 Histopathological Effects of Copper. Histological examination of the rainbow trout employed in the bioassays of copper at pH 6, 7, and 8 demonstrated that some of these fish were suffering from nephrocalcinosis. Since this infection would interfere with interpretation of toxicant effects, histopathological analyses were discontinued. The fish employed in copper bioassays at pH 9.0, however, were free of nephrocalcinosis and were subject to histological examination.

5.2.3.2.1 Kidney Histopathology. The lesions observed in the kidneys of trout exposed to copper (Table 30) were similar but generally more severe than those observed in vanadium-exposed fish. Cloudy swelling (Figure 21, Sections 19 and 20) and hydropic degeneration (Figure 21,

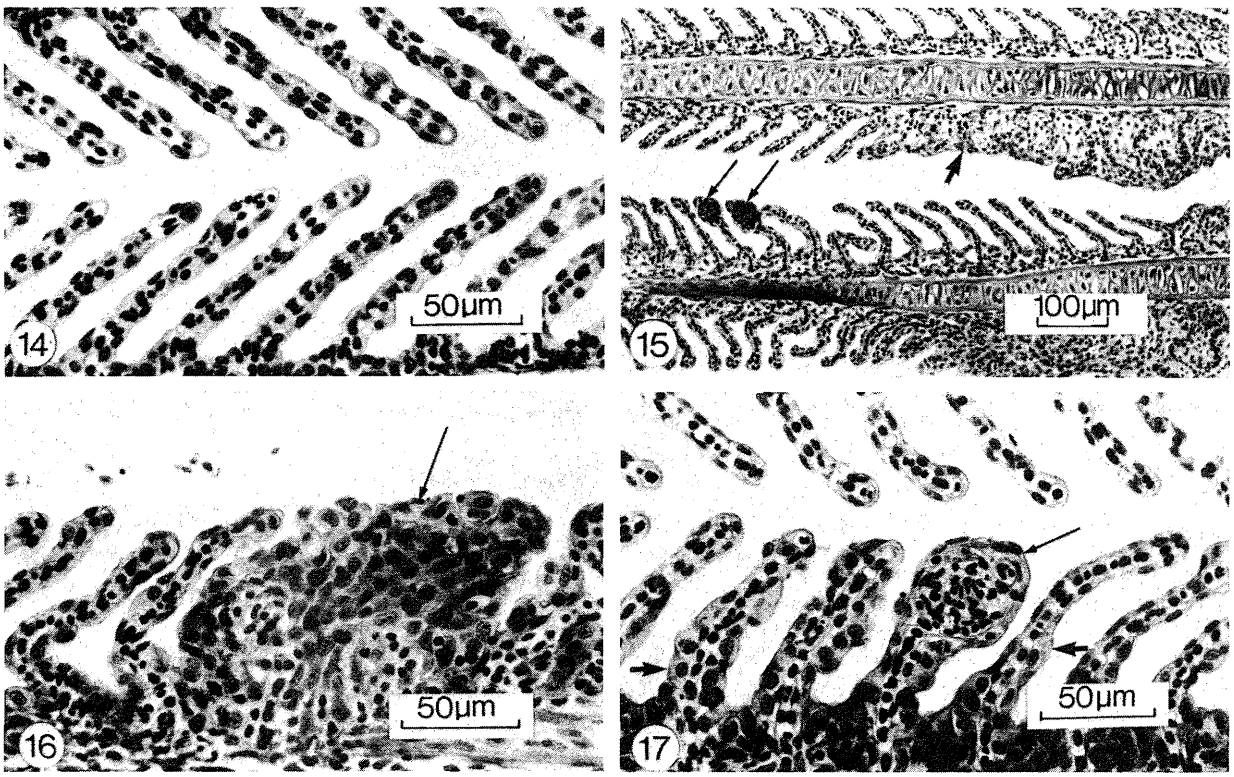

Figure 20. Longitudinal section of the secondary gill lamellae of rainbow trout showing: 14. normal morphology; 15. gross interlamellar hyperplasia (large arrows) and hematoma (small arrows); 16. interlamellar hyperplasia (higher magnification); and 17. hematomas (small arrow) and increased epithelial cell size (large arrows). Sections 15 to 17 are from trout exposed to vanadium (7-13 mg/L) for 96 h.

Table 29. Histopathological effects of vanadium on gills of rainbow trout (*Salmo gairdneri*) surviving a 96 h exposure period. One gill arch from each fish was examined and the results are expressed as the number of fish exhibiting histological effects/number of fish examined.

	Vanadium Concentration (mg/L)								
	Control	3.0	4.0	5.3	7.1	9.5	12.6	16.8	22.5
pH 6.0									
Epithelial Cell Thickening	0/2	1/1	1/1	1/1	1/1	1/1	3/3	3/3	3/3
Hyperplasia	0/2	0/1	1/1	1/1	0/1	0/1	3/3	3/3	3/3
Secondary Lamellar Hematomas	0/2	1/1	0/1	1/1	0/1	1/1	0/3	1/3	0/3
pH 7.0									
Epithelial Cell Thickening	0/2	1/1	2/2	2/2	3/3	3/3	3/3	ND[a]	ND
Hyperplasia	0/2	1/1	2/2	1/2	2/3	2/3	3/3	ND	ND
Secondary Lamellar Hematomas	0/2	1/1	1/1	0/2	1/3	0/3	0/3	ND	ND
pH 8.0									
Epithelial Cell Thickening	0/4	2/2	2/2	2/2	4/4	6/6	6/6	3/3	ND
Hyperplasia	0/4	2/2	2/2	1/2	3/4	5/6	6/6	3/3	ND
Secondary Lamellar Hematomas	0/4	1/2	0/2	2/2	1/4	1/6	2/6	2/3	ND
pH 9.0									
Epithelial Cell Thickening	0/2	0/1	1/1	1/1	2/2	3/3	3/3	3/3	ND
Hyperplasia	0/2	0/1	1/1	1/1	2/2	3/3	3/3	3/3	ND
Secondary Lamellar Hematomas	0/2	0/1	0/1	0/1	1/2	0/3	2/3	1/3	ND

[a] ND indicates no data.

Table 30. Histopathological effects of copper on kidney of rainbow trout (*Salmo gairdneri*) after 96 h exposure at pH 9.0.

Copper (mg/L)	Number of Fish Examined/# Fish Total Survivors	Relative Degree[a] of Cloudy Swelling			Hyaline Degeneration			Hydropic Degeneration			Tubule Dilation			Enlarged Macrophages		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0.356	1/1	3			1			2			0			3		
0.200	3/3	3	3	2	1	1	0	3	3	1	1	1	0	3	3	3
0.150	2/10	3	3		1	0		2	2		1	1		3	3	
0.113	3/10	3	3	2	1	0	0	3	3	2	1	1	1	1	1	0
Control	3/10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] Ranking code as in Table 27.

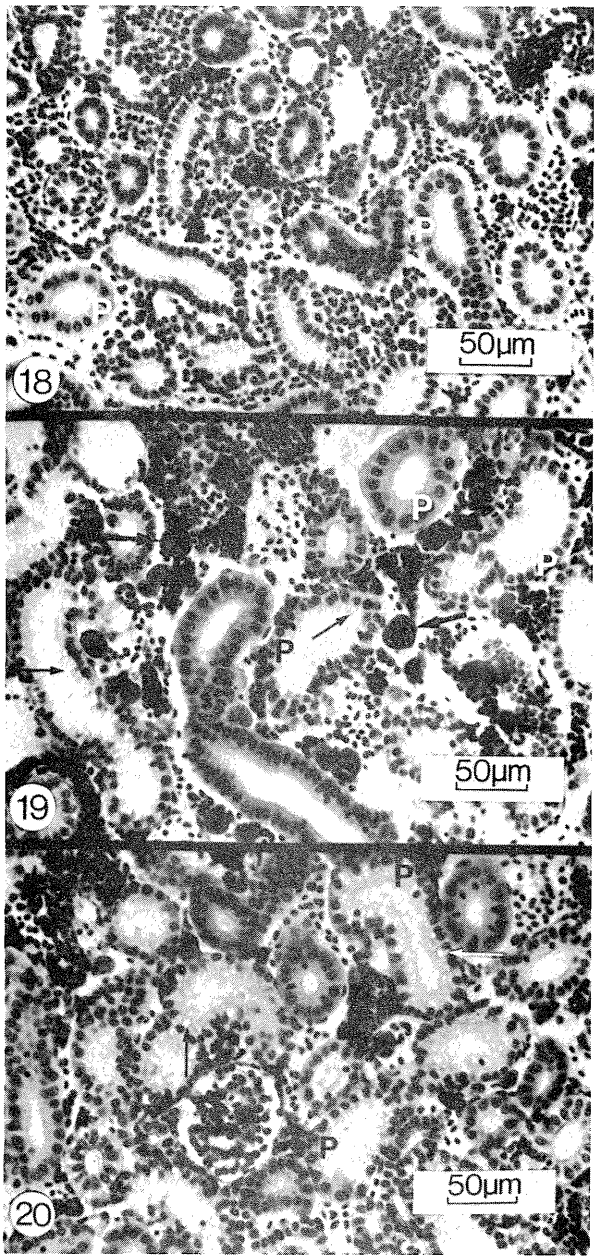

Figure 21. Longitudinal section of the posterior kidney of rainbow trout showing: 18. normal morphology; 19. cloudy swelling (small arrows) and enlarged macrophytes (large arrows); and 20. hydropic degeneration (arrows) among proximal tubule cells with cloudy swelling. All sections are from trout exposed to pH 9.0 with Sections 19 and 20 from fish exposed to copper (0.15-0.20 mg/L) for 96 h.

Section 20) were widespread in copper-exposed fish at all copper concentrations tested (Table 30). Enlarged macrophages (Figure 21, Section 19) were very prominent around the kidney tubules, and tubule dilation was observed in seven of the nine test fish examined. None of the histological aberrations exhibited a consistent dose-effect relationship with copper concentration (Table 30).

5.2.3.2.2 Gill Histopathology. The most prominent alteration in the gills of fish exposed to 0.113 to 0.356 mg Cu/L was an increase in the incidence of mucous-secreting goblet cells. Interlamellar hyperplasia was observed in three fish exposed to 0.20 mg Cu/L and one fish exposed to 0.113 mg Cu/L. These lesions were minor and probably of little physiological consequence. No lamellar hematomas were observed in treated fish and slight epithelial cell thickening occurred only in fish exposed to 0.20 mg Cu/L.

5.2.3.2.3 Liver Histopathology. Histopathological effects were not observed in the livers of rainbow trout exposed to 0.113 to 0.356 mg Cu/L.

5.2.4 Discussion

The results of the histological studies clearly demonstrate that concentrations of both vanadium and copper which were insufficient to kill rainbow trout within the 4 and 15 d exposure periods did produce significant histopathological aberrations in the kidneys and gills. In general, the lesions observed in vanadium-exposed fish were similar over the range of pH 6.0 to 8.0 and slightly more severe at pH 9.0. Consistent dose-effect relationships between vanadium concentration and histopathological effect were not observed. It would appear, however, that this lack of a dose-response relationship may have resulted from exposure of fish to a limited range of toxicant concentrations. In this regard it is clear that both time of exposure and toxicant concentrations must be considered in future histological bioassay studies since the degree of histopathological damage appears to be related to the product of these two factors. This observation

supports the conclusions of the acute lethality studies (Section 5.1) in that LC50 continues to decline with time of exposure without reaching a well-defined incipient lethal concentration.

Extrapolition of observed histological changes to specific physiological effects is difficult, but in general it would be expected that exposure to vanadium may increase the diffusion distance and lower the diffusion gradient for exchange of respiratory gases across the gill surface as a result of epithelial cell thickening and hyperplasia of the secondary lamellae. Copper may produce a similar effect by stimulating the production of mucous-secreting cells in the respiratory epithelium (Carpenter 1927; Brown 1957). Histological changes of gill epithelium in response to both copper and vanadium would be expected to have an effect on the efficiency of the transport of sodium and potassium ions from the water to the plasma, thereby producing a stress on the osmoregulatory system.

The major physiological effects of kidney damage resulting from exposure to vanadium and copper would be loss of nutrients and various protein and lipid constituents from the blood, and an impairment of the sodium resorption mechanisms of the kidney tubule. The resultant loss of sodium ions would add to the stress on the sodium transport mechanisms of the gills. In view of the manner in which histological aberrations appear to accumulate during prolonged exposure to sublethal concentrations of vanadium, further experiments encompassing chronic exposure to low toxicant levels and co-ordinated physiological and histopathological bioassays would appear desirable.

5.3 EFFECTS OF VANADIUM AND COPPER ON EMBRYOLOGICAL DEVELOPMENT OF RAINBOW TROUT

(Principal Investigator: W.A. MacDonald)

5.3.1 Introduction

Although most aquatic toxicant tests investigate toxic effects on juvenile or adult life stages, it is apparent that information on the relative senstivities of all life stages is required to assess adequately the impact of the toxicants on natural populations

of organisms. Many aquatic organisms, including fish, exhibit widely varying responses to environmental stresses such as temperature, dissolved oxygen, etc. at different developmental stages, and a similar diversity of responses to toxicants has also been observed (McKim and Benoit 1971; Olson and Marking 1973). The present experiments were conducted to assess the effects of acute exposure to copper and vanadium on eyed eggs of rainbow trout in terms of survival times and success of hatching. The toxicity of these heavy metals to embryonic and juvenile trout is compared.

5.3.2 Methods

The static tests were conducted using soft water reconstituted from distilled water according to the formula of Stephan (1975). Ion concentrations are given in Table 75. Reagent grade copper sulphate was used to prepare six test solutions ranging from 0.03 to 4.78 mg Cu/L. Reagent grade vanadium pentoxide and sodium hydroxide (in a 2:1 ratio) were used to prepare six test solutions ranging from 25 to 595 mg V/L. The 12 test waters plus a stock of control water were stored in reagent bottles at 10^{o}C.

Eyed rainbow trout eggs were received from a commerical source on 5 November 1976. Five hundred eggs were acclimated in control water for 60 h at 10^{o}C in a low temperature incubator. Samples of 10 eggs were then transferred to glass petri dishes (90 mm ID) containing 40 mL of test water. Metal concentrations and controls were run in duplicate. Mean length of a sample of embryos dissected at this time was 12.0 mm. Periodic observations were made during the 96 h of exposure. Specimens exhibiting no detectable heart beat were recorded as dead and removed. The numbers of hatched and partially hatched embryos were also recorded.

Test waters were renewed daily. Dissolved oxygen and pH of used test water (from the exposure) and unused test water (from the reagent bottles) were determined daily (Table 76). Ammonia, nitrate, and nitrite concentrations were determined at 48 and 96 h (Table 77). Samples of unused test water were acidified with 5 mL/L HNO_3 at zero and 72 h. Used test water was sampled at 48 and 96 h.

Toxicant concentrations were analyzed on a Perkin Elmer 403 AA spectrophotometer with an air acetylene flame and a 324.8 mm resonance line for copper and nitrous oxide flame and a 318.5 resonance line for vanadium (Table 78). Toxicant concentrations are taken to be the mean of the analyzed values.

At the end of 96 h of exposure, surviving fish were transferred to clean control water for recovery. After a further 3 d, sub-samples of the survivors were selected for further holding. The mean length of a sample of control alevins at this time was 14.1 mm. Twelve alevins from each toxicant concentration having 12 or more survivors were placed in 250 mL capacity dishes of clean control water. There were three samples of copper-treated fish (0.03, 0.08, and 0.24 mg/L), two samples of vanadium-treated fish (25 and 44 mg/L), plus two control samples. The excess survivors were fixed with Bouin fluid and later examined for histological damage. The living survivors were held to the eleventh day after exposure. They were then photographed for length determinations. During the post-exposure holding period, the water changed and dissolved oxygen and pH measurmeents were obtained every second day. Ammonia, nitrate, and nitrite were determined on the last day (Table 79).

5.3.3 Results

The calculated LC50 values for copper and vanadium (Table 80) are plotted against time in Figures 22 and 23, respectively. Computer program BMD03S (Dixon 1970) was used except for the 96 h LC50 estimates in Figure 22, where the straight line graphical interpolation estimate for the LC50 was used (APHA et al. 1971). There was some mortality during the 3 d post-exposure period which lowered the second estimate of the 96 h LC50 for both copper and vanadium. Median survival times, calculated by the methods of Litchfield (1949) are given in Table 31.

Hatching occurred in the control dishes near the end of the exposures. The effect of copper on hatching is illustrated in Figure 24. There was no hatch at 1.76 mg/L of copper and above and

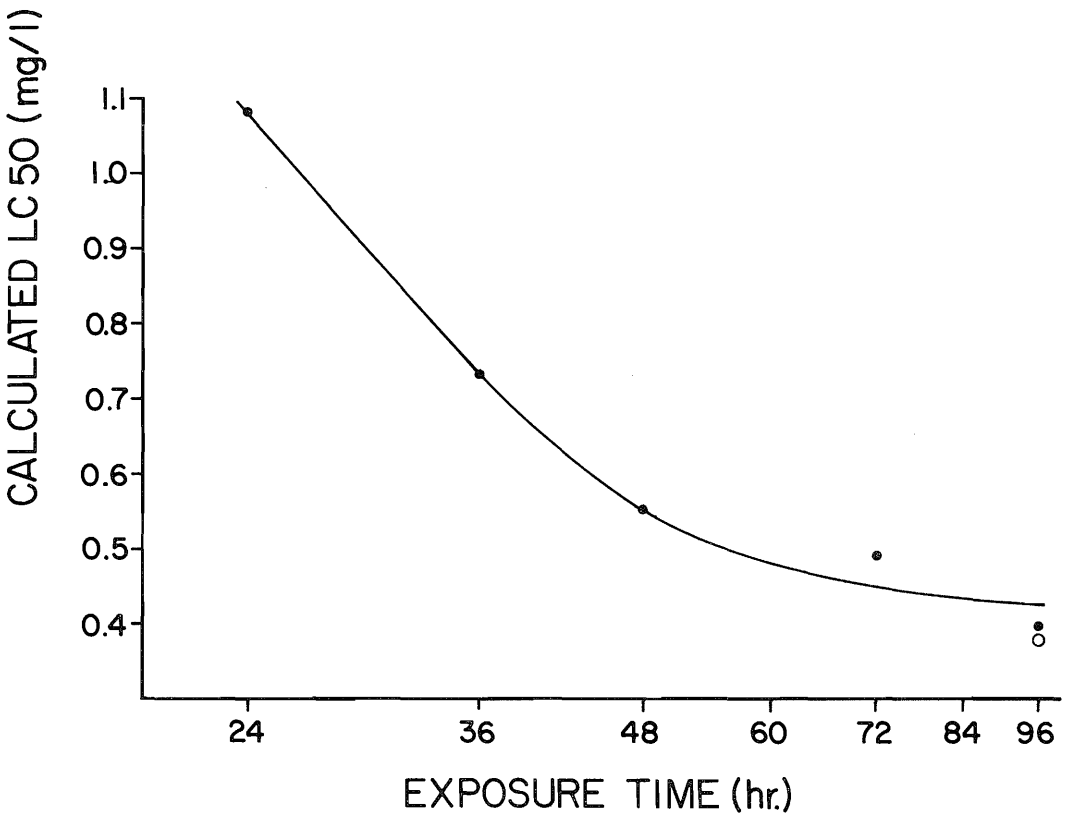

Figure 22. Acute lethal toxicity of copper to eyed eggs of rainbow trout *(Salmo gairdneri)*. The open circle at 96 h exposure refers to the LC50 estimate during the 3 d recovery period after exposure to copper was discontinued.

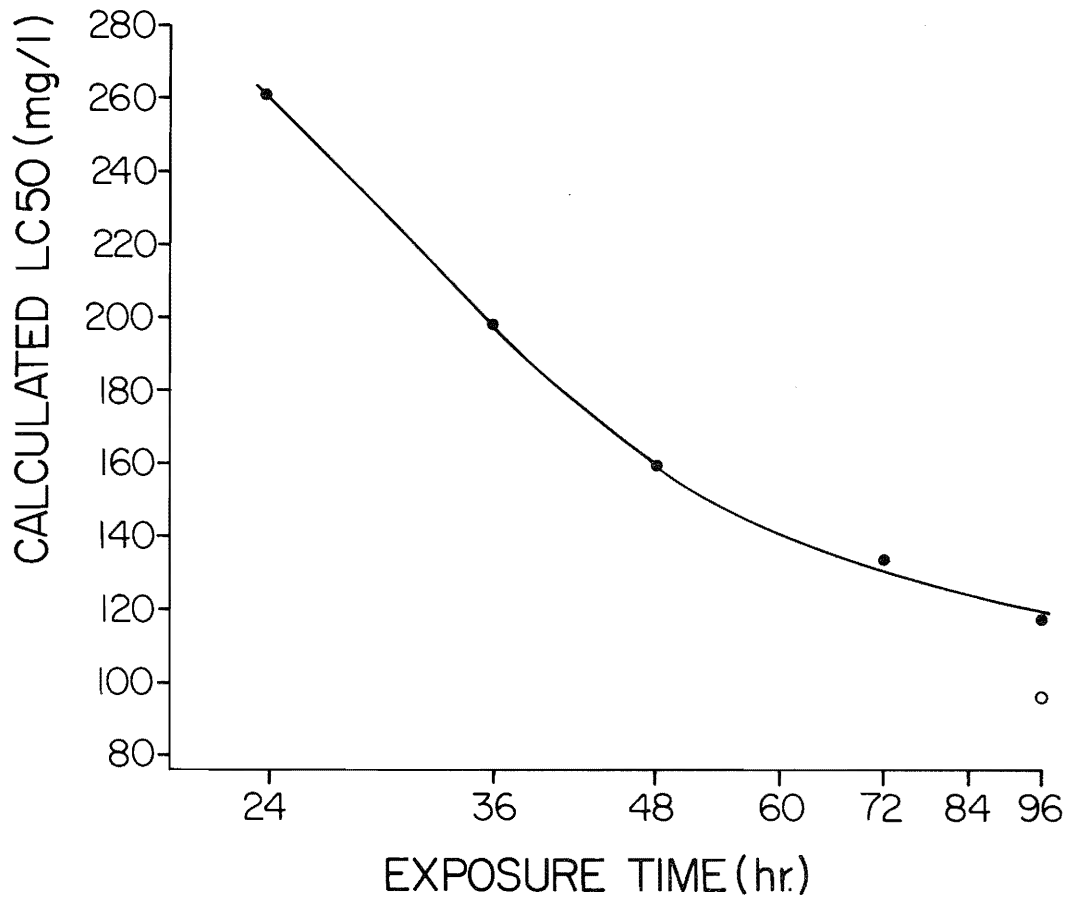

Figure 23. Acute lethal toxicity of vanadium to eyed eggs of rainbow trout *(Salmo gairdneri)*. The open circle at 96 h exposure refers to the LC50 estimate during the 3 d recovery period after exposure to vanadium was discontinued.

Table 31. Median survival times of rainbow trout embryos in copper and vanadium solutions.

Metal Concentration (mg/L)	MST (h)	95% C.I. (h)	St. Dev.	95% C.I.
Copper				
0.24	>96	34.0 to 47.1	1.45	1.10 to 1.92
0.86	40.0	10.6 to 13.1	1.2[illegible]	1.18 to 1.36
1.76	11.8	5.6 to 6.7	1.24	1.16 to 1.33
Vanadium				
86	>96			
181	48.0			
334	18.9	13.5 to 26.5	2.17	1.17 to 2.76
595	3.4			

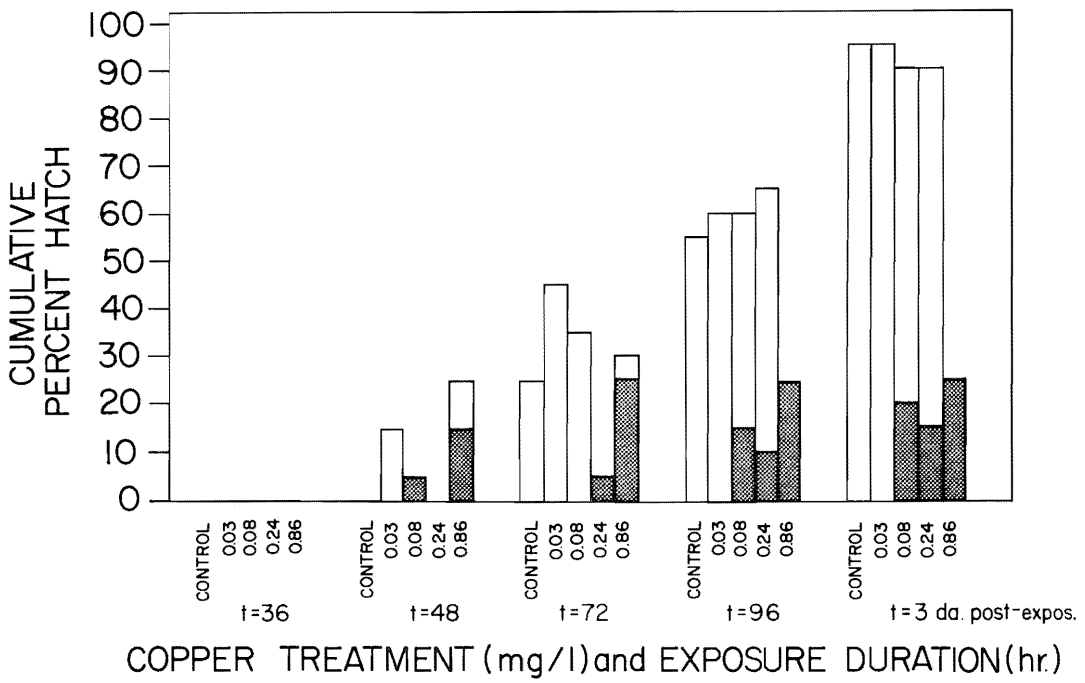

Figure 24 . The effect of copper on hatching of rainbow trout eggs. The height of each bar represents the cumulative percent of hatched and partially hatched fish. The cross-hatched area represents the proportion dead.

hatching was reduced by approximately 80% at 0.86 mg/L of copper. Although the success of hatching in controls and eggs exposed to 0.03, 0.08, and 0.24 mg Cu/L was similar, approximately 10 to 15% of the embryos in the latter two copper concentrations died immediately after hatching. Vanadium induced early hatching but most of the emerging alevins died at 181 mg/L of vanadium and above (Figure 25).

There was no further mortality in alevins selected at 3 d post-exposure and held to 11 d. Mean length at the end of the experiment was 18.0 mm and there were no significant differences in length between the treated and control alevins.

There was no histological damage observed in alevins fixed at 3 d post-exposure.

5.3.4 Discussion

Based on the calculated LC50 values shown in Figures 22 and 23, eyed rainbow trout eggs are about 250 to 300 times more resistant to the acute lethal effect of vanadium than of copper. A qualitative difference in responses to copper and to vanadium shows up in the effects on hatching (Figures 24 and 25). The higher levels of copper killed the embryo within the chorion, whereas the higher vanadium levels caused rupture of the chorion before the death of the embryo. The latter response was reported by McKim and Benoit (1971) during long term exposure of brook trout to copper. The relatively brief exposure periods employed in the present study may account for the differences in response to copper observed in the two experiments. Long term exposures will be needed to ascertain the importance of the observed tendency for vanadium to cause early hatch.

With respect to both copper and vanadium, the eggs are more resistant than fingerlings (Section 5.1). Based on 96 h LC50 values at comparable pH, eyed eggs are two to three times more resistant to copper and 10 to 15 times more resistant to vanadium than fingerlings. A comparison of MST's of eyed eggs in copper (Table 31) with those reported by Shaw and Brown (1974) for small fingerlings confirms that

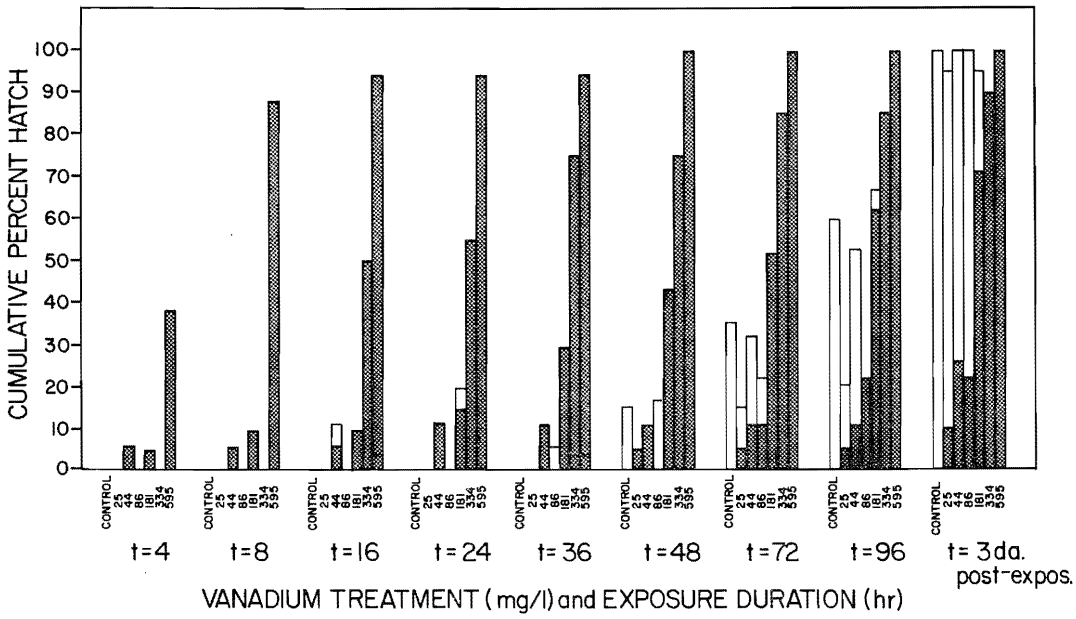

Figure 25. The effect of vanadium on hatching of rainbow trout eggs. The height of each bar represents the cumulative percent of hatched and partially hatched fish. The cross-hatched area represents the proportion dead.

eggs are several times more resistant. McKim and Benoit (1971) report similar results with brook trout where alevin-juveniles were the most sensitive stage in long term exposures to copper. Hazel and Meith (1970) exposed eyed chinook salmon eggs to copper levels of 0.08 mg/L and lower. Percent hatch was not affected but sac fry mortality was proportional to copper concentration and all sac fry were killed at the highest level.

The common finding that fish eggs are relatively resistant to toxicants such as those found in this study suggests that the chorion may protect the embryo by acting as a barrier. This hypothesis is not supported by the work of Skidmore (1966) who found that removal of the chorion of zebrafish embryos increased their resistance to zinc. Only a slight decrease in resistance to zinc was observed following the removal of the chorion of whitefish embryos (MacDonald unpublished observations). If the chorion does not protect the embryo, then an explanation of the resistance of eggs may have to await elucidation of the mechanisms of toxicity in various life stages of the fish. In the case of both copper and vanadium, this work is yet to be done.

5.4 EFFECTS OF VANADIUM AND COPPER ON LIVER ENZYMES OF RAINBOW TROUT

(Principal Investigator: S. Harrison)

5.4.1 Introduction

Several attempts have been made to correlate toxicity with enzyme inhibition (Holland et al. 1967; Jackim et al. 1970; Racicot et al. 1975; Ahokas et al. 1976; Mukherjee and Bhattacharya 1977). Detection of enzyme activity changes in reponse to exposure of fish to toxicants in the laboratory could help to predict long-term toxicity of sublethal concentrations of toxicants in the environment. Christensen (1972) studies in vitro effects of metal cations on glutamic oxalacetic transaminase (GOT) and lactic dehydrogenase (LDH) in blood plasma of white suckers. He detected a correlation between inhibitory effect and both the electronegativity

and the equilibrium constants of metal sulphates. Jackim et al. (1970) demonstrated that the activity of several liver enzymes of the killifish (*Fundulus heteroclitus*) was modified by the presence of heavy metal ions in vitro and in vivo. In vivo, cupric ion caused a decrease in zanthine oxidase, but did not affect catalase activity. In vitro, fish surviving lethal cupric ion levels exhibited an increase in zanthine oxidase activity and a decrease in catalase activity. Such a response could be useful in verifying copper toxicity in a field situation, and consequently was chosen for investigation. Vanadium was the second metal chosen for study because little information was available on its toxicity to fish. The object was to determine the effect of copper and vanadium on the activity of catalase and xanthine oxidase in the livers of rainbow trout fingerlings and to attempt to correlate enzyme effects to lethality.

5.4.2 Methods

5.4.2.1 In Vitro Studies. In vitro studies with liver homogenate were performed to determine the PI50s (concentration causing 50% inhibition) of hydroxylamine (the specific inhibitor for catalase), of allopurinol (the specific inhibitor for xanthine oxidase), and of the ions of copper and vanadium.

Rainbow trout (*Salmo gairdneri*) fingerlings were held at 10°C in a 45 gal fiberglass tank receiving Winnipeg city tap water dechlorinated by sodium thiosulphate. The fish were fed Silver Cup pellet food to satiation once daily.

The method of Cohen et al. (1970) was used to measure catalase activity. Three to six trout were anesthetized in 5 mL 2-phenoxy-ethanol/L of dechlorinated tap water. The fish were rinsed in fresh water, weighed, and the livers were removed and placed in isotonic solution (Cohen et al. 1970) on ice. The livers were blotted on tissue, weighed, and placed in cold centrifuge tubes. Sufficient cold isotonic solution was added to each centrifuge tube to yield a concentration of 300 mg of liver/mL homogenate, and the

livers were homogenized at 20 000 RPM for 20 sec in a Polytron tissue homogenizer. After the homogenate was centrifuged at 700 g for 10 min, 10 µL of ethanol were added to 1 mL of supernatant and the mixture was incubated for 30 min on ice. One hundred microlitres of 100% Triton X-100 were then added and the mixture was incubated on ice for 5 min. The homogenate was diluted 1:100 with isotonic solution containing the toxic chemical and the mixture incubated 10 min at $0^{o}C$ before the catalase activity was determined. Triplicate analyses were performed by adding 5 mL of 6 mM hydrogen peroxide to 0.5 mL homogenate samples, stopping the reactions after 3 min with 1 mL 6 N H_2SO_4, and measuring the residual hydrogen peroxide with 7 mL 0.01 N potassium permanganate. Results were expressed in terms of k, the first-order reaction rate constant, which is the preferred unit (Aebi 1974).

The method of Fried and Fried (1974) was used to measure xanthine oxidase activity. The livers from three to six rainbow trout fingerlings were removed and placed in phosphate buffer (Fried and Fried 1974) on ice. Sample treatment was identical to that for catalase samples until the centrifugation step. Xanthine oxidase homogenate was centrifuged at 15 000 RPM for 30 min at $0^{o}C$. The reagent mixture was modified by adding extra phosphate buffer in place of the EDTA to avoid possible chelation of the metal ions by EDTA. In the assay mixture, 2 mL of reagent containing tetrazolium colour reagent, 0.5 mL of buffer solution, and 0.5 mL of xanthine solution were combined. For in vitro studies, the 0.5 mL of buffer solution contained the toxic chemical whose concentration was calculated for the incubation period. The assay mixture was incubated for 10 min at $24^{o}C$ before adding xanthine and the change in absorbance at 540 nm was monitored at 2 min intervals over a 10 min period. Results were expressed as µmol of substrate converted per minute per gram wet weight of liver tissue. The activity values reported for both in vivo and in vitro studies are means of two to three separate experiments.

5.4.2.2 In Vivo Studies. A modified Mount and Brungs (1967) proportional diluter described in Harrison et al. (1975) was used to deliver 60 mL/min of each of 10 toxicant concentrations plus dilution water control to 20 L polyethylene test containers. Six rainbow trout (*Salmo gairdneri*) fingerlings were acclimated in ultra-violet dechlorinated tap water (Armstrong and Scott, 1974) at 10°C in each tank for 24 h before the toxicant addition. At the start of the bioassay, concentrated toxicant was added to bring the concentrations in the test tanks to the desired level, and toxicant addition was begun by the proportional diluter. The highest concentration chosen for each toxicant exposure approximated the 48 h LC50 value (Section 5.1.3.1). After 48 h exposure, survivors (if two or more in a tank) were removed, and were handled as were the fish in the in vitro studies. Dissolved oxygen, temperature, and pH were monitored daily in each tank.

5.4.3 Results

The data from the in vitro studies are summarized in Table 32. The PI50 for copper on catalase was five times the PI50 for vanadium and 659 times the PI50 for hydroxylamine, the specific inhibitor of catalase. The PI50 for vanadium on xanthine oxidase was 209 times the PI50 for allopurinol, the specific inhibitor of xanthine oxidase, while copper had little effect on the enzyme. The PI50 for copper on catalase was 1206 times the 48 h LC50 concentration of 0.213 mg Cu^{++}/L. Copper concentrations 298 times the 48 h LC50 concentrations produced only 4.5% inhibition of xanthine oxidase. The PI50 for vanadium to catalase was five times the 48 h LC50 of 24.68 mg V/L (Section 5.1), while the PI50 to xanthine oxidase was four times the 48 h LC50.

The data from the in vivo studies are summarized in Table 33. There appears to be no relation between cupric or vanadium ion concentration and xanthine oxidase activity. Catalase activity appeared to increase with increasing vanadium concentration. Detailed statistical analyses of this trend are hampered by the high degree of variability and small sample sizes, but the consistency

Table 32. In vitro effects of copper and vanadium upon the activity of ctalase and xanthine oxidase on liver of rainbow trout (*Salmo gairdneri*). Catalase activity expressed as k per gram wet weight of liver. Xanthine oxidase expressed as µmol of substrate converted per minute per gram wet weight of liver.

	PI50	n
Catalase		
Hydroxylamine	1.41×10^{-5}M (980 µg/L)	3
Copper	4.05×10^{-3}M (257 mg/L)	2
Vanadium	2.38×10^{-3}M (121 mg/L)	2
Xanthine Oxidase		
Allopurinol	3.51×10^{-6}M (478 µg/L)	3
Copper	-	2
Vanadium	1.97×10^{-3}M (100 mg/L)	3

Table 33. Effects of 48 h exposure to vanadium and copper on liver catalase and xanthine oxidase activities of rainbow trout (*Salmo gairdneri*).

	CATALASE ACTIVITY ($kx10^3$)			XANTHINE OXIDASE ACTIVITY [a]		
Cupric Ion Concentration (µg/L)	n	Mean	Standard Deviation	n	Mean	Standard Deviation
600	3	833.9	67.6	4	5.17	0.38
450	3	827.6	108.0	2	4.79	N.A.[b]
338	3	981.7	263.1	3	5.17	0.63
254	3	907.9	161.4			
190	2	967.4	N.A.	3	5.04	0.32
106				2	5.10	N.A.
60				2	4.79	N.A.
Control	3	896.2	143.6	9	5.29	0.97
Vanadium Ion Concentration (mg/L)	n	Mean		n	Mean	
30	2	971.2	N.A.	2	9.58	N.A.
22.5	2	971.2	N.A.	2	7.56	N.A.
16.9	2	943.0	N.A.	2	9.58	N.A.
12.7	2	822.3	N.A.			N.A.
Control	3	815.7		12	7.94	0.716

[a] µmol of substrate converted per minute per gram of wet liver tissue.

[b] N.A. Not Applicable.

of the observed relationships may warrant further investigation.

5.4.4 Discussion

It is apparent that neither cupric ion nor vanadium is a potent inhibitor in vitro of either catalase or xanthine oxidase activity because the metals produced an effect at a many times higher concentration than the specific inhibitors did. The in vitro cupric ion concentrations were many times higher than would be likely to occur in the environment, and could only occur in fish livers through a high degree of bio-accumulation, which has not been demonstrated in rainbow trout (Majewski et al. 1978).

Jackim et al. (1970) found that cupric ion in vitro was a strong inhibitor of xanthine oxidase but had little effect on catalase. Physiological differences between the killifish (*Fundulus heteroclitus*) used by Jackim et al. (1970) and the rainbow trout (*Salmo gairdneri*) used in this study may account for the differences in results.

Under the present experimental conditions, exposure of rainbow trout fingerlings to vanadium or cupric ions did not produce a consistent dose-related effect on xanthine oxidase activity. A consistent dose-related effect of vanadium on catalase was observed only at toxicant levels exceeding the 96 h LC50. Since histological studies (Section 5.2) of livers of vanadium- and copper-exposed rainbow trout fingerlings failed to demonstrate any significant degree of cell aberrations, this lack of response of liver enzymes is not surprising. It is clear, however, that the present study does not rule out the possibility that chronic exposure to substances such as vanadium, which appear to induce toxic effect over long periods of time, may produce deleterious histological and enxymological changes in the livers of fish.

5.5 PHYSIOLOGICAL EFFECTS OF VANADIUM AND COPPER ON ADULT RAINBOW TROUT

(Principal Investigators: V. Blouw and H. Majewski)

5.5.1 Introduction

As outlined previously (Section 4.4.1), physiological responses of organisms to toxicants have been employed as the basis for various bioassay procedures as well as to provide information on the modes of toxic action. In view of the published effects of copper on fish respiration (Drummond et al. 1973; Morgan and Kuhn 1974) and the observation that both copper and vanadium induce major histopathological changes in the gills of juvenile trout (Section 5.2), experiments were conducted to examine the acute effects of acute exposure to these two heavy metals upon nine major cardio-vascular-respiratory parameters in adult trout. The usefulness of this approach as a potential sublethal field bioassay procedure is assessed.

5.5.2 Methods

For sublethal testing with $CuSO_4$, 17 rainbow trout (*Salmo gairdneri*) of either sex, with a mean (+S.D.) weight of 555.6 g $\pm$ 53.7, and a mean (+S.D.) length of 34.4 cm $\pm$ 1.5, were used. For testing with V_2O_5, 17 rainbow trout of either sex, with a mean ($\pm$S.D.) weight of 420.4 g $\pm$ 64.6, and a mean ($\pm$S.D.) length of 31.4 cm $\pm$ 1.6, were used. These fish were held in dechlorinated (sodium thiosulphate 1 to 5 mg/L) Winnipeg city water (Table 61) at 10°C for at least 1 month prior to use.

Methods for obtaining buccal amplitude, respiration rate, and ECG data have been described previously (Section 4.4.2). Dorsal aortic blood pressure was obtained by means of a length of PE 50 tubing inserted into the dorsal aorta with an 18G. Soverign (#8890-707816) indwelling canine catheter. After these procedures, which required approximately 20 min, fish were placed into restraining chambers (Figure 26), and were held at 10°C $\pm$ 0.5 in city of Winnipeg ultra-violet dechlorinated water for 24 h prior to introduction of

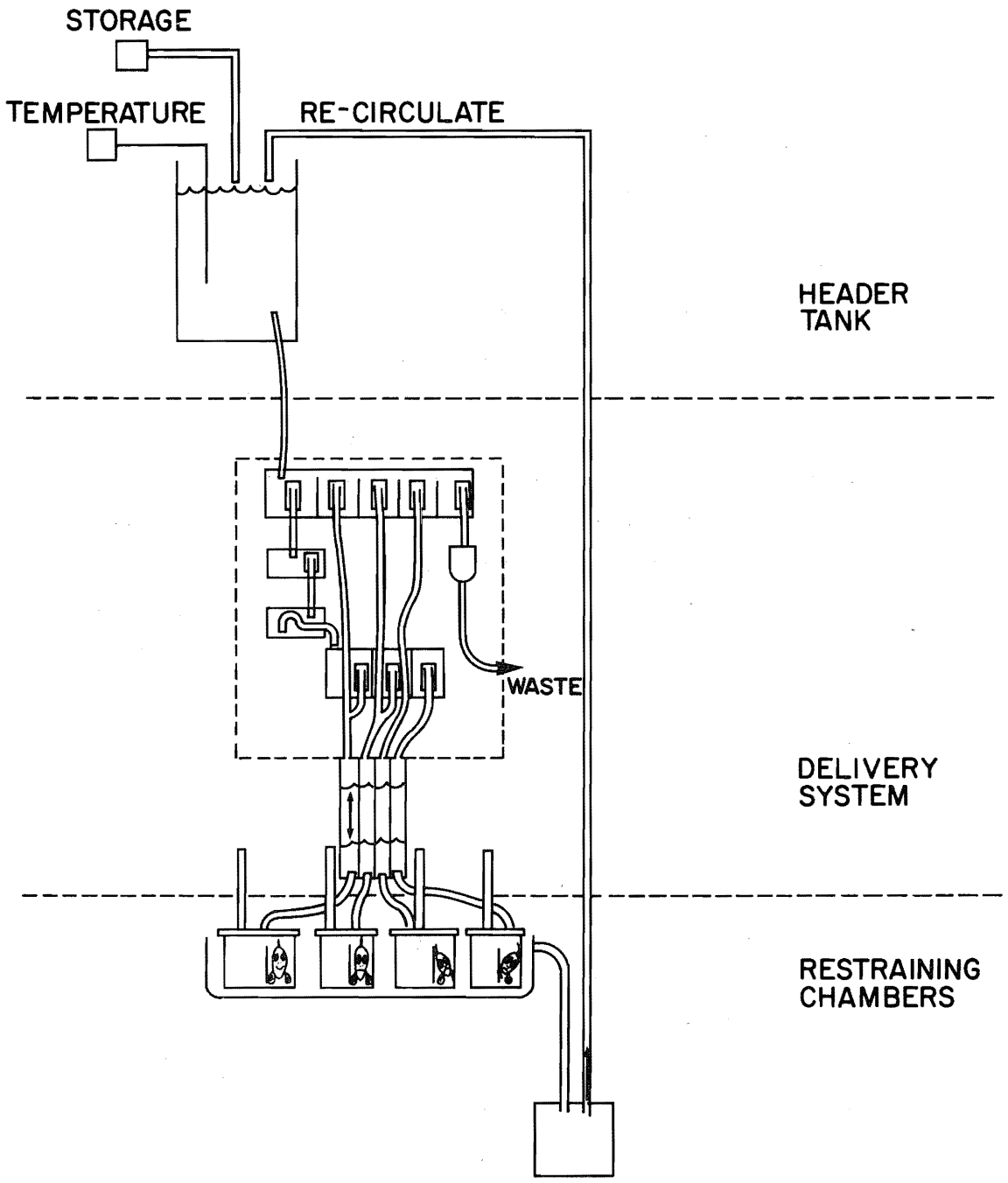

Figure 26. Schematic representation of the toxicant delivery system employed in the studies of physiological responses of adult rainbow trout to saline groundwater.

toxicant. An intermittent-flow dilutor system (Harrison et al. 1975) was employed to maintain three toxicant concentrations and a control. Flow rate was maintained at 0.5 L/min (99% replacement time = 1.5 h), and dissolved oxygen was maintained at near saturation. Buccal respiratory traces, ECG, and dorsal aorta blood pressure traces were recorded at 1 h intervals for 2 h prior to toxicant introduction, and again at 0.25, 0.5, 1, 2, 4, 8, 12, 21, adn 24 h after treatment. Blood samples for pO_2, pH, hematocrit, and lactic acid were taken 1 h before toxicant introduction, and again at 4 and 24 h after treatment. Blood and water pO_2, and blood pH were measured with a Radiometer-Copenhagen MBS3 Mk 2 Blood Microsystem in conjunction with a PHM72 Mk2 Digital Acid-Base Analyzer. Blood lactic acid was measured using the lactate dehydrogenase method (Mattenheimer 1971).

Instrumentation used to record physiological data and the procedure for normalizing some of the vanadium data have been described previously (Section 4.4.2).

5.5.3 Results

5.5.3.1 Copper. Physiological data obtained from 17 adult rainbow trout exposed to 0.1, 0.14, and 0.21 mg/L copper are presented in Tables 34 to 43. These copper concentrations represent 52, 73, and 110%, respectively, of the 96 h LC50 for juvenile rainbow trout at similar pH and 15°C. No statistically significant responses to copper were observed in any of the measured parameters. Although ventilation rate (Table 34), heart rate (Table 38), blood pH (Table 41), and hematocrit (Table 42) remained relatively stable in the experimental fish throughout the exposure period, considerable variability was observed in buccal amplitude (Table 35), oxygen uptake (Table 36), cough frequency (Table 37), dorsal aortic blood pressure (Table 39), dorsal aortic pO_2 (Table 40), and blood lactate concentration (Table 43).

It is possible that the high variability in these parameters may have masked statistically significant responses but no trends were

Table 34. Ventilation rate (buccal beats/min) in adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of Cu over a 24 h period. Results expressed as $\bar{x} \pm 1$ S.D. for sample size N.

Time (h)	Copper Concentration (mg/)			
	Control (N=4)	0.10 (N=3)	0.14 (N=5)	0.21 (N=5)
-2	68.8 ±12.6	66.0 ±5.3	69.2 ±12.5	63.8 ±2.2
-1	67.0 ±11.7	71.7 ±1.5	67.2 ±13.4	64.6 ±2.9
0	70.5 ±9.1	62.3 ±6.1	69.4 ±10.6	64.4 ±4.6
0.25	70.7 ±12.1	–	–	59.0 ±7.1
0.5	66.7 ±11.7	68.0 ±11.3	66.0 ±12.3	70.5 ±13.2
1	65.8 ±10.2	61.7 ±4.7	66.0 ±11.6	69.6 ±12.9
2	76.3 ±3.5	62.5 ±9.2	58.0 ±1.8	66.3 ±10.1
4	68.8 ±11.0	62.3 ±4.5	64.0 ±8.0	64.2 ±8.2
8	63.8 ±5.2	61.0 ±4.4	62.2 ±5.9	65.0 ±5.5
12	75.0 ±5.6	65.0 ±9.9	61.5 ±1.9	69.3 ±10.6
21	76.3 ±7.1	61.0 ±8.2	66.4 ±7.3	66.2 ±4.2
24	70.3 ±12.3	57.0 ±2.7	66.4 ±8.4	68.2 ±7.8

Table 35. Buccal amplitude (mm Hg) in adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of Cu over a 24 h period. Results expressed as $\bar{x}\pm 1$ S.D. for sample size N.

Time (h)	Copper Concentration			
	0.05 mg/L Control (N=4)	0.10 mg/L (N=3)	0.14 mg/L (N=5)	0.21 mg/L (N=5)
-2	1.80 ±.43	2.13 ±1.11	2.18 ±1.04	1.60 ±.64
-1	1.48 ±.15	1.83 ±.85	1.54 ±.54	1.82 ±.74
0	1.68 ±.36	1.63 ±.38	2.38 ±1.00	1.76 ±.86
0.25	1.75 ±.07		2.60 ±1.70	1.35 ±.35
0.5	1.50 ±.36	2.40 ±1.84	2.30 ±1.94	2.45 ±1.73
1	1.55 ±.41	1.43 ±.31	1.96 ±1.38	2.80 ±1.57
2	2.07 ±.67	1.4	1.65 ±.78	1.90 ±1.22
4	1.58 ±.17	1.50 ±.10	2.32 ±.70	1.68 ±.56
8	1.70 ±.48	1.37 ±.06	1.76 ±.61	1.90 ±1.20
12	2.20 ±.72	1.60 ±.28	1.58 ±.66	2.50 ±1.46
21	2.20 ±.61	1.37 ±.32	1.62 ±.46	1.64 ±.55
24	1.53 ±.31	1.17 ±.15	2.02 ±1.10	1.98 ±.42

Table 36. Oxygen uptake (mg O_2/h/kg) in adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of Cu over a 24 h period. Results expressed as $\bar{x}$±1 S.D. for sample size N.

Time (h)	Copper Concentration (mg/L)			
	Control (N=4)	0.10 (N=3)	0.14 (N=5)	0.21 (N=5)
-2	86.8 ±38.8	82.3 ±8.1	115.6 ±47.7	80.8 ±20.4
-1	77.3 ±12.6	77.7 ±25.0	88.4 ±30.9	100.4 ±39.3
0	76.8 ±18.2	52.0 ±10.6	101.6 ±38.7	96.8 ±47.6
0.25			163.0 ±75.0	
0.5	64.0 ±11.3		142.0 ±65.1	144.7 ±111.2
1	68.5 ±6.4	57.0 ±15.6	104.0 ±55.8	118.6 ±78.0
2	72.5 ±33.7	66.0 ±19.1	84.2 ±28.7	112.8 ±91.8
4	56.3 ±14.1	59.3 ±13.7	87.6 ±37.3	93.0 ±60.6
8	81.0 ±44.0	66.3 ±15.1	92.4 ±23.1	85.8 ±26.3
12	81.7 ±22.0	93.5 ±41.7	82.3 ±13.6	106.8 ±47.3
21	104.5 ±13.8	79.3 ±16.6	82.2 ±34.4	89.4 ±25.7
24	61.0 ±3.7	72.7 ±23.0	83.2 ±38.8	99.6 ±29.9

Table 37. Cough frequency (coughs/min) in adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of Cu over a 24 h period. Results expressed as $\bar{x}\pm 1$ S.D. for sample size N.

Time (h)	Concentration (mg/L)			
	Control (N=4)	0.10 (N=3)	0.14 (N=5)	0.21 (N=5)
-2	1.17 ±.29	0.44 ±.51	0.80 ±.57	1.50 ±1.00
-1	1.21 ±.25	0.59 ±.53	0.67 ±.59	1.25 ±.57
0	1.13 ±.25	0.58 ±.38	0.68 ±.49	1.32 ±.92
0.25	0.84 ±.23			2.35 ±1.20
0.5	1.28 ±.25	0.21 ±.06	1.04 ±.67	1.38 ±.80
1	1.21 ±.63	0.48 ±.45	1.42 ±2.01	1.83 ±.87
2	0.83 ±.14	1.17 ±.71	0.96 ±.34	1.88 ±1.21
4	1.25 ±.50	0.89 ±.96	1.50 ±1.07	1.57 ±1.28
8	1.08 ±.17	1.61 ±1.78	1.50 ±1.43	1.80 ±1.32
12	1.0	1.34 ±.94	0.75 ±.29	2.12 ±1.59
21	1.13 ±.37	0.80 ±.61	2.00 ±1.62	2.20 ±2.18
24	1.25 ±.50	0.75 ±1.09	1.36 ±1.69	1.73 ±1.86

Table 38. Heart rate (beats/min) in adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of Cu over a 24 h period. Results expressed as $\bar{x}\pm1$ S.D. for sample size N.

Time (h)	Concentration (mg/L)			
	Control (N=4)	0.10 (N=3)	0.14 (N=5)	0.21 (N=5)
-2	55.5 ±9.3	61.0 ±5.6	55.8 ±10.0	54.6 ±8.5
-1	54.8 ±9.6	61.0 ±7.0	55.8 ±10.7	54.2 ±8.6
0	53.5 ±6.7	61.0 ±6.6	57.2 ±9.4	53.8 ±8.4
0.25	57.5 ±6.4			
0.5	51.7 ±8.7	59.5 ±5.0	56.4 ±12.5	53.8 ±8.6
1	52.8 ±7.1	61.7 ±7.5	56.8 ±13.0	55.8 ±8.7
2	53.0 ±9.5	64.0 ±4.2	55.0 ±14.9	53.5 ±7.0
4	55.3 ±7.1	61.3 ±8.5	58.2 ±12.6	56.0 ±8.0
8	54.5 ±7.4	61.7 ±6.7	62.0 ±9.6	57.6 ±9.8
12	54.3 ±7.5	66.0 ±5.7	59.8 ±15.9	53.3 ±9.0
21	54.3 ±8.4	57.7 ±10.0	53.8 ±15.5	55.6 ±6.9
24	52.3 ±5.7	53.3 ±4.2	56.6 ±12.4	54.4 ±7.1

Table 39. Dorsal aorta blood pressure (mm Hg) in adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of Cu over a 24 h period. Results expressed as $\bar{x}\pm 1$ S.D. for sample size N. S and D represent systolic and diostolic pressures, respectively.

Time (h)	Concentration (mg/L)							
	Control (N=4)		0.10 (N=3)		0.14 (N=3)		0.21 (N=4)	
	S	D	S	D	S	D	S	D
-2	41.5 ±3.1	33.0 ±2.2	41.7 ±3.8	32.3 ±2.3	45.7 ±12.3	32.7 ±8.4	43.0 ±2.9	31.5 ±3.0
-1	41.5 ±1.7	34.0 ±2.8	40.0 ±1.7	32.0 ±2.0	38.0 ±13.9	28.3 ±12.7	42.3 ±8.2	29.3 ±6.1
0	40.0 ±4.6	31.0 ±4.7	40.3 ±4.2	32.0 ±2.7	40.3 ±7.2	30.7 ±5.1	44.8 ±8.1	32.0 ±6.8
0.25	44.0 ±2.8	35.0 ±1.4	- -	- -	- -	- -	41.5 ±7.8	29.5 ±6.4
0.5	41.0 ±1.7	32.3 ±2.5	43.5 ±9.2	34.0 ±5.7	48.0 ±25.5	32.0 ±14.1	48.7 ±5.5	35.0 ±5.0
1	42.8 ±5.3	34.0 ±5.7	39.7 ±3.8	31.7 ±2.9	47.3 ±13.0	34.3 ±8.1	46.3 ±10.4	34.0 ±7.9
2	41.7 ±2.1	32.7 ±2.1	44.0 ±4.2	36.0 ±5.7	37.7 ±13.6	29.0 ±10.4	43.0 ±9.3	31.8 ±7.6
4	40.0 ±4.1	31.8 ±3.3	42.7 ±0.6	33.7 ±1.2	45.0 ±3.6	32.7 ±2.3	42.8 ±9.8	31.5 ±7.2
8	37.8 ±2.5	29.0 ±1.2	42.7 ±3.1	32.3 ±2.5	38.0 ±5.3	29.3 ±2.9	37.8 ±2.2	26.5 ±2.4
12	43.5 ±2.1	33.5 ±2.1	43.5 ±2.1	32.0 ±1.4	41.3 ±4.9	29.3 ±4.0	39.7 ±8.1	29.7 ±8.3
21	40.8 ±2.6	32.3 ±2.6	37.7 ±2.5	29.0 ±1.7	46.7 ±9.5	34.0 ±7.8	40.8 ±6.4	30.0 ±4.2
24	38.8 ±3.0	30.3 ±3.2	37.7 ±1.2	29.3 ±1.5	41.3 ±3.2	31.0 ±3.6	41.5 ±5.9	31.3 ±6.2

Table 40. Dorsal aorta CO_2 (mm Hg) in adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of Cu over a 24 h period. Individual values are listed, as well as x±1 S.D.

Copper Concentration (mg/L)	Time (h)		
	-1	+4	+24
[0.05]	102.3	92.3	107.5
	95.8	88.0	76.4
	81.9	77.9	94.5
	-	-	-
	85.1	91.1	90.4
	91.28	87.33	92.2
	±9.45	±6.54	±12.81
[0.10]	107.0	105.9	115.5
	102.0	96.7	96.9
	111.5	96.2	107.8
	-	-	-
	-	-	-
	106.83	99.60	106.73
	±4.75	±5.46	±9.35
[0.14]	-	-	-
	97.0	-	-
	89.3	95.4	107.2
	85.5	100.7	94.3
	94.1	83.8	46.0
	91.48	93.3	82.5
	±5.09	±8.64	±32.26
[0.21]	-	-	-
	105.6	97.2	105.9
	96.7	80.1	85.4
	79.8	41.8	68.6
	91.4	78.1	95.7
	93.38	74.3	88.9
	±10.78	±23.30	±15.91

Table 41. Dorsal aorta pH in adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of Cu over a 24 h period. Individual values are listed, as well as $\bar{x}\pm1$ S.D.

Copper Concentration (mg/L)	Time (h)		
	-1	+4	+24
[0.05]	7.960	7.942	7.986
	7.938	7.920	7.957
	7.912	7.877	7.925
	-	-	-
	7.861	7.855	7.905
	7.918	7.899	7.943
	±.043	±.040	±.036
[0.10]	7.944	7.896	7.853
	7.987	7.965	8.068
	8.015	8.032	8.000
	-	-	-
	-	-	-
	7.982	7.964	7.974
	±.036	±.068	±.110
[0.14]	-	-	-
	7.972	7.858	7.843
	7.880	7.899	7.900
	7.990	8.001	8.010
	7.910	7.954	7.892
	7.938	7.928	7.911
	±.052	±.063	±.070
[0.21]	-	-	-
	7.874	7.876	7.960
	7.998	7.866	7.905
	8.071	7.996	8.001
	7.982	7.892	7.977
	7.981	7.908	7.961
	±.081	±.060	±.041

Table 42. Hematocrit (%) values for adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of Cu for a 24 h period. Individual values are listed, as well as $\bar{x} \pm 1$ S.D.

Copper Concentration (mg/L)	Time (h)		
	-1	+4	+24
[0.05]	11	7	12
	20	13	12
	25	26	22
	-	-	-
	16	12	10
	18.0	14.5	14.0
	±5.9	±8.1	±5.4
[0.10]	15	13	12
	7	12	4
	10	16	19
	-	-	-
	-	-	-
	10.7	13.7	11.7
	±4.0	±2.1	±7.5
[0.14]	-	-	-
	12	28	22
	26	23	19
	12	16	19
	24	18	17
	18.5	21.3	19.3
	±7.5	±5.4	±2.1
[0.21]	-	-	-
	15	20	19
	18	25	12
	22	30	26
	9	8	7
	16.0	20.8	16.0
	±5.5	±9.4	±8.3

Table 43. Blood lactate concentrations (mg/100 mL) for adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of Cu over a 24 h period. Individual values are listed, as well as $\bar{x}\pm 1$ S.D.

Copper Concentration (mg/L)	Time (h)		
	-1	+4	+24
[0.05]	19.6	12.5	13.7
	14.4	14.7	10.2
	9.9	20.6	6.4
	-	-	-
	8.8	9.3	9.2
	13.2	14.3	9.9
	±4.9	±4.8	±3.0
[0.10]	32.4	30.3	14.6
	15.6	19.6	18.7
	59.6	43.7	14.9
	-	-	-
	10.3	7.8	6.9
	29.5	25.4	13.8
	±22.2	±15.3	±4.9
[0.14]	20.7	18.6	16.6
	17.2	18.1	27.4
	18.8	7.8	11.4
	10.6	12.8	7.9
	19.3	25.9	17.2
	17.3	16.6	16.1
	±4.0	±6.8	±7.4
[0.21]	-	-	-
	13.1	10.6	9.1
	19.4	35.3	23.7
	6.7	7.3	7.9
	-	-	-
	13.1	17.7	13.6
	±6.4	±15.3	±8.8

evident upon graphical examination of normalized data.

5.5.3.2 Vanadium. Of the 10 physiological parameters (Tables 44 to 53) recorded from adult rainbow trout during exposure at 6.25, 12.0, and 25.0 mg/L of vanadium, only ventilation rate and cough frequency exhibited a response to the toxicant. Cough frequency in fish exposed to 25 mg V/L (Table 45) increased progressively during the initial eight hours of exposure to rates which were three-fold higher than control values. During the next 16 h of exposure, the cough frequency in these fish declined somewhat but remained significantly higher than that of control animals. Vanadium concentrations of 6.25 and 12.5 mg/L did not induce significant changes in cough frequency over the 24 h exposure period. Since the 96 h LC50 of vanadium for juvenile rainbow trout is 6.4 µg/L, the significance of the response in cough frequency at 25 mg V/L is questionable.

Ventilation rate (Table 44) was elevated within 1 h of exposure to vanadium and the magnitude of the response increased with increasing concentrations of vanadium (Figure 27). The elevated ventilation rates observed after 1 h of exposure to vanadium were maintained over the remainder of the 24 h exposure period. The slight decline in all ventilation rates during the 8 to 21 h period (Figure 27) occurred overnight and probably reflects a decrease in the amount of disturbance arising from general laboratory activity. Considering the variability in control data, the increase in ventilatory rate is approximately proportional to the log of the vanadium concentration at exposure times of 1, 8 and 24 h (Figure 27, Table 44).

5.5.4 Discussion

No consistent response to copper was observed in any of the 10 physiological parameters examined in this study. These results conflict with published data in which significant increases in cough frequency and ventilation rate of brook trout (*Salvelinus fontinalis*), largemouth bass (*Micropterus salmoides*), and bluegill

Table 44. Ventilation rate (buccal beats per min) in adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of vanadium over a 24 h period. Results are expressed as beats per minute in $\bar{x}\pm 1$ S.D. and normalized form as a % deviation from ventilation frequency at T=0. N = Number of fish.

	Vanadium Concentration (mg/L)							
	Control	(N=4)	6.25	(N=5)	12.5	(N=3)	25	(N=4)
Time (h)	Beats/ Min	Normalized (% Dev)	Beats/ Min	Normalized (% Dev)	Beats/ Min	Normalized (% Dev)	Beats/ Min	Normalized (% Dev)
-3	62.0 ±21.2	-8.1%	67.0 ±12.3	0	ND[a] ND	ND ND	69.0 ±7.8	+3.0%
-2	68.3 ±9.3	+1.2%	70.8 ±8.0	+5.7%	69.7 ±10.7	-2.8%	63.7 ±6.4	-4.9%
-1	77.0 ±4.2	+14.1%	66.3 ±4.7	-1.0%	69.3 ±5.1	-3.3%	63.5 ±6.4	-5.2%
0	67.5 ±12.4	0	67.0 ±7.5	0	71.7 ±7.6	0	67.0 ±5.5	0
0.25	64.7 ±12.2	-4.1%	64.0 ±4.2	-4.5%	ND ND	ND ND	ND ND	ND ND
0.5	64.0 ±12.1	-5.2%	69.8 ±5.6	+4.2%	80.7 ±6.1	+12.6%	73.0 ±11.8	+9.0%
+1	68.8 ±10.6	+1.9%	73.0 ±3.5	+9.0%	81.3 ±9.9	+13.4%	82.0 ±14.0	+22.4%
+2	63.0 ±15.6	-6.7%	70.2 ±5.8	+4.8%	84.3 ±4.5	+17.6%	84.0 ±14.7	+25.4%
+4	73.5 ±17.7	+8.9%	72.6 ±8.6	+8.4%	84.0 ±3.6	+17.2%	83.5 ±17.0	+24.6%

Continued...

Table 44. Concluded.

Time (h)	Vanadium Concentration (mg/L)							
	Control	(N=4)	6.25	(N=5)	12.5	(N=3)	25	(N=4)
	Beats/ Min	Normalized (% Dev)	Beats/ Min	Normalized (% Dev)	Beats/ Min	Normalized (% Dev)	Beats/ Min	Normalized (% Dev)
+8	65.0 ±14.9	-3.7%	70.4 ±6.9	+5.1%	81.0 ±2.6	+13.0%	82.5 ±15.9	+23.1%
+12	63.8 ±11.3	-5.5%	70.2 ±10.4	+4.8%	77.3 ±3.8	+7.8%	76.0 ±15.4	+13.4%
+21	62.8 ±13.2	-7.0%	72.6 ±11.1	+8.4%	78.7 ±1.2	+9.8%	79.5 ±15.3	+18.7%
+24	65.7 ±15.4	-2.7%	69.8 ±13.1	+4.2%	79.3 ±6.8	+10.6%	83.3 ±15.7	+24.3%

[a] ND indicates no data.

Table 45. Cough frequency (coughs/min) in rainbow trout (*Salmo gairdneri*) exposed to three concentrations of vanadium over a 24 h period. Results are expressed as $\bar{x}\pm 1$ S.D. and in normalized form as a % deviation from cough frequency at T=0. N = number of fish.

	Vanadium Concentration (mg/L)							
	Control	(N=4)	6.25	(N=5)	12.5	(N=3)	25	(N=4)
Time (h)	Coughs/ Min	Normalized (% Dev)	Coughs/ Min	Normalized (% Dev)	Coughs/ Min	Normalized (% Dev)	Coughs/ Min	Normalized (% Dev)
-3	1.34 ±.94	+1%	2.23 ±1.53	+62.8%	ND[a] ND	ND ND	1.07 ±.12	-1.8%
-2	1.05 ±.49	-21.1%	.81 ±.81	-40.9%	1.10 ±.17	+10%	.97 ±.46	-11.0%
-1	1.25 ±.35	-6.0%	.64 ±.34	-53.3%	1.02 ±.28	0	.55 ±.07	-50.0%
0	1.33 ±.65	0	1.37 ±.61	0	1.0 0	0	1.09 ±.63	0
0.25	1.17 ±.76	-12%	1.84 ±1.65	+34.3%	ND ND	ND ND	ND ND	ND ND
0.5	1.58 ±.38	+18.8%	1.58 ±1.07	+15.3%	2.06 ±1.73	+106%	.94 ±.31	-13.8%
+1	.92 ±.17	-30.8%	1.33 ±.70	-2.9%	.77 ±.49	-23%	1.53 ±.89	+40.4%
2	1.30 ±.48	-2.3%	1.31 ±1.06	-4.4%	.93 ±.40	-7%	1.84 ±.96	+68.8%
4	1.04 ±.34	-21.8%	1.45 ±.95	+5.8%	.66 ±.35	-34%	2.26 ±1.74	+107.3%

Continued...

Table 45. Concluded.

Time (h)	Vanadium Concentration (mg/L)							
	Control	(N=4)	6.25	(N=5)	12.5	(N=3)	25	(N=4)
	Coughs/ Min	Normalized (% Dev)	Coughs/ Min	Normalized (% Dev)	Coughs/ Min	Normalized (% Dev)	Coughs/ Min	Normalized (% Dev)
8	1.15 ±.40	-13.5%	1.40 ±1.03	+2.2%	.99 ±.89	-1%	4.67 ±6.27	+328.4%
12	1.74 ±.81	+30.8%	1.53 ±1.53	+11.7%	2.46 ±2.05	+146%	2.95 ±1.42	+170.6%
21	1.00 ±.27	-24.8%	1.33 ±1.06	-2.9%	1.10 ±.85	+10%	2.77 ±1.02	+154.1%
24	1.00 ±.33	-24.8%	1.08 ±.74	-21.2%	.90 ±.17	-10%	4.14 ±1.63	+279.8%

[a] ND indicates no data.

Table 46. Oxygen uptake (mg O_2/h/kg) in adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of vanadium over a 24 h period. Results expressed as x±1 S.D. for sample size N.

Time (h)	Vanadium Concentration (mg/L) L			
	Control (N=5)	6.25 (N=5)	12.5 (N=4)	25 (N=4)
-3	ND[a]	ND	ND	ND
	ND	ND	ND	ND
-2	71.3	68.0	70.3	77.3
	±21.2	±11.3	±6.0	±33.1
-1	93.0	79.4	92.8	78.3
	±13.7	±13.3	±29.6	±31.0
0	111.3	66.5	111.3	82.0
	±47.0	±8.02	±60.6	±19.1
0.25	97.0	ND	ND	ND
	±42.4	ND	ND	ND
0.5	ND	77.0	90.5	ND
	ND	±24.0	±34.6	ND
+1	86.2	90.0	135.3	124.5
	±33.7	±42.8	±71.8	±26.6
2	88.6	69.0	134.8	150.3
	±24.0	±17.8	±59.6	±27.3
4	115.4	80.4	118.3	81.3
	±34.8	±30.7	±56.2	±9.8
8	82.2	56.4	71.3	68.8
	±40.0	±12.5	±11.9	±25.3
12	57.4	49.8	88.3	68.0
	±15.4	±8.8	±15.9	±20.9
21	84.3	69.5	120.3	83.7
	±19.2	±18.7	±64.7	±3.1
24	78.6	53.2	116.3	78.8
	±25.6	±22.8	±13.5	±8.1

[a]ND indicates no data.

Table 47. Buccal amplitude (mm Hg) in adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of vanadium over a 24 h period. Results expressed as $\bar{x}\pm 1$ S.D. for sample size N.

Time (h)	Vanadium Concentration (mg/L)			
	0 (N=4)	6.25 (N=5)	12.5 (N=3)	25.0 (N=4)
-3	ND[a] ND	2.0 ±2.2	ND ND	1.2 ±.5
-2	1.5 ±1.1	2.5 ±2.0	.7 ±.1	1.0 ±.1
-1	1.0 ±0.6	1.3 ±1.3	.8 ±.3	1.2 ±.3
0	1.5 ±1.0	1.1 ±.8	1.0 ±.4	1.3 ±.2
0.25	0.9 ±0.2	1.6 ±.9	ND ND	ND ND
0.5	1.9 ±.8	2.0 ±1.2	1.8 ±1.5	1.6 ±.5
+1	1.5 ±.9	1.8 ±1.4	1.6 ±1.6	2.2 ±1.6
2	1.4 ±1.0	1.7 ±.7	2.4 ±2.2	2.3 ±.7
4	2.0 ±.6	2.0 ±1.2	1.5 ±1.4	.9 ±.3
8	1.2 ±.5	1.8 ±1.5	.9 ±.4	1.0 ±.3
12	1.5 ±1.0	1.8 ±1.5	.9 ±.4	.8 ±.2
21	1.4 ±.9	2.2 ±1.2	1.1 ±.3	1.0 ±.3
24	1.3 ±1.1	1.6 ±1.1	1.8 ±1.5	1.1 ±.4

[a] ND indicates no data.

Table 48. Heart rate (beats/min) in adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of vanadium over a period of 24 h. Results expressed as x±1 S.D. for sample size N.

Time (h)	Vanadium Concentration (mg/L)			
	Control (N=5)	6.25 (N=5)	12.50 (N=4)	25.0 (N=4)
-3	54 ±11.0	55 ±7	ND[a] ND	61 ±8
-2	52 ±10	58 ±6	52 ±13	57 ±5
-1	47 ±7	55 ±6	53 ±12	56 ±6
0	53 ±10	55 ±6	56 ±12	54 ±13
0.25	51 ±9	53 ±9	ND ND	ND ND
0.5	50 ±11	57 ±7	55 ±7	52 ±13
+1	53 ±8	59 ±6	55 ±9	60 ±7
-2	52 ±10	59 ±9	60 ±4	63 ±9
4	58 ±10	57 ±5	60 ±5	59 ±16
8	54 ±10	58 ±4	56 ±8	57 ±16
12	43 ±24	55 ±5	57 ±5	59 ±13
21	47 ±12	54 ±4	56 ±3	56 ±13
24	49 ±11	53 ±5	62 ±4	63 ±9

[a] ND indicates no data.

Table 49. Dorsal aorta blood pressure (mm Hg) in adult rainbow trout (*Salmo gairdneri*) exposed to concentrations of vanadium over a 24 h period. Results expressed as x±1 S.D. for sample size N. S and D represent systolic and diatolic pressures, respectively.

Time (h)	Vanadium Concentration (mg/L) L							
	Control (N=4)		6.25 (N=4)		12.5 (N=3)		25.0 (N=2)	
	S	D	S	D	S	D	S	D
-3	37	28	37	28	41	33	ND[a]	ND
	±8	±6	±3	±1	±1	±1	ND	ND
-2	38	29	36	27	40	31	44	35
	±4	±4	±11	±0	±2	±1	ND	ND
-1	43	29	28	20	32	25		
	±4	±5	±14	±9	±10	±11	ND	ND
0	35	27	36	27	43	35	46	35
	±8	±5	±3	±2	±3	±0	ND	ND
0.25	30	26	ND	ND	41	35	ND	ND
	±6	±5	ND	ND	±3	±1	ND	ND
0.5	37	28	40	28	44	35	45	34
	±10	±7	±10	±8	±6	±3	ND	ND
1	35	27	34	24	50	38	42	31
	±9	±4	±7	±6	±13	±2	ND	ND
2	37	25	32	23	42	35	44	35
	±17	±10	±7	±8	±4	±4	ND	ND
4	35	24	27	19	42	31	33	27
	±13	±9	±5	±4	±4	±5	ND	ND
8	31	20	29	21	38	29	37	26
	±10	±7	±6	±3	±4	±4	ND	ND
12	31	19	27	18	ND	ND	39	26
	±9	±7	±10	±7	ND	ND	ND	ND
21	33	23	25	16	44	36	43	30
	±17	±11	±11	±9	±2	±1	ND	ND
24	29	19	22	14	42	34	40	27
	±19	±14	±13	±11	±1	±2	ND	ND

[a] ND indicates no data.

Table 50. Blood lactate (mg/100 mL) values for adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of vanadium over a 24 h period. Individual values are listed, as well as $\bar{x}\pm1$ S.D.

Vanadium Concentration (mg/L)	Time (h)		
	-1	+4	+24
Control			
	4.63	7.98	5.23
	7.93	6.75	8.64
	20.42	21.56	36.35
	6.22	12.66	6.23
	9.80	11.02	14.11
	±7.21	±6.58	±14.89
[6.25]			
	12.23	16.91	12.77
	37.73	28.75	44.79
	67.39	53.19	-
	-	-	-
	39.12	32.95	28.78
	±27.61	±18.50	±22.64
[12.5]			
	20.86	39.74	46.25
	9.56	15.24	32.60
	10.87	13.15	21.34
	13.76	22.71	33.40
	±6.18	±14.79	±12.47
[25.0]			
	5.39	13.52	18.65
	-	-	-
	8.70	11.29	10.75
	6.60	19.23	21.76
	6.90	14.68	17.05
	±1.67	±4.10	±5.68

Table 51. Hematocrit (%) values for adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of vanadium for a 24 h period. Individual values are listed as well as $\bar{x}\pm 1$ S.D.

Vanadium Concentration (mg/L)	Time (h)		
	-1	+4	+24
Control			
	18.0	16.0	14.0
	17.0	11.0	14.0
	4.0	4.0	4.0
	9.0	6.0	6.0
	12.0	9.3	9.5
	±6.7	±5.4	±5.3
[6.25]			
	3.5	3.0	17.0
	12.0	12.0	6.0
	12.0	9.0	12.0
	9.2	8.0	11.7
	±4.9	±4.6	±5.5
[12.5]			
	19.0	22.0	20.0
	23.0	18.0	23.0
	16.0	11.0	18.0
	19.3	17.0	20.3
	±3.5	±5.6	±2.5
[25]			
	11.0	12.0	11.0
	13.0	13.0	13.0
	11.0	9.0	9.0
	11.7	11.3	11.0
	±1.2	±2.1	±2.0

Table 52. Dorsal aorta pH in rainbow trout (*Salmo gairdneri*) exposed to three concentrations of vanadium over a 24 h period. Individual values are listed, as well as $\bar{x}\pm1$ S.D.

Vanadium Concentration (mg/L)	Time (h)		
	-1	+4	+24
Control			
	7.883	7.956	7.855
	7.850	7.910	7.876
	7.927	7.997	7.933
	7.917	7.888	7.812
	7.894	7.938	7.869
	±.035	±.050	±.050
[6.25]			
	7.932	7.908	7.981
	7.952	-	8.061
	7.856	7.965	7.861
	7.913	7.937	7.968
	±.051	±.040	±.101
[12.5]			
	7.704	7.700	8.160
	7.920	7.952	8.030
	7.929	8.040	8.066
	7.851	7.897	8.085
	±.127	±.176	±.067
[25]			
	7.839	7.967	8.014
	7.889	8.073	8.124
	7.908	8.152	8.138
	7.879	8.064	8.092
	±.036	±.093	±.068

Table 53. Dorsal aorta pO_2 (mm Hg) in adult rainbow trout (*Salmo gairdneri*) exposed to three concentrations of vanadium over a 24 h period. Individual values are listed, as well as $\bar{x} \pm 1$ S.D.

Vanadium Concentration (mg/L)	Time (h)		
	-1	+4	+24
Control			
	100.8	108.8	102.2
	91.0	97.5	96.6
	103.2	97.5	122.1
	98.3	101.3	107.0
	±6.5	±6.5	±13.4
[6.25]			
	126.1	123.0	128.6
	110.6	115.4	113.1
	112.3	108.0	97.8
	-	-	-
	116.3	115.5	113.2
	±8.5	±7.5	±15.4
[12.5]			
	100.7	95.1	101.9
	99.0	88.6	94.2
	99.9	91.9	98.1
[25]			
	83.7	77.6	84.5
	103.6	62.7	91.3
	93.7	70.2	87.9

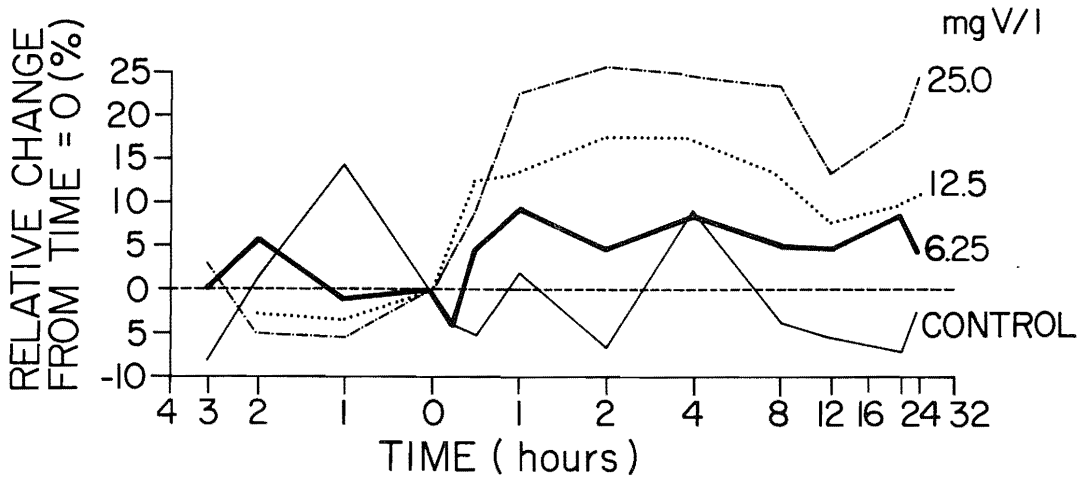

Figure 27. Effect of vanadium on the cough frequency of adult rainbow trout *(Salmo gairdneri)*. The cough frequencies are expressed as percent change from time = 0 to reduce the effects of individual variation of the test animals.

sunfish (*Lepomis megalates*) have been observed upon exposure to 0.009 to 0.5 mg/L of copper (Spoor et al. 1971; Drummond et al. 1973; Morgan and Kuhn 1974). Although it is possible that the rainbow trout employed in the present study were physiologically abnormal, the control values for all parameters were within published ranges for this species (Stevens and Randall 1967; Hughes and Roberts 1970; Wood 1974; Lunn et al. 1976; Majewski et al. 1977). A more likely explanation relates to background levels of copper in the dilution water employed in this study. Copper levels ranging from 50 to 70 µg/L were recorded in the ultra-violet dechlorinated water during the experimental period. In addition, the tissue concentration of copper in the experimental fish is relatively high and related to fish size (Majewski et al. 1978). Davis (1973) has demonstrated that, following an additional cardiovascular-respiratory response to Kraft mill effluent, sockeye salmon (*Oncorhynchus nerka*) cough frequency rapidly (within 20 to 60 h) returns to near normal levels. Presumably such an acclimatory response could have occurred in the present study and account for the lack of significant experimental effects with copper.

Vanadium at concentrations one to three times the 96 h LC50 to juvenile rainbow trout induced substantial increases in ventilation rate and, at the highest concentration, in cough frequency of adult trout. All other parameters were essentially unaffected by exposure to vanadium although variability was extremely high. Since histopathological changes observed in the gills of treated fish would indicate that some impairment of gas exchange should occur, it is probable that the increase in ventilation rate offset the reduction in gas exchange efficiency. No reduction in the oxygen partial pressure or decrease in pH of aortic blood was observed, demonstrating that oxygen and carbon dioxide exchange was not adversely affected.

Considering the short exposure period and high degree of histological damage observed in the gills of vanadium-exposed trout, the lack of a cough response at 6.25 and 12.5 mg V/L is surprising.

Increased cough frequency upon exposure to gill irritants and in response to decreases in transport of oxygen and carbon dioxide across the gill is commonly observed in rainbow trout and is often associated with an increase in ventilation rate. It is possible that the degree of gas-transport impairment during these short exposure times in restrained fish was not sufficient to elicit a cough response at the highest concentration.

Although a dose-response relationship between vanadium concentration and ventilation frequency was observed, the usefulness of this relationship in a potential field bioassay procedure is questionable. In the present study, doses greater than the 96 h LC50 were required to produce a quantifiable response. Even if the sensitivity of the response could be improved by an order of magnitude to include sublethal toxicant concentrations, the relative magnitude of stress induced by the experimental protocol (especially severe restraining of swimming activity) to that induced by the toxicant under study would complicate the interpretation of responses, especially at sublethal levels of toxicant. It would appear advantageous, therefore, to develop a "physiological bioassay" in which the test animals are able to maintain relatively normal physical activities and measure the effects of toxicants upon the organisms' ability to maintain some degree of homeostasis. The additional metabolic costs of maintaining hemeostatic or the degradation of hemeostatic mechanisms upon exposure to toxicants could then be more realistically related to other lethal and sublethal tests in terms of modes of toxic action. Such procedures would be applicable to field toxicity testing and monitoring functions as well as laboratory protocols. With toxicants such as vanadium which may not exhibit a well defined incipient lethal level, it would appear that an exposure time factor may be required to assess adequately the dose-response relationships.

5.6 AVOIDANCE-PREFERENCE REACTIONS OF WHITEFISH (*Coregonus clupeaformis*) TO VANADIUM AND COPPER

(Principal Investigators: D. Hodgins and G. Watts)

5.6.1 Introduction

As indicated previously (Section 4.3), behavioural bioassays are used to investigate the effects of toxicants at one of the highest levels of biological organization. Behavioural responses represent the integrated output of various systems including sensory perception and neuromuscular co-ordination, which in turn are supported by several levels of physiological and biochemical organization. The results of avoidance-preference bioassays have been extrapolated to field situations to explain responses observed in natural fish populations with varying success.

Since either avoidance or preference responses to a toxicant may be expected to have a major impact on the time and severity of exposure of aquatic organisms, behavioural information can be of major value in assessing the impact of a toxicant upon an ecosystem. No published information is currently available on the behavioural responses of fish to vanadium. The following experiments were undertaken, therefore, to examine the avoidance-preference responses of whitefish (*Coregonus clupeaformis*) to vanadium and, for purposes of comparison, to copper.

5.6.2 Methods

Lake whitefish (*C. clupeaformis*) were obtained from the Manitoba provincial hatchery at Anama Bay and raised in a natural pothole lake at Erickson, Manitoba. The three-month-old whitefish were transported to the Freshwater Institute where they were acclimated to ultra-violet dechlorinated (Armstrong and Scott 1974) Winnipeg city water at $10 \pm 2^{\circ}C$ for 2 wk prior to the first test. The mean weight and fork length of the whitefish were 21.398 ± 6.995 g and 13.7 ± 0.1 cm, respectively. No mortalities occurred during the acclimation period.

The lethal toxicity of vanadium as V_2O_5 and copper as $CuSO_4$ to whitefish was determined in continuous-flow toxicity tests at 10°C. Modified Mount and Brungs (1967) dilutors delivered 100 mL/L minute to duplicate 20 L polyethylene-lined test vessels, each containing five whitefish, with a loading density of 2.61 L/g/day. Dissolved oxygen, pH, and temperature were measured daily for the 96 h toxicity test (Table 81). Mortality checks followed a geometric series and the LC50's were calculated by the logit method (Ashton 1972).

The avoidance-preference response of whitefish to vanadium and copper was measured by a rectangular counter-current chamber (Scherer and Nowak 1973). Rotameters and adjustable needle valves controlled the flow rate to 3.9 L/min (velocity = 0.55 cm/s). The remaining test producers and statistical analysis of results employed in these studies were identical to those described in Section 4.5.2.

5.6.3 Results

The 96 h LC50 estimates for vanadium and copper were 17.38 $\pm$ 3.09 and 1.2442 $\pm$ 0.127 mg/L, respectively. No whitefish died in concentrations less than 12.5 mg/L vanadium and no mortality occurred in the controls.

No statistically significant preference occurred for one end of the chamber with vanadium ($t=0.46$, d.f.=59, $p>0.05$) or copper ($t=0.35$, d.f.=49, $p>0.05$); nor was there a statistically significant difference in the results of water-water trials for different concentrations of vanadium ($F=1.505$, d.f.=5, $p>0.05$) and copper ($F=0.689$, d.f.=5, $p>0.05$).

Whitefish displayed a statistically significant increased avoidance response at concentrations of 0.05, 1.0, 5, 10, and 17 mg/L vanadium and 0.05, 0.1, 0.5, and 1.0 mg/L copper (Table 54). No statistically significant response was shown by whitefish at concentrations of 0.1 mg/L vanadium and 0.01 mg/L copper. The avoidance (greater than 50% time spent in pure water) and preference (less than 50% time spent in pure water) of whitefish to vanadium and

Table 54. The avoidance-preference responses of whitefish (*Coregonus clupeaformis*) to various sublethal concentrations of vanadium and copper.

Toxicant Conc. (mg/L)L	% Time Spent on Side Vanadium and Copper Introduced			
Vanadium	Water-water trial	Toxicant-water trial	Calculated paired t-test	Significance p=0.05 t=2.262 p=0.01 t=3.240
0.1	54.92	54.57	-0.2780	p>0.05; no significant difference
0.5	50.58	28.13	-9.1955	p<0.01; increased avoidance
1.0	53.33	34.48	-2.5228	p<0.05; increased avoidance
5.0	52.68	30.62	-8.1053	p<0.01; increased avoidance
10.0	54.17	38.05	-3.2884	p<0.01; increased avoidance
17.0	56.38	33.15	-5.8935	p<0.01; increased avoidance
Copper				p=0.05 t=2.262 p=0.01 t=3.240
0.01	54.03	46.68	-2.2609	p>0.05; no significant difference
0.05	56.35	35.50	-4.6882	p<0.01; increased avoidance
0.10	51.83	34.33	-2.6070	p<0.05; increased avoidance
0.50	53.78	17.57	-5.7672	p<0.01; increased avoidance
1.00	55.46	30.83	-2.8613	p<0.05; increased avoidance

copper are shown in Figures 28 and 29, respectively. The threshold concentration for avoidance of vanadium was between 0.1 and 0.5 mg/L and the relative degree of avoidance did not appear to increase with concentration above the threshold (Figure 28). With copper, however, a gradual increase in the degree of avoidance was observed as the concentration increased from 0.05 to 0.5 mg Cu/L.

5.6.4 Discussion

The 96 h LC50 values of copper and vanadium for whitefish observed in the present study are in general agreement with those published for related species (Lloyd 1961; Brown and Dalton 1970; Sprague et al. 1978; Section 5.1). Slight variations in toxicity of both these metals may be expected since water pH and hardness influence the valence state and/or rates of free to bound metal in both these materials.

The minimum concentrations of vanadium and copper at which avoidance reactions were observed (threshold concentrations) occurred at 0.6 to 29% and 0.8 to 4.0% of their respective 96 h LC50 values. The concentration of copper in saline groundwater (Section 4.1) is within this range of threshold concentration. The concentration of vanadium in groundwater, however, is 14 to 70 times less than the threshold concentration required for an avoidance response. The observation that whitefish do not avoid saline groundwater (Section 4.5) may reflect the degradation of this effluent during storage.

5.7 TOXICITY OF VANADIUM AND COPPER TO *Daphnia magna* AND *Artemia salina*

(Principal Investigators: S. Leonhard and M. Friesen)

Several bioassays using *Artemia salina*, *Daphnia magna*, and *D. pulex* to assess the toxicity of vanadium and of copper were done. The preliminary results indicated the 96 h LC50 for *Daphnia* sp. was less than 0.11 µg/L Cu and less than 0.16 µg/L V. The literature indicates the 48 h LC50 for Cu in soft water is 9.8 µg/L

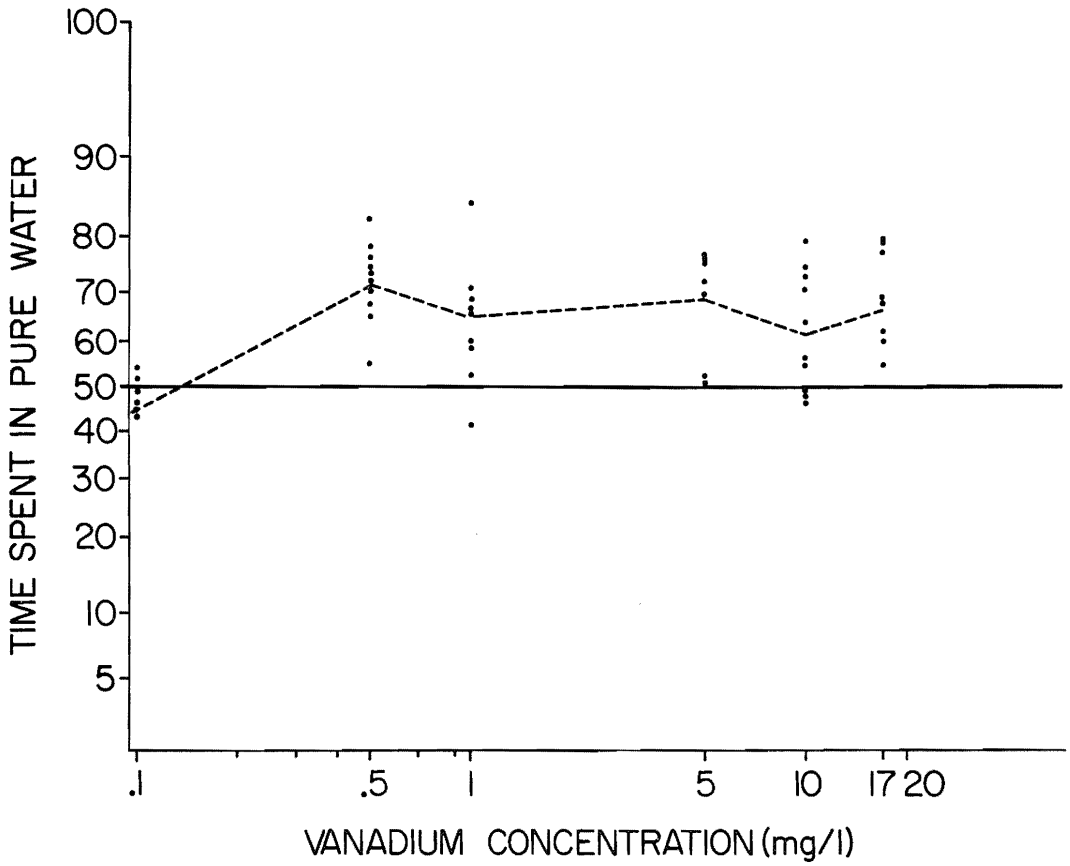

Figure 28. Avoidance-preference response to whitefish (*Coregonus clupeaformis*) to vanadium.

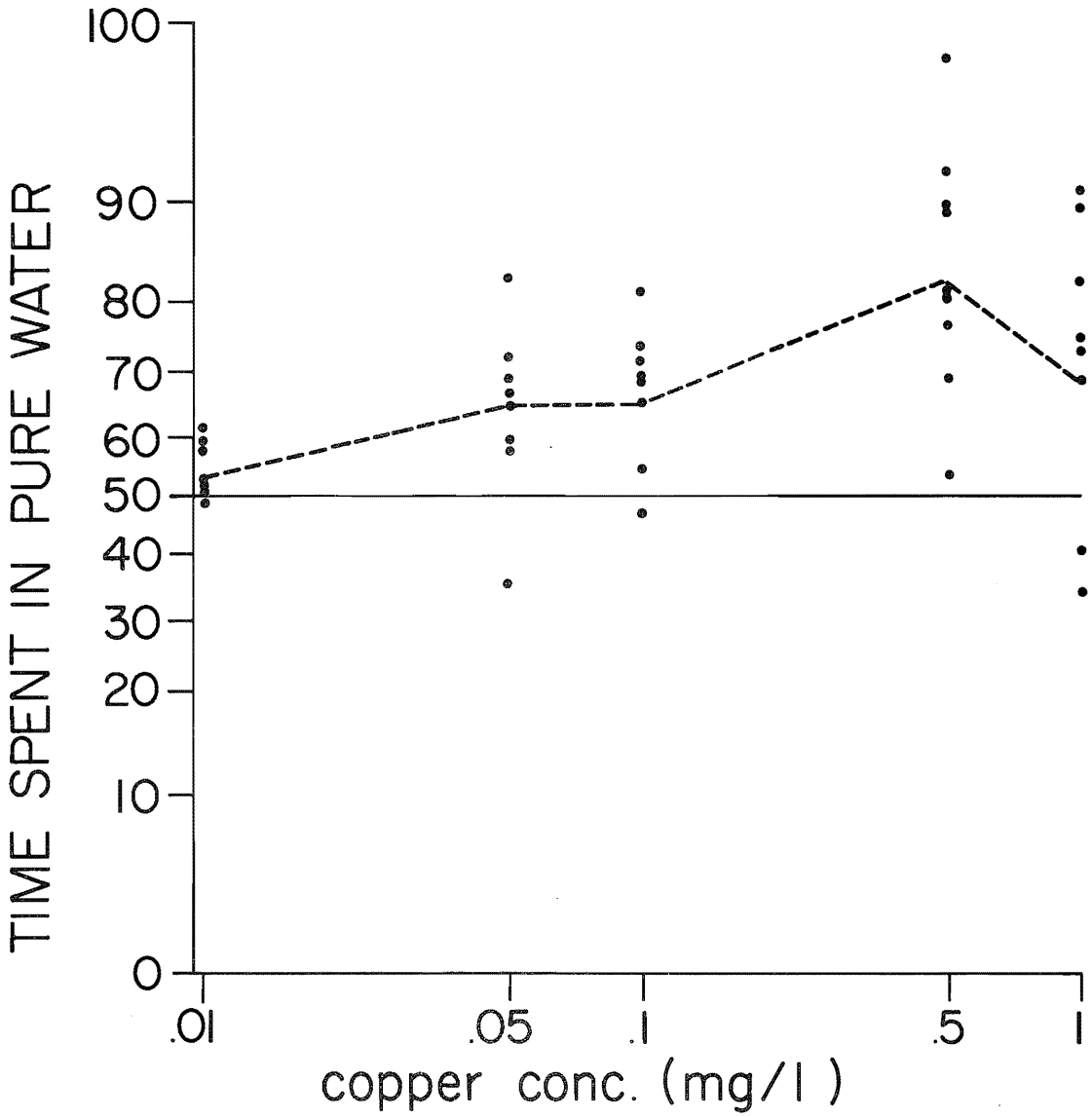

Figure 29. Avoidance-preference responses of whitefish (*Coregonus clupeaformis*) to copper.

Biesenger and Christensen 1972).

High mortality among controls indicated either that two effects were being observed, or that stock cultures of *Daphnia* were declining in vitality.

However, *Daphnia* sp. grown in reconstituted medium were normal with respect to life span and growth characteristics.

Chemical analysis showed that copper concentrations in incoming dechlorinated water ranged from 3.1 µg/L to 12.3 µg/L during the testing period.

Further tests have been discontinued until copper-free water is available in reasonable supply for use in continuous flow bioassays.

6. CONCLUSIONS

1. Major chemical changes in the composition of saline groundwater were observed during transport and storage of the effluent. These changes occurred in both the organic and inorganic components of the effleunt.

2. Considerable seasonal variation in the chemical composition of effluent from several individual depressurization wells was observed. The magnitude and direction of the seasonal variation for many effluent components was not consistent among individual wells.

3. The 10 d LC50 of groundwater ranged from 20 to 35% effluent for fingerling rainbow trout (*Salmo gairdneri*) in tests conducted on two composite samples of effluent after different periods of storage. Although the 10 d LC50 remained reasonably consistent, different composite effluents and storage times produced major changes in the pattern of mortality during the bioassays.

4. In two bioassays of the effluent collected in October 1976, the rate mortality appeared to increase during the latter stages of the bioassay and an incipient concentration was not observed.

5. Saline groundwater at sublethal concentrations induces severe histopathological lesions in the gill and kidney of fingerlings rainbow trout but has little apparent histological effect on the liver. The lowest concentration of groundwater inducing histopathological lesions is undefined but less than 6.25%.

6. Acute (24 h) exposure of adult rainbow trout did not result in measurable effects on the cardiovascular-respiratory systems.

7. A highly significant avoidance response to saline groundwater concentrations of >0.5 to 35% was observed in *Gammarus lacustris* but not in whitefish (*Coregonus clupeaformis*). Neither species preferred saline groundwater at any concentration from 0.1 to 35%.

8. Five species of aquatic invertebrates exposed to saline groundwater exhibited the following ranking of increasing

tolerance to the effluent; *Daphnia magna<<Chironomus tentans< Hexagenia rigida<<Orconectes viriles<Artemia salina*. The 96 h LC50 of two composites of groundwater to *Daphnia magna* differed greatly (<6.25% to 20-50%), possibly reflecting changes in chemical composition of the effluent.

9. No significant differences in the toxicity of saline groundwater when dechlorinated Winnipeg city water or Athabasca River water was employed as diluent were observed in any of the acute lethal or sublethal toxicity tests with fish or invertebrates.

10. Vanadium is moderately toxic to rainbow trout and whitefish (96 h LC50 of 8 to 22 mg/L) with the toxicity varying slightly with pH. The log time-log LC50 relationship is linear to 96 h exposure and a definite incipient lethal level was not observed within a 10 d bioassay period.

11. Pronounced histopathological lesions were observed in the gills and kidneys of trout exposed to sublethal concentrations of vanadium and the degree of damage increased with increasing time of exposure to the toxicant.

12. In terms of lethal concentrations, the eyed eggs of rainbow trout are 250 to 300 times more resistant to vanadium than fingerling trout. No histological aberrations were observed in trout embryos which survived exposure to vanadium.

13. No major responses to acute exposure to lethal concentrations of vanadium were observed in cardiovascular-respiratory parameters or the activities of liver catalase and xanthine oxidase enzymes.

14. Juvenile whitefish (*Coregonus clupeaformis*) avoid vanadium concentration of 0.5 mg/L and greater. The lowest toxicant concentration avoided was 3% of the 96 h LC50.

7. IMPLICATIONS AND RECOMMENDATIONS

It is difficult, if not impossible, to separate the implications and recommendations arising from this study into those pertaining to matters of general scientific importance and those relating specifically to oil sands developments. The main purpose of this study was to assess the acute toxicity of saline groundwater by means of a broad toxicological screening system, to provide a basis for further research on the toxicity of individual components to aquatic organisms. By integrating information on the chemical behaviour of these components in the Athabasca River drainage system and research on the chronic toxicity of major toxic components of the effluent, a reasonable assessment of the ecological impact of saline groundwater could be derived. This approach is indicated in the research proposal developed through a series of sequential investigations which identifies useful avenues of continued study and eliminates protocols which are not pertinent.

The results of the first stage of this sequential approach have produced several definite implications regarding the scientific approaches required for further study of the ecological effects of saline groundwater on the biota Athabasca River system. These implications and recommendations arising from them are listed below.

1. The chemical composition of saline groundwater changes drastically during transport and storage and the responses of fish and aquatic invertebrates to acute exposure to stored effluent also change with time. These observations imply that toxicity tests of stored effluent may not be valid and strongly suggest that future chronic toxicity studies would be difficult to interpret. It is recommended that future acute toxicity testing of saline groundwater be conducted on-site near one or preferably several groundwater wells, using fresh effluent in a continuous flow bioassay. This would circumvent problems of chemical degradation and facilitate use of aquatic organisms endemic to the Athabasca drainage system.

2. In view of the strong possibility that saline groundwater produces a direct lethal toxic response in fish over an extended period of time (greater than 10 d), the relevance of a 96 h

or 240 h lethal bioassay is questionable. Longer term (perhaps 21 to 30 d) bioassays should be conducted as part of the on-site toxicology program. Such tests may precisely define the range of incipient lethal concentrations of groundwater for endemic aquatic organisms.

3. Certain of the saline groundwater toxicity studies and the vanadium study described in this report indicate that the sublethal toxic responses may be cumulative over the exposure period. If so, the usual application factors used to set effluent criteria may not be valid. It is recommended that sensitive sublethal responses of one or more species of fish and aquatic invertebrates be examined during chronic exposure to saline groundwater and selected toxic components, with concurrent measurement of the degree of toxicant bioaccumulation.

4. Since the validity of laboratory-based toxicity testing of stored saline groundwater is questionable, and substantial information on the chemical composition of this effluent is currently becoming available, increased emphasis on the toxicity of individual components of the effluent and their toxic interactions is appropriate. Such studies should employ a range of increasingly sensitive acute and chronic tests to generate toxicological data using species endemic to the AOSERP study area.

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9. APPENDIX

The relevant routine chemical analyses and the cumulative mortality data from toxicity tests performed in this study are presented in Tables 55 and 81.

Table 55. Summary of routine chemical analyses from fish acute lethality bioassays of saline groundwater.

SGW (%)	Temperature (°C) N	Mean	±S.D./(Range)	Dissolved Oxygen (mg/L) N	Mean	±S.D./(Range)	Conductivity (µMmho/cm) N	Mean	±S.D./(Range)	pH N	Mean	±S.D./(Range)	Un-ionized Ammonia µg/L N	Mean	±S.D./(Range)
STATIC BIOASSAY/WINNIPEG WATER DILUENT/20-30 JULY 1976															
100	2	15.2	(15.0-15.4)	1	10.4		1	20000		1	8.7			2765	
90	2	15.2	(15.0-15.4)	1	10.3		1	18700		1	8.7		1	2404	
80	2	15.15	(15.1-15.2)	1	10.2		1	16000		1	8.7		1	2043	
70	2	15.1	(15.1-15.1)	1	10.2		1	15000		1	8.7		1	1803	
60	2	14.75	(14.5-15.0)	1	10.2		1	13000		1	8.6		1	1273	
50	4	15.1	(15.0-15.1)	5	10.1	(10.0-10.2)	4	10075	(9500-10750)	6	8.6	(8.0-9.1)	3	1837	±672.7
40	5	15.1	(15.0-15.2)	6	9.9	(10.0-10.3)	5	9230	(8600- 9600)	5	8.9	(8.6-9.2)	2	1504.5	(1504.5-1504.5)
30	10	15.2	(14.8-15.5)	10	9.9	(9.4-10.3)	10	6595	(6200- 6900)	10	8.9	(8.5-9.1)	5	1355.2	±458.0
20	10	15.3	(15.0-15.8)	10	9.9	(9.3-10.3)	10	4594	(4120- 4980)	10	8.7	(8.4-8.9)	5	762.7	±201.6
10	10	15.3	(15.0-15.9)	10	9.9	(9.3-10.2)	10	2457	(2300- 2620)	10	8.4	(8.2-8.6)	5	267.3	±140.8
Control	10	15.1	(15.0-15.8)	10	10.0	(9.2-10.8)	10	139	(50- 150)	10	8.0	(7.8-8.3)	5	23.8	±30.8
STATIC BIOASSAY/ATHABASCA R. WATER DILUENT/20-30 JULY 1976															
100	2	15.0	(15.0-15.0)	1	10.4		1	20000		1	8.9		1	4927	
90	2	15.0	(15.0-15.0)	1	10.2		1	18200		1	8.8		1	3083	
80	2	15.0	(15.0-15.0)	1	10.2		1	16500		1	8.8		1	2496	
70	2	15.0	(15.0-15.0)	1	10.2		1	14700		1	8.8		1	2055	
60	2	15.0	(15.0-15.0)	1	10.2		1	13000		1	8.8		1	1835	
50	2	15.0	(15.0-15.0)	2	10.3	(10.2-10.4)	2	10750	(10700-10800)	3	8.9	(8.8-9.1)	2	1550.5	(1550.5-1550.5)
40	5	15.0	(15.0-15.0)	5	9.9	(9.4-10.4)	5	8390	(8250- 8900)	5	9.0	(8.7-9.2)	4	1695.2	±429.8
30	8	15.0	(15.0-15.0)	9	9.8	(9.4-10.2)	8	6380	(6000- 6800)	11	9.1	(8.7-9.3)	6	1740.2	±445.7
20	8	15.0	(15.0-15.1)	9	9.8	(9.2-10.2)	8	4424	(4150- 4750)	11	8.9	(8.6-9.2)	6	1033.8	±327.0
10	8	15.0	(15.0-15.1)	9	9.8	(9.1-10.1)	8	2431	(2300- 2580)	11	8.5	(7.6-9.2)	5	374.6	±300.2
Control	8	15.0	(14.8-15.4)	9	9.8	(9.1-10.3)	8	180	(100- 220)	10	8.4	(7.8-9.1)	6	173.8	±192.5

Continued ...

Table 55. Continued.

SGW (%)	Temperature (°C) N	Mean	±S.D./(Range)	Dissolved Oxygen (mg/L) N	Mean	±S.D./(Range)	Conductivity (µMmho/cm) N	Mean	±S.D./(Range)	pH N	Mean	±S.D./(Range)	Un-ionized Ammonia µg/L N	Mean	± S.D./(Range)
REPLACEMENT BIOASSAY, 27 OCTOBER TO 6 NOVEMBER 1976															
100	4	15.1	0.12	2	9.8	(9.0 -10.6)	2	17750	(17375- 1825)	2	8.45	(8.225-8.675)	2	922	(911 -933)
80	4	15.1	0.12	2	9.9	(9.0 -10.8)	2	14100	(14050-14150)	2	8.65	(8.325-8.975)			
60	9	15.0	0.10	4	9.8	0.20	6	10200	167	7	8.6	0.11			
50	13	14.9	0.13	5	9.96	0.26	11	8336	120	7	8.7	0.09	6	685.8	±113.9
40	19	15.0	0.10	8	9.8	0.32	18	6365	165	10	8.7	0.16			
30	20	15.0	0.10	8	9.4	0.83	18	4475	213	10	8.6	0.16			
20	20	14.9	0.12	8	9.6	0.51	18	4274	67	10	8.6	0.09	7	287.4	70.5
Control	20	15.0	0.10	8	9.8	0.23	18	147	16	10	7.8	0.09	8	12.2	7.3
STATIC BIOASSAY, 27 OCTOBER TO 6 NOVEMBER 1976															
100	4	15.5	0.46	2	9.5	(9.0 -10.0)	2	18250	(18000-18500)	2	8.6	(8.3 -8.9)			
90	4	15.5	0.44	2	9.5	(9.0 -10.0)	2	16250	(16000-16500)	2	8.7	(8.35 -9.05)			
80	6	15.9*	1.1	3	9.7	0.32	4	14525	450	4	8.6	0.14			
70	5	15.4	0.44	3	9.7	0.40	4	12725	359	4	8.6	0.15			
60	4	15.5	0.44	2	9.5	0.42	2	10500	0	2	8.75	0.07			
50	5	15.4	0.46	3	9.9	0.21	4	8650	300	4	8.8	0.09			
40	11	15.7*	0.84	5	9.4	0.51	9	6567	166	7	8.7	0.13			
30	13	15.5*	0.83	6	9.8	0.25	11	4541	124	7	8.75	0.13			
20	20	15.5*	0.79	8	9.7	0.43	19	4364	96	11	8.7	0.11			
10	20	15.5*	0.70	8	9.7	0.27	20	2270	59	11	8.5	0.10			
Control	20	15.5*	0.70	8	9.8	0.39	20	162	14	11	7.9	0.10			

Continued ...

Table 55. Concluded.

SGW (%)	Temperature (°C) N	Mean	±S.D./(Range)	Dissolved Oxygen (mg/L) N	Mean	±S.D./(Range)	Conductivity (µMmho/cm) N	Mean	±S.D./(Range)	pH N	Mean	±S.D./(Range)	Un-ionized Ammonia µg/L Conc.%	N	Mean
STATIC BIOASSAY 16-25 NOVEMBER 1976															
100	2	15.2	(15.0 -15.4)	2	9.8	(9.8 -9.8)	2	14800	(14800 -14800)	1	8.9				
90	2	15.2	(15.0 -15.4)	2	9.7	(9.7 -9.7)	2	13150	(13115 -13185)	1	8.9				
80	2	15.1	(15.1 -15.1)	2	9.8	(9.8 -9.8)	2	11750	(11728 -11772)	1	8.9				
70	4	15.1	(15.1 -15.1)	2	9.7	(9.7 -9.7)	2	10150	(10115 -10185)	1	8.9	-	100	1	1545*
60	4	15.2	(15.2 -15.2)	2	9.8	(9.8 -9.8)	2	8900	(8900 - 8900)	1	8.9	-	50	1	626
50	4	14.9	0.10	2	9.8	(9.765-9.835)	2	7000	(7000 - 7000)	1	8.8	-	20	1	169
40	4	14.8	0.11	2	9.9	(9.865-9.935)	2	5300	(5300 - 5300)	1	8.7	-	Control	1	14
30	4	14.9	0.13	2	9.7	(9.665-9.735)	2	3887	17.7	1	8.7	-			
20	4	14.8	0.20	8	9.7	0.24	20	4197	208	10	8.6	0.10			
10	20	15.1	0.19	8	9.3	0.86	20	2145	91	10	8.2	0.17			
Control	20	15.0	0.05	8	9.6	0.33	20	164	17	10	7.9	0.11			

Table 56. Cumulative mortality of rainbow trout (*Salmo gairdneri*) in saline groundwater in a static bioassay, 20-30 July 1976, using dechlorinated Winnipeg City water diluent. Saline groundwater collected 8 July 1976.

SGW (%)	N	Hours of Exposure																			
		0.5	1.0	2.0	2.5	3	3.5	4	9	13	24	32	48	72	96	120	144	168	192	216	240
100	5	0	0	5																	
90	5	0	0	4	5																
80	5	0	0	3	5																
70	5	0	0	2	5																
60	5	0	0	1	3	3	3	3	5												
50	5	0	0	0	0	0	0	1	2	3	3	3	4	5							
40	5	0	0	0	0	0	0	0	0	0	0	0	0	3	5						
30	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	4	4	5		
20	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LC50 (% Groundwater)[a]		-	-	75	59	59	59	57	51	48	48	48	47	39	35	30.5	28	28	25	25	25

[a]estimated from eye-fitted graph.

Table 57. Cumulative mortality of rainbow trout (*Salmo girdneri*) in saline groundwater in a static bioassay, 20-30 July 1976, using Athabasca River water diluent. Saline groundwater collected 8 July 1976.

SGW (%)	N	Hours of Exposure																			
		0.5	1.0	2	2.5	3.0	3.5	4	9	13	24	32	48	72	96	120	144	168	192	216	240
100	5	0	0	5																	
90	5	0	0	2	5																
80	5	0	0	2	5																
70	5	0	0	1	1	3	4	4	5												
60	5	0	0	4	4	4	5														
50	5	0	0	0	0	0	1	1	2	4	4	5									
40	5	0	0	0	0	0	0	0	0	1	1	1	1	2	3	5					
30	5	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2	2	2	2	2
20	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LC50 (% Groundwater)[a]		-	-	-	-	-	-	-	51	44	44	40	40	40	37	30	30	30	30	30	30

[a]LC50 fitted by eye.

Table 58. Cumulative mortality of rainbow trout[a] (*Salmo gairdneri*) in saline groundwater in a daily replacement bioassay, 27 October to 6 November 1976. Saline groundwater collected 19 October 1976.

SGW (%)	N	Hours of Exposure																	
		0.25	0.5	1	2	4	8	16	24	32	48	72	96	120	144	168	192	216	240
100	10	0	0	0	7	7	9	10											
80	10	0	0	0	4	5	6	9	10										
60	10	0	0	0	0	2	2	6	7	7	7	7	8	9	10				
50	10	0	0	0	0	2	2	2	2	2	2	2	3	8	10				
40	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5	5	10
30	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2
20	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LC50 (% groundwater)					87.9	80.2	71.9	59.2	55.9	55.9	55.9	55.9	53.8	46.0	45*	42*	38.3	34.5	32.8
95% Confidence					61.4	50.9	51.4	47.4	47.1	47.1	47.1	47.1	45.5	41.4			30.7	29.5	30.9
Interval of LC50					125.8	126.5	100.7	73.9	66.3	66.3	66.3	66.3	63.6	51.9			47.7	42.9	34.8
Slope Function							1.3	1.2	1.1	1.1	1.1	1.1	1.1	1.2			1.2	1.2	1.1

[a] fish weight, 11.16±3.57 g; fork length 10.1±1.0 cm.

[b] LC50 fitted by eye.

Table 59. Cumulative mortality of rainbow trout[a] (*Salmo gairdneri*) in saline groundwater in a static bioassay, 27 October to 6 November 1976. Saline groundwater collected 19 October 1976.

SGW (%)	N	Hours of Exposure																	
		0.25	0.5	1	2	4	8	16	24	32	48	72	96	120	144	168	192	216	240
100	10	0	0	1	7	9	10												
90	10	0	0	2	8	9	10												
80	10	0	0	0	5	6	6	8	8	8	8	9	10						
70	10	0	0	0	4	5	5	5	5	5	5	10							
60	10	0	0	0	8	9	10												
50	10	0	0	0	6	7	8	8	8	8	8	10							
40	10	0	0	0	0	0	1	1	1	1	2	5	5	6	9	10			
30	10	0	0	0	0	0	0	0	0	0	0	1	2	9	10				
20	10	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	4	6	6
10	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LC50 (% Groundwater)					62.4	54.8	52.4	50.9	50.9	50.9	49.6	39.0	37.1	29.5[b]	24.7	21.7	20.5	19.5	19.5
95% Confidence Interval of LC50						35.6 84.3	36.3 75.5	37.3 69.5			35.5 69.2	30.5 49.7	28.2 48.7		19.0 32.1	18.9 25.0	18.1 23.2	17.3 22.0	
Slope Function													1.15			1.18	1.13		

[a]fish weight: 13.54±3.94 g
length: 10.5 ±1.1 cm

Table 60. Cumulative mortality of rainbow trout[a] (*Salmo gairdneri*) in saline groundwater under static bioassay conditions. Bioassay performed 16-25 November 1976; saline groundwater collected 19 October 1976.

SGW (%)	N	Hours of Exposure																		
		0.5	1.0	2	2.5	3	4	8	16	24	32	48	72	96	120	144	168	192	216	240
100	10	0	10																	
90	10	0	9	10																
80	10	0	8	10																
70	10	0	5	10																
60	10	0	6	10																
50	10	0	6	10																
40	10	0	0	9	10															
30	10	0	0	4	8	9	10													
20	10	0	0	0	0	0	1	2	2	2	2	2	2	2	2	2	2	2	2	2
10	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LC50 (% groundwater)				31.5	28	27	22	21.5												

[a] fish weight: 11.1±5.0 g
length: 9.8±1.3 cm

[b] LC50 fitted by eye.

Table 61. Chemical characteristics of Winnipeg City water.

Parameter	Concentration (mg/L)
hardness (as $CaCO_3$)	100
iron (Fe)	0.32
free ammonia nitrogen (NO_3)	<0.01
calcium (Ca)	23.2
chloride (Cl^-)	5.8
copper (Cu)	<0.005
nickel (Ni)	<0.005
cadmium (Cd)	<0.005
chromium (Cr)	<0.005
zinc (Zn)	0.038
lead (Pb)	<0.05
pH	7.6

Table 62. Summary of routine chemical analyses from static bioassays with whitefish (*Coregonus clupeaformis*) and *Gammarus lacustris*.

SGW (%)	Temperature °C			Dissolved Oxygen (mg/L)			Conductivity umho/cm			pH			NH_3 (mg/L)		
	#	Range	Mean	#	Range	Mean	#	Range	Mean	#	Range	Mean	#	Range	Mean
Coregonus clupeaformis															
100	1	15.0	15.0	1	9.6	9.6	2	17500-18000	17750	3	8.7 -8.8	8.75	3	0.918-1.589	1.14
50	9	14.8-16.2	15.4	4	7.7-10.2	9.5	9	8000-8500	8111.11	10	8.5 -8.8	8.7	8	0.557-0.852	0.711
25	10	14.9-16.2	15.3	4	9.5-10.1	9.8	10	2800-3400	2997.5	10	8.5 -8.8	8.8	7	0.200-0.410	0.287
12.5	10	14.9-16.2	15.3	4	9.7-10.3	9.9	10	2650-2780	271	10	8.5 -8.8	8.7	-	-	-
6.25	10	15.0-16.1	15.3	4	9.5-10.4	10.0	10	1380-1430	1402	10	8.4 -8.8	8.5	-	-	-
Control	10	14.3-16.0	15.1	4	9.6-10.7	10.1	10	125-150	138.65	10	7.9 -8.1	8.0	8	0.004-0.210	0.036
Gammarus lacustris															
I. 80	10	13.8-15.9	15.1	10	8.0-11.6	10.3	10	18290-24000	20750	10	7.85-9.0	8.5	NOT MEASURED		
51.2	10	14.0-16.2	15.3	10	8.3-12.0	10.8	10	12400-14040	13365.0	10	7.9 -8.95	8.6			
26.2	10	14.5-16.1	15.3	10	8.1-11.6	10.4	10	6510-7560	6433.0	10	7.8 -8.9	8.6			
13.42	10	15.0-16.0	15.4	10	8.2-11.4	10.2	10	3300-4500	3828.0	10	8.0 -8.7	8.5			
3.52	10	15.0-16.5	15.6	10	8.0-10.8	9.6	10	1200-1320	1223.6	10	7.8 -8.35	8.1			

Continued ...

Table 62. Concluded.

SGW (%)	Temperature °C #	Range	Mean	Dissolved Oxygen (mg/L) #	Range	Mean	Conductivity umho/cm #	Range	Mean	pH #	Range	Mean	NH_3 (mg/L) #	Range	Mean
Gammarus lacustris															
I. Control	10	15.0-17.0	15.5	10	7.2- 9.8	8.7	8	126-252	184.3	10	7.6-8.1	7.8	NOT MEASURED		
II. 100	10	13.0-16.0	15.2	10	8.1-11.0	9.8	10	23940-27000	24968	10	7.9-9.45	8.8	NOT MEASURED		
80	10	14.5-16.0	15.3	10	7.8-10.6	9.6	10	19840-21720	20086.6	10	7.9-9.6	8.9			
51.2	10	15.5-16.0	15.8	10	7.1-10.1	9.4	10	12810-14400	13324.5	10	8.5-9.4	8.9			
13.42	10	15.0-16.5	15.8	10	9.2-10.0	9.1	10	3480-3960	3702.0	10	7.3-9.0	8.4			
3.52	10	15.3-16.5	15.9	10	6.2-10.0	9.2	10	1032-1200	1150.4	10	7.0-9.15	8.03			
Control	10	15.3-17.0	16.1	10	6.8-10.2	9.1	10	122-290	172.8	10	7.1-8.1	7.69			

Table 63. Conductivity of water and saline groundwater in avoidance-preference trials with whitefish (*Coregonus clupeaformis*) and *Gammarus lacustris*.

SGW %	Water		Saline Groundwater		Number of Measurements
	Range	Mean	Range	Mean	
Coregonus clupeaformis					
0.1	140	140	165	165	1
1.0	140	140	410	410	1
10.0	140	140	1750	1750	1
20.0	140	140	3600	3600	1
35.0	140	140	4250	4250	1
Gammarus lacustris					
0.5	138.6-189.0	141.1	283.5-315.0	294.5	4
1.0	157.5-226.8	196.6	409.5	409.5	5
5.0	189.0	180.0	1285.2-1480.5	1369.62	5
15.0	189.0-283.5	212.63	2772 -3906.0	3496.5	4
25.0	189.0-352.8	245.7	5292.0-6489.0	6142.5	4

Table 64. Composition of NC-2 culture media used in the *Daphnia magna* bioassays.

Chemical	Stock Solution (g/L)	Final Concentration in Media (mg/L)
KCl	50.0	50.0
$MgSO_4 \cdot 7H_2O$	40.0	40.0
K_2HPO_4	6.0	6.0
KH_2PO_4	6.0	6.0
Na_2SiO_3	20.0	20.0
$NaNO_3$	50.0	50.0
$CaCl_2 \cdot 6H_2O$[a]	21.92	153.44

[a] pH adjusted to 6.65 with 1.2 NHCl before addition of $CaCl_2$ and maintained below 7.0 with HCl to prevent precipitation of calcium carbonates.

Table 65. Summary of routine chemical parameters recorded during the acute lethal bioassay of the "December composite" of saline groundwater to *Daphnia magna*. Test period 5 to 10 January 1977.

SGW %	Conductivity (umho/cm^3)		pH		Temperature (^oC)		Un-ionized NH_3 (µg/L)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Control	400	400	7.82	8.29	19°C	19°C	0.226	3.41
0.01%	430	430	7.86	8.27	19°C	19°C	0.283	1.36
0.05%	450	450	7.85	8.29	19°C	19°C	0.566	1.36
0.10%	490	490	8.36	8.35	19°C	19°C	1.68	1.68
0.50%	550	550	8.04	8.02	19°C	19°C	0.703	0.708
1.00%	700	650	8.27	8.52	19°C	19°C	1.26	3.12
5.00%	1,800	1,800	8.63	8.66	19°C	19°C	15.28	13.9
10.00%	3,200	3,200	9.24	9.03	19°C	19°C		29.5
50.00%	13,000	13,000	9.44	9.58	19°C	19°C		
100.00%	23,500	23,500	9.43	9.40	19°C	19°C		

Table 66. Summary of routine chemical parameters recorded during the acute exposure of *Artemia salina* cysts to saline groundwater.

SGW %	Conductivity (umho/cm^3)		pH		Temperature (°C)		Un-ionized NH_3 (µg/L)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Control	30,000	41,000	8.72	8.94	20°C	19°C	1.98	11.28
0.01% SGW		39,000	8.71	8.92	19.8°C	19°C	1.98	9.02
0.05%		38,000	8.72	8.90	19.5°C	19°C	1.98	9.02
0.10%		39,000	8.72	8.93	19.5°C	19°C	1.98	9.02
0.50%		38,000	8.71	8.94	19.5°C	19°C	3.98	9.02
1.00%		39,000	8.71	8.93	19.5°C	19°C	7.29	9.02
5.00		33,000	9.07	8.95	20.0°C	19°C	139.50	29.53
10.00		33,000	8.92	9.10	20.0°C	19°C	237.79	83.23
50.00		35,000	8.79	9.47	19.5°C	19°C	1057.68	59.07
100.00	20,100	38,000	8.74	9.60	19.0°C	19°C	1647.73	

Table 67. Initial and final conductivity and pH readings from the early-stage egg (*Hexagenia rigida*) bioassays of the July saline groundwater composite using Winnipeg city or Athabasca River water as diluents.

SGW %		Conductivity Day 0(15°C)	Conductivity Day 10(21°C)	pH Day 0(15°C)	pH Day 10(21°C)
WINNIPEG DILUENT					
Control	i[a]		1,200		
	ii	370	1,130	8.26	7.55
	iii		1,140		
20%	i		6,800		
	ii	5,300	7,000	8.76	8.96
	iii		7,000		
40%	i		11,900		
	ii	9,500	12,100	8.80	9.28
	iii		12,700		
60%	i		17,000		
	ii	13,300	17,100	8.84	9.45
	iii		17,300		
80%	i		22,000		
	ii	17,000	22,000	8.83	9.59
	iii		22,200		
100%	i		27,000		
	ii	26,500	27,300	8.83	9.63
	iii		27,000		
ATHABASCA DILUENT					
Control	% i				
	ii	308	1,280	8.19	8.39
	iii		1,280		
20%	i		6,700		
	ii	5,300	7,500	8.81	9.23
	iii		7,000		
40%	i		12,500		
	ii	9,450	12,200	8.85	9.45
	iii		12,500		
60%	i		18,000		
	ii	13,550	17,200	8.85	9.58
	iii		17,800		

Continued...

Table 67. Concluded.

SGW %	Conductivity Day 0(15°C)	Conductivity Day 10(21°C)	pH Day 0(15°C)	pH Day 10(21°C)
ATHABASCA DILUENT (Continued)				
80% i		22,600		
ii	16,800	22,100	8.85	9.64
iii		22,100		
100% i		27,100		
ii	22,000	27,600	8.83	9.74
iii		27,600		

[a] i to iii = replicate number

Table 68. Initial and final conductivity and pH readings from the late-stage egg (*Hexagenia rigida*) bioassay of the July composite of saline groundwater using Winnipeg city or Athabasca River water as diluent.

SGW %		Conductivity Day 0(15°C)	Conductivity Day 10(21°C)	pH Day 0(15°C)	pH Day 10(21°C)
WINNIPEG DILUENT					
Control	i[a]	138	625	7.75	7.53
	ii		925		
20%	i	4,860	5,750	8.40	8.98
	ii		6,000		
40%	i	9,250	12,000	8.56	9.33
	ii		12,000		
60%	i	13,000	16,000	8.62	9.51
	ii		ND		
80%	i	16,000	21,000	8.70	9.61
	ii		22,000		
100%	i	20,000	ND	8.74	9.63
	ii		26,000		
ATHABASCA DILUENT					
Control					
%	i	180	575	8.84	8.51
	ii		275		
20%	i	4,700	5,750	8.67	9.19
	ii		6,000		
40%	i	8,900	11,000	8.73	9.40
	ii		11,250		
60%	i	13,000		8.75	9.57
	ii		16,000		
80%	i	16,500	21,000	8.79	9.63
	ii		21,000		
100%	i	20,000	24,500	8.86	9.70
	ii		26,000		

[a] i to ii = replicate number

Table 69. Percent hatch of early-stage eggs of *Hexagenia rigida* incubated in SGW concentrations using Winnipeg water as diluent. Mean percent is calculated from mean number of eggs hatched.

SGW %		Number of Eggs Counted	Days From Start of Bioassay															
			15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
						% HATCH	% HATCH			% HATCH	% HATCH	% HATCH		% HATCH				% HATCH
Control	i[a]	500	–[b]	+[c]		93.8				97.6								
	ii	500	–	+		91.6				98.2								
	iii	500	–	+		92.6				98.2								
Mean		500	–	+		92.7				98.0								
20%	i	500	–	–	+		92.8				94.0							
	ii	240	–	–	+		97.5				98.8							
	iii	500	–	–	+		96.0				97.2							
Mean		413	–	–	+		95.0				96.2							
40%	i	500	–	–	–	+		87.8				91.0						
	ii	500	–	–	–	+		88.8				91.2						
	iii	500	–	–	–	+		88.2				91.8						
Mean		500	–	–	–	+		88.3				91.3						
60%	i	500	–	–	–	–	–	+	10.2					45.0				43.6
	ii	500	–	–	–	–	–	+	18.2					48.4				47.2
	iii	500	–	–	–	–	–	+	15.8					35.2				46.8
Mean		500	–	–	–	–	–	+	14.7					42.9				45.9
80%	i	500	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	ii	500	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	iii	500	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mean		500	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Continued...

Table 69. Concluded.

SGW %		Number of Eggs Counted	Days From Start of Bioassay															
			15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
						% HATCH	% HATCH			% HATCH	% HATCH	% HATCH		% HATCH				% HATCH
100%	i	500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ii	500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	iii	500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean		500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] i to iii = replicate number

[b] - indicates no hatch

[c] + indicates first hatch

Table 70. Percent hatch of early-stage eggs of *Hexagenia ridiga* incubated in SGW concentrations using Athabasca water as diluent. Mean percent hatch is calculated from mean number of eggs hatched.

SGW %		Number of Eggs Counted	Days From Start of Bioassay															
			15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
						% HATCH	% HATCH	% HATCH		% HATCH	% HATCH	% HATCH		% HATCH				% HATCH
Control	i[a]	500	-[b]	+[c]		79.2				98.0								
	ii	500	-	+		93.4				97.2								
	iii	500	-	+		96.2				97.6								
Mean		500	-	+		89.6				98.4								
20%	i	500	-	-	+		95.2				97.4							
	ii	451	-	-	+		94.0				97.8							
	iii	337	-	-	+		94.7				98.2							
Mean		429	-	-	+		94.6				97.7							
40%	i	500	-	-	-	+		90.2				94.6						
	ii	500	-	-	-	+		88.6				92.8						
	iii	500	-	-	-	+		91.6				92.2						
Mean		500	-	-	-	+		90.1				93.2						
60%	i	500	-	-	-	-	-	+		25.2				41.6				42.4
	ii	500	-	-	-	-	-	+		18.0				45.8				42.4
	iii	500	-	-	-	-	-	+		21.4				45.4				51.2
Mean		500	-	-	-	-	-	+		21.5				44.3				45.3
80%	i	500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ii	500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	iii	500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean		500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Continued...

Table 70. Concluded.

SGW %		Number of Eggs Counted	Days From Start of Bioassay															
			15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
						% HATCH	% HATCH	% HATCH		% HATCH	% HATCH	% HATCH		% HATCH				% HATCH
100%	i	500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ii	500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	iii	500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean		500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] i to iii = replicate number

[b] - indicates no hatch

[c] + indicates first hatch

Table 71. Percent hatch of late-stage eggs of *Hexagenia rigida* incubated in SGW concentrations using Winnipeg water as diluent. Mean percent hatch is calculated from mean number of eggs hatched.

SGW %		DAYS AFTER BIOASSAY									
		0		4		8		13		18	
		# Eggs Counted	% Hatch	# Eggs Counted	% Hatch	# Eggs Counted	% Hatch	# Eggs Counted	% Hatch	# Eggs Counted	% Hatch
Control											
	i	500	79.2	500	92.8	615[a]	92.3				
	ii	500	81.4	500	90.4	503[a]	93.0				
Mean		500	80.3	500	91.6	559	92.7				
20%	i	500	83.3	500	92.8	729[a]	92.6				
	ii	500	82.2	416	90.4	416[a]	91.6				
Mean		458	83.1	458	91.7	573	92.2				
40%	i	500	54.1	500	93.0	822[a]	89.4				
	ii	500	55.2	500	90.0	605[a]	91.1				
Mean		500	54.8	500	91.5	714	90.1				
60%	i	500	21.6	500	84.8	559[a]	84.8				
	ii	500	18.6	500	80.8	619[a]	82.1				
Mean		500	20.1	500	82.8	589	83.4				
80%	i	500	0.0	500	45.4	500	68.8	617[a]	69.7	617[a]	74.7
	ii	500	0.0	500	58.0	500	72.8	1078[a]	75.4	1078[a]	78.7
Mean		500	0.0	500	51.7	500	70.8	848	73.3	848	77.2
100%	i	500	0.0	500	0.4	500	23.4	1065[a]	28.9	1065[a]	30.8
	ii	320	0.0	320	0.6	320	27.2	320[a]	29.1	320[a]	33.1
Mean		410	0.0	410	0.5	410	24.8	693	29.0	693	31.4

[a] Number given represents all the eggs in test vessel.

Table 72. Percent hatch of late-stage eggs of *Hexagenia rigida* incubated in SGW concentrations using Athabasca water as diluent. Mean percent hatch is calculated from mean number of eggs hatched.

SGW %		DAYS AFTER BIOASSAY									
		0		4		8		13		18	
		# Eggs Counted	% Hatch	# Eggs Counted	% Hatch	# Eggs Counted	% Hatch	# Eggs Counted	% Hatch	# Eggs Counted	% Hatch
Control											
	i	500	85.6	500	92.4	824[a]	94.2				
	ii	500	87.8	500	93.2	517[a]	93.6				
Mean		500	86.7	500	92.8	671[a]	94.0				
20%	i	500	88.0	500	92.2	597[a]	92.6				
	ii	500	91.4	500	91.4	1042[a]	94.7				
Mean		500	89.7	500	91.8	820	94.0				
40%	i	500	71.4	500	91.6	537[a]	91.1				
	ii	270	59.3	270	88.8	270[a]	87.8				
Mean		385	67.1	385	90.1	404	90.0				
60%	i	500	12.2	500	79.2	655[a]	78.3				
	ii	415	15.2	415	82.4	415[a]	87.0				
Mean		457	13.6	457	80.7	535	81.7				
80%	i	500	0.0	500	31.0	500	68.6	672[a]	72.2	672[a]	74.7
	ii	500	0.0	500	28.0	500	74.6	693[a]	78.6	693[a]	78.8
Mean		500	0.0	500	29.5	500	71.6	683	75.5	683	76.8
100%	i	500	0.0	500	0.4	500	28.6	864[a]	32.2	864[a]	36.1
	ii	483	0.0	483	0.2	483	16.1	483	27.7	483[a]	30.0
Mean		492	0.0	492	0.3	492	22.5	674	30.6	674	33.6

[a] Number given represents all the eggs in test vessel.

Table 73. Summary of chemical parameters monitored during the acute vanadium bioassays at pHs 6, 7, 8, and 9 with rainbow trout (*Salmo gairdneri*).

Conc. Theoretical ppm	Conc. Actual ppm	pH Mean	pH + S.D.	O_2 Mean	O_2 + S.D.	Temperature Mean	Temperature + S.D.	NH_3-N µg/L	PO_4-P µg/L	CO_2 µ mole/L	CL mg/L	SO_4 mg/L	Na mg/L	K mg/L	Ca mg/L	Fe mg/L	Mg mg/L
Exp. - Vanadium pH 6 - Date 22 Nov. 1976- Species - Salmo gairdneri (Nesquali) Length 8.9±1.1 - Wt. 8.22±3.00																	
40.0	40.2	6.04	0.794	9.6	0.44	15.1	0.11	20	19	80	11.4	111	22.5	1.07	25.2	0.08	5.79
30.0	30.5	6.02	0.264	9.6	0.27	15.0	0.15	130	23	100	9.2	93.0	16.8	1.12	25.1	0.04	5.66
22.5	24.1	5.99	0.206	9.6	0.33	15.0	0.15	140	20	145	9.2	85.0	13.3	1.14	25.4	0.08	6.10
16.8	19.1	6.19	0.198	9.6	0.28	15.0	0.15	220	31	95	9.0	80.0	10.6	1.16	25.0	0.15	5.85
12.6	15.5	6.05	0.249	9.6	0.28	14.9	0.11	210	27	180	8.8	75.5	8.45	1.12	25.1	0.04	5.66
9.5	12.7	6.24	0.198	9.7	0.33	14.9	0.04	170	21	90	8.4	73.0	6.84	1.16	25.4	0.08	5.60
71.0	10.3	6.16	0.187	9.6	0.33	14.9	0.08	160	23	110	8.4	71.5	6.03	1.20	25.6	0.04	6.10
53.0	10.1	6.11	0.197	9.4	0.38	14.9	0.05	180	26	140	7.8	71.5	5.55	1.16	25.1	0.15	5.79
4.0	6.8	6.34	0.196	9.6	0.31	14.9	0.09	160	18	65	8.0	70.5	4.32	1.18	25.6	0.04	6.16
3.0	5.17	6.21	0.192	9.6	0.36	15.0	0.09	180	20	115	7.4	69.0	3.61	1.16	26.3	0.04	6.10
Control	<1.0	6.45	0.426	9.6	0.28	15.0	0.10	150	21	95	8.2	66.0	1.74	1.18	24.8	0.11	5.72
Exp. - Vanadium pH 7 - Date 22 Nov. 1976- Species - Salmo gairdneri (Nesquali) Length 9.1±1.22 - Wt. 9.02±3.53																	
40.0	36.8	7.29	0.132	9.7	0.42	15.0	0.04	30	16	75	8.0	42.0	22.9	1.22	25.7	0.08	6.16
30.0	29.9	7.47	0.097	9.7	0.39	15.0	0.05	80	12	50	8.0	41.5	17.3	1.29	24.9	0.04	5.85
22.5	23.8	7.26	0.105	9.7	0.46	14.9	0.08	100	14	80	9.2	41.0	13.4	1.26	25.6	0.08	5.79
16.8	18.4	7.14	0.099	9.7	0.48	14.8	0.08	140	17	100	8.6	41.0	10.5	1.31	25.4	0.04	5.72
12.6	14.3	7.26	0.099	9.7	0.49	14.8	0.04	170	16	75	8.0	41.0	8.05	1.29	25.6	0.08	5.66
9.5	12.3	7.17	0.109	9.6	0.39	14.7	0.29	190	22	90	8.6	40.0	7.40	1.35	25.8	0.04	5.79
7.1	9.2	7.03	0.103	9.6	0.33	14.8	0.04	180	21	120	8.6	40.0	5.87	1.33	25.5	0.23	5.79
5.3	7.2	7.27	0.154	9.7	0.27	14.8	0.08	150	20	70	8.6	40.0	4.39	1.35	25.8	0.11	5.85
4.0	5.1	7.26	0.115	9.7	0.31	14.8	0.04	170	21	65	8.0	41.0	3.63	1.33	25.7	0.04	6.16
3.0	4.6	7.13	0.120	9.6	0.35	14.9	0.07	220	32	80	8.6	40.0	3.54	1.35	26.4	0.04	5.79
Control	<1.0	7.20	0.114	9.7	0.42	15.0	0.04	150	23	75	9.2	40.0	1.74	1.35	25.8	0.11	5.85

Continued ...

Table 73. Continued.

Conc. Theoretical ppm	Conc. Actual ppm	pH Mean + S.D.	O_2 Mean + S.D.	Temperature Mean + S.D.	NH_3-N µg/L	PO_4-P µg/L	CO_2 µ mole/L	CL mg/L	SO_4 mg/L	Na mg/L	K mg/L	Ca mg/L	Fe mg/L	Mg mg/L
Exp. - Vanadium pH 8 - Date 15 Nov. 1976- Species - Salmo gairdneri (Nesquali) Length 18.8±0.9 - Wt. 7.87±2.55														
40.0	35.07 ±0.47	7.93 ± 0.023	9.72 ± 0.41	15.24 ± 0.20	70	30	55	6.2	5.8	23.3	1.31	23.5	0.11	6.16
30.0	27.80 ±0.2	7.96 ± 0.050	9.68 ± 0.26	15.16 ± 0.11	80	23	45	6.0	5.6	17.7	1.35	23.9	0.08	5.85
22.5	21.47 ±0.45	7.89 ± 0.046	9.83 ± 0.33	15.06 ± 0.05	140	16	50	5.4	5.6	13.6	1.29	24.2	0.08	5.91
16.8	17.13 ±0.29	7.97 ± 0.036	9.78 ± 0.29	15.06 ± 0.05	210	13	40	5.4	5.6	10.7	1.31	24.2	0.08	5.97
12.6	13.97 ±0.23	7.99 ± 0.033	9.76 ± 0.23	15.04 ± 0.09	180	12	40	5.2	5.8	8.77	1.29	24.3	0.04	5.54
9.5	11.33 ±1.02	7.93 ± 0.046	9.84 ± 0.30	15.04 ± 0.09	140	16	40	5.2	5.8	6.84	1.33	24.4	0.08	5.54
7.1	9.13 ±0.75	7.91 ± 0.042	9.24 ± 0.22	15.04 ± 0.69	130	24	40	5.4	5.8	5.71	1.29	23.9	0.11	5.72
5.3	7.37 ±0.93	7.93 ± 0.050	9.72 ± 0.22	15.04 ± 0.09	120	12	35	5.2	6.0	5.55	1.29	23.8	0.04	5.91
4.0	5.43 ±0.64	8.00 ± 0.024	9.78 ± 0.18	15.06 ± 0.05	150	11	35	5.2	5.8	4.02	1.26	24.1	0.04	5.60
3.0	3.23 ±2.04	7.88 ± 0.046	9.74 ± 0.17	15.06 ± 0.05	110	14	50	5.2	6.0	1.76	1.22	24.1	0.04	5.66
Control	<1.0	7.88 ± 0.04	9.74 ± 0.19	15.04 ± 0.09	110	12	45	5.2	6.0	1.73	1.26	25.1	0.08	5.66

Continued ...

Table 73. Continued.

Conc. Theoretical ppm	Conc. Actual ppm	pH Mean + S.D.	O_2 Mean + S.D.	Temperature Mean + S.D.	NH_3-N µg/L	PO_4-P µg/L	CO_2 µ mole/L	CL mg/L	SO_4 mg/L	Na mg/L	K mg/L	Ca mg/L	Fe mg/L	Mg mg/L
Exp. - Vanadium pH 9 - Date 29 Nov. 1976- Species - Salmo gairdneri (Nesquali) Length 10.0±0.8 - Wt. 11.50±3.15														
40.0	35.07 ±1.16	8.91 ± 0.08	9.90 ± 0.37	14.82 ± 0.41	130	18	10	6.4	5.8	39.5	1.62	23.9	<0.04	6.34
30.0	27.10 ±0.46	8.85 ± 0.12	10.0 ± 0.29	14.66 ± 0.42	150	36	10	5.8	5.8	32.1	1.68	23.6	<0.04	6.25
22.5	21.83 ±0.64	8.92 ± 0.09	9.96 ± 0.19	14.72 ± 0.41	210	13	10	5.2	5.8	27.6	1.62	24.2	<0.04	6.39
16.8	16.97 ±0.67	8.93 ± 0.07	9.94 ± 0.26	14.62 ± 0.39	190	9	10	4.8	5.8	23.4	1.60	24.5	<0.04	6.44
12.6	13.03 ±0.97	8.89 ± 0.07	9.94 ± 0.24	14.64 ± 0.40	220	9	10	4.4	5.6	20.0	1.59	24.6	<0.04	6.48
9.5	11.37 ±0.45	8.99 ± 0.07	9.98 ± 0.29	14.56 ± 0.43	170	10	10	4.2	5.6	19.3	1.57	25.2	<0.04	6.39
7.1	8.27 ±0.70	9.03 ± 0.09	9.84 ± 0.56	14.58 ± 0.37	130	13	5	4.4	5.4	16.7	1.57	25.4	<0.04	6.53
5.6	6.10 ±0.40	8.95 ± 0.06	9.82 ± 0.44	14.60 ± 0.37	130	9	10	4.2	5.4	15.5	1.59	23.8	<0.04	6.48
4.0	4.40 ±0.20	8.96 ± 0.08	9.86 ± 0.38	14.66 ± 0.36	120	12	10	4.2	5.4	14.2	1.59	24.9	<0.04	6.44
3.0	4.33 ±0.12	9.04 ± 0.06	9.86 ± 0.32	14.64 ± 0.40	130	10	10	4.0	5.4	14.2	1.56	23.6	<0.04	6.34
Control		9.02 ± 0.05	9.90 ± 0.25	14.68 ± 0.39	100	6	10	4.2	5.4	11.6	1.54	24.2	<0.04	6.44

Continued ...

Table 73. Concluded.

Conc. Theoretical ppm	Conc. Actual ppm	pH Mean + S.D.	O_2 Mean + S.D.	Temperature Mean + S.D.	NH_3-N µg/L	PO_4-P µg/L	CO_2 µ mole/L	CL mg/L	SO_4 mg/L	Na mg/L	K mg/L	Ca mg/L	Fe mg/L	Mg mg/L
Exp. - Vanadium (14 day) pH 8 - Date 21 March 1977-Species - Salmo gairdneri (Idaho) Length - 7.2±0.7 - Wt. 4.40±1.5														
10.0	10.56 ±0.31	7.84 ± 0.09	9.80 ± 0.17	15.51 ± 0.43	65	7	43	3.6	4.8	6.87	1.63	27.1	<0.04	6.62
7.50	8.15 ±0.28	7.83 ± 0.09	9.77 ± 0.17	15.47 ± 0.42	75	14	45	3.6	4.9	5.73	1.68	27.5	0.04	6.62
5.63	6.23 ±0.37	7.86 ± 0.07	9.82 ± 0.21	15.36 ± 0.41	60	6	40	3.7	4.9	4.71	1.58	26.7	0.07	6.64
4.22	4.63 ±0.21	7.91 ± 0.09	9.81 ± 0.20	15.31 ± 0.39	55	6	43	3.7	4.8	3.96	1.65	26.7	0.08	6.62
3.16	3.41 ±0.19	7.79 ± 0.07	9.75 ± 0.21	15.24 ± 0.35	45	6	43	3.7	4.8	3.38	1.59	26.8	<0.04	6.67
2.37	3.06 ±0.16	7.87 ± 0.06	9.72 ± 0.22	15.41 ± 0.40	35	8	43	3.8	4.9	3.26	1.59	26.7	<0.04	6.56
1.78	2.05 ±0.15	7.85 ± 0.12	9.75 ± 0.19	15.37 ± 0.38	55	7	40	3.7	4.9	2.76	1.58	26.9	<0.04	6.64
1.33	1.48 ±0.09	7.91 ± 0.08	9.79 ± 0.19	15.33 ± 0.39	65	11	43	3.7	4.9	2.57	1.59	26.9	<0.04	6.52
1.00	1.03 ±0.09	7.93 ± 0.11	9.68 ± 0.19	15.40 ± 0.40	65	10	43	3.7	4.9	2.28	1.58	26.4	<0.04	6.55
0.75	0.81 ±0.11	7.87 ± 0.14	9.71 ± 0.23	15.43 ± 0.39	65	6	40	3.6	4.8	2.24	1.59	27.1	<0.04	6.60
Control	<0.1	7.81 ± 0.09	9.65 ± 0.21	15.44 ± 0.41	65	8	38	3.8	4.9	1.90	1.63	26.8	0.04	6.52

Table 74. Summary of chemical parameters monitored during the acute copper bioassays at pHs 6, 7, 8, and 9 with rainbow trout (*Salmo gairdneri*).

Conc. Theoretical ppm	Conc. Actual ppm	pH Mean + S.D.	O_2 Mean + S.D.	Temperature Mean + S.D.	NH_3-N µg/L	PO_4-P µg/L	CO_2 µ mole/L	Cl mg/L	SO_4 mg/L	Na mg/L	K mg/L	Ca mg/L	Fe mg/L	Mg mg/L
Exp. - Copper pH 6 - Date 25 March 1977-Species - Salmo gairdneri (Idaho) Length 6.9±0.6 - Wt. 3.86±1.06														
0.8	0.693 ±0.051	5.92 ± 0.23	10.03 ± 0.47	15.38 ± 0.56	50	5	185	8.4	2.04	1.47	25.7	0.09	0.09	6.76
0.6	0.545 ±0.033	5.86 ± 0.17	10.00 ± 0.27	15.78 ± 0.48	40	4	125	8.2	74.5	1.94	1.37	25.8	0.04	6.96
0.45	0.394 ±0.021	6.01 ± 0.16	9.96 ± 0.26	15.32 ± 0.39	30	3	80	7.4	73.5	1.92	1.37	25.1	0.09	6.60
0.34	0.293 ±0.015	6.02 ± 0.17	10.00 ± 0.23	15.68 ± 0.34	40	4	75	8.2	73.5	1.88	1.37	25.3	0.04	6.71
0.25	0.238 ±0.015	6.05 ± 0.17	9.92 ± 0.27	15.82 ± 0.47	70	13	75	9.2	73.5	2.01	1.43	25.0	0.09	6.81
0.19	0.188 ±0.005	5.96 ± 0.16	9.90 ± 0.30	15.80 ± 0.44	110	10	115	8.8	73.5	1.95	1.41	25.2	0.04	6.66
0.14	0.138 ±0.005	5.92 ± 0.17	9.88 ± 0.23	15.34 ± 0.50	50	5	115	8.8	73.5	1.94	1.39	25.4	0.04	6.81
0.11	0.118 ±0.017	6.13 ± 0.19	9.88 ± 0.19	15.66 ± 0.44	70	7	45	8.8	73.5	1.88	1.37	25.4	0.04	6.81
0.080	0.078 ±0.005	6.09 ± 0.14	9.82 ± 0.23	15.68 ± 0.41	80	8	65	10.0	73.5	1.94	1.41	25.3	0.09	6.76
0.060	0.063 ±0.015	6.00 ± 0.17	9.78 ± 0.22	15.60 ± 0.53	100	11	95	10.4	73.5	1.90	1.33	25.7	0.04	7.01
0.0	0.02±0	6.14 ± 0.14	9.80 ± 0.21	15.74 ± 0.50	120	8	55	8.6	75.1	1.94	1.41	24.9	0.04	6.66

Continued ...

Table 74. Continued.

Conc. Theoretical ppm	Conc. Actual ppm	pH Mean + S.D.	O_2 Mean + S.D.	Temperature Mean + S.D.	NH_3-N µg/L	PO_4-P µg/L	CO_2 µ mole/L	Cl mg/L	SO_4 mg/L	Na mg/L	K mg/L	Ca mg/L	Fe mg/L	Mg mg/L
Exp. - Copper pH 7 - Date 4 April 1977 - Species - Salmo gairdneri (Idaho) Length 7.5±0.7 - Wt. 5.09±1.46														
0.8	0.86 ±0.07	7.03 ± 0.10	9.92 ± 0.27	15.64 ± 0.37	10	1	40	4.8	70	1.72	1.52	25.9	0.47	7.11
0.6	0.69 ±0.02	7.00 ± 0.13	9.92 ± 0.41	15.38 ± 0.33	20	2	45	6.0	71	1.61	1.49	25.7	0.08	7.19
0.45	0.49 ±0.02	7.00 ± 0.10	9.94 ± 0.31	15.34 ± 0.28	20	2	45	5.2	71	1.60	1.47	25.9	0.08	7.24
0.34	0.38 ±0.01	7.03 ± 0.11	10.00 ± 0.20	15.40 ± 0.40	20	2	45	4.4	72	1.61	1.49	25.7	0.04	6.98
0.25	0.29 ±0.01	7.03 ± 0.11	10.00 ± 0.20	15.34 ± 0.25	30	3	40	5.8	73	1.61	1.52	26.4	0.04	7.06
0.19	0.22 ±0.01	7.03 ± 0.31	9.88 ± 0.28	15.22 ± 0.46	50	5	60	5.8	73	1.61	1.52	26.1	0.04	6.76
0.14	0.17 ±0.01	6.90 ± 0.13	9.52 ± 0.23	15.10 ± 0.22	80	10	65	5.6	73	1.66	1.54	26.4	0.04	6.76
0.11	0.12 ±0.01	6.97 ± 0.12	9.72 ± 0.11	15.04 ± 0.23	110	9	60	4.6	74	1.66	1.54	26.0	0.16	7.06
0.08	0.09 ±0.01	0.98 ± 0.12	9.80 ± 0.07	15.38 ± 0.34	100	6	50	5.0	74	1.61	1.52	25.6	<0.04	7.06
0.06	0.07 ±0.01	6.93 ± 0.11	9.66 ± 0.05	15.12 ± 0.34	90	5	60	4.4	75	1.61	1.52	25.7	<0.04	7.19
0.0	≈0.01	7.03 ± 0.09	9.92 ± 0.13	15.58 ± 0.34	90	3	35	4.6	75	1.64	1.54	26.0	<0.04	7.45

Continued ...

Table 74. Continued.

Conc. Theoretical ppm	Conc. Actual ppm	pH Mean + S.D.	O_2 Mean + S.D.	Temperature Mean + S.D.	NH_3-N µg/L	PO_4-P µg/L	CO_2 µ mole/L	Cl mg/L	SO_4 mg/L	Na mg/L	K mg/L	Ca mg/L	Fe mg/L	Mg mg/L
Exp. - Copper pH 8 - Date 21 February 1977- Species Salmo gairdneri (Idaha) Length 5.9±0.7 - Wt. 2.45±0.99														
2.0	1.87 ±0.07	7.93 ± 0.06	9.98 ± 0.04	14.84 ± 0.43	40	11	40	4.0	8.0	2.15	1.77	28.9	<0.04	6.95
1.5	1.49 ±0.03	8.13 ± 0.07	9.98 ± 0.13	14.64 ± 0.36	30	7	35	3.8	6.4	1.97	1.64	28.7	<0.04	6.86
1.13	1.09 ±0.05	8.03 ± 0.04	10.00 ± 0.11	14.48 ± 0.35	30	7	40	3.8	7.7	2.10	1.66	28.0	<0.04	6.86
0.84	0.83 ±0.05	8.08 ± 0.04	10.08 ± 0.11	14.30 ± 0.31	30	7	40	3.8	7.0	2.13	1.68	28.4	<0.04	6.82
0.63	0.65 ±0.03	8.12 ± 0.04	10.02 ± 0.15	14.30 ± 0.31	30	9	35	4.0	6.6	2.02	1.66	28.5	0.12	6.68
0.47	1.41 ±0.07	8.04 ± 0.04	10.04 ± 0.15	14.26 ± 0.23	30	8	40	4.0	8.0	2.08	1.66	28.7	0.08	7.32
0.36	0.40 ±0.00	8.10 ± 0.07	10.07 ± 0.17	14.45 ± 0.37	30	7	35	4.0	6.4	2.03	1.66	2.03	<0.04	28.7
0.27	0.35 ±0.01	8.05 ± 0.05	10.12 ± 0.21	14.42 ± 0.34										
0.20	0.27 ±0.03	8.08 ± 0.05	10.00 ± 0.20	14.38 ± 0.33	50	9	40	3.8	6.2	2.10	1.68	28.9	<0.04	6.55
0.15	0.26 ±0.03	8.14 ± 0.04	9.96 ± 0.21	14.40 ± 0.36	50	10	40	4.2	6.2	2.17	1.66	29.8	0.04	6.50
0.0	0.06 ±0.01	8.09 ± 0.05	10.00 ± 0.20	14.38 ± 0.36	60	18	45	4.2	6.0	2.37	1.66	28.8	0.04	7.09

Continued ...

Table 74. Concluded.

Conc. Theoretical ppm	Conc. Actual ppm	pH Mean + S.D.	O_2 Mean + S.D.	Temperature Mean + S.D.	NH_3-N µg/L	PO_4-P µg/L	CO_2 µ mole/L	Cl mg/L	SO_4 mg/L	Na mg/L	K mg/L	Ca mg/L	Fe mg/L	Mg mg/L
Exp. - Copper pH 9 - Date 31 January 1977-Species - Salmo gairdneri (Idaho) Length 6.1±0.5 - Wt. 2.66±0.59														
2.00	1.92 ±0.07	9.07 ± 0.07	10.08 ± 0.08	14.94 ± 0.60	40	9	<10	4.0	8.0	13.9	1.64	27.5	<0.04	7.06
1.50	0.52 ±0.03	9.00 ± 0.08	10.02 ± 0.13	14.64 ± 0.51	40	8	10	3.6	6.2	13.4	1.56	27.8	0.18	7.11
1.13	1.12 ±0.05	9.09 ± 0.08	10.01 ± 0.10	14.52 ± 0.46	40	7	<10	3.6	7.2	13.6	1.60	26.1	<0.04	6.89
0.80	0.44 ±0.03	9.11 ± 0.06	10.08 ± 0.15	14.40 ± 0.43	20	7	<10	3.4	7.0	13.4	1.58	25.8	0.30	6.98
0.63	0.66 ±0.01	9.03 ± 0.07	10.06 ± 0.11	14.32 ± 0.46	20	7	<10	3.4	6.6	13.5	1.62	27.7	1.09	7.11
0.47	1.44 ±0.06	9.05 ± 0.07	10.06 ± 0.09	14.40 ± 0.43	20	7	<10	4.0	8.0	13.5	1.56	27.5	0.11	7.06
0.36	0.42 ±0.01	9.11 ± 0.05	10.04 ± 0.15	14.42 ± 0.46	20	7	<10	3.4	6.6	13.4	1.58	27.5	0.23	7.15
0.27	0.38 ±0.02	9.16 ± 0.05	10.08 ± 0.13	14.38 ± 0.45	40	9	<10	3.4	6.4	13.3	1.60	27.3	<0.04	7.06
0.20	0.30 ±0.02	9.09 ± 0.06	10.00 ± 0.14	14.42 ± 0.47	60	9	10	3.4	6.2	13.4	1.60	27.6	0.18	7.11
0.15	0.25 ±0.01	9.02 ± 0.06	10.00 ± 0.14	14.50 ± 0.49	50	7	10	3.4	6.2	13.4	1.58	27.1	0.18	6.94
0	0.07 ±0.01	9.11 ± 0.06	10.02 ± 0.11	14.54 ± 0.51	40	7	<10	3.4	6.0	13.4	1.58	27.4	<0.04	7.06

Table 75. Chemical composition and reconductivity of reconstituted water utilized for incubation of eyed eggs of rainbow trout (*Salmo gairdneri*).

	Cl^- (mg/L)	$SO_4^=$ (mg/L)	Na^+ (mg/L)	K^+ (mg/L)	Mg^{++} (mg/L)	Ca^{++} (mg/L)	Conductivity (umho/L)
17 Sept.	1.6	30.0	13.7	0.97	3.11	9.37	140
22 Oct.	1.4	26.5	11.8	0.91	2.43	8.15	130
Mean	1.5	28.2	12.8	0.94	2.77	8.67	135

Table 76. Dissolved oxygen (DO) and pH readings of test water employed in the study of the toxic effects of copper and vanadium to rainbow trout eggs. f = fresh test water taken from a reagent flask. u = used test water measured after use for 24 h.

	t = 0 (f)		t = 24 (u)		t = 24 (f)		t = 48 (u)		t = 48 (f)		t = 72 (u)		t = 72 (f)		t = 96 (u)		t = 3 d. post. (u)	
	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)
COPPER																		
Control	7.82	9.4	7.40	8.8	7.59	9.8	7.40	10.0			7.35	9.2	7.49	10.4	7.23	9.3	7.28	8.1
			7.36	8.6							7.18	8.6						
0.03	7.70	8.8	7.48	8.8	7.72	9.8	7.30	9.5	7.65	10.6	7.19	8.9			7.36	9.8	7.25	7.8
			7.40	8.6							7.21	8.8						
0.08	7.72	8.6	7.48	9.3	7.72	9.6	7.46	10.2	7.67	10.5	7.30	9.0	7.44	9.1	7.28	9.6	7.35	8.2
			7.45	8.9							7.20	9.2						
0.24	7.65	9.0	7.38	8.8	7.70	10.0	7.39	10.1	7.61	10.7	7.20	9.1	7.47	10.1	7.43	9.6	7.35	8.5
			7.44	8.8							7.18	9.0						
0.86	7.42	8.9	7.41	9.0	7.40	9.9	7.36	9.9	7,44	10.8	7.33	9.4	7.44	10.2				
			7.35	8.8							7.41	9.7						
1.76	7.06	9.2	7.67	9.5														
			7.59	9.6														
4.78	6.75	9.0																

Continued ...

Table 76. Concluded.

	t = 0 (f)		t = 24 (u)		t = 24 (f)		t = 48 (u)		t = 48 (f)		t = 72 (u)		t = 72 (f)		t = 96 (u)		t = 3 d. post. (u)	
	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)
VANADIUM																		
Control	7.55	9.3	7.39	8.8	7.56	9.9	7.26	11.2	7.15	11.2	7.19	8.8	7.56	10.3	7.16	9.3	7.19	8.0
			7.42	8.8							7.20	8.5						
25	7.35	9.4	7.45	8.9	7.52	9.6	7.38	10.2	7.56	11.4	7.29	8.9	7.50	10.0	7.36	9.6	7.19	8.2
			7.46	8.9							7.31	8.9						
44	7.58	9.5	7.50	8.6	7.74	9.6	7.42	10.2	7.69	11.0	7.22	8.3	7.64	10.1	7.39	10.0	7.19	7.6
			7.59	8.8							7.35	9.1						
86	7.85	9.4	7.64	8.9	7.91	9.8	7.52	10.4	7.80	11.1	7.47	9.3	7.75	10.2			7.27	9.6
			7.50	8.8														
181	7.90	9.4	7.54	9.0	8.02	9.6	7.64	10.1	8.00	10.8	7.54	9.4	7.95	9.8	7.63	9.8	7.37	9.3
			7.59	8.8							7.54	9.4						
334	8.10	9.4	7.75	9.6	8.25	9.7	7.89	10.6	8.20	11.0	7.64	10.0	8.40	10.1			7.35	10.4
			7.78	9.8							7.69	9.1						
595	8.35	9.4	8.04	10.6	8.50	9.8	8.16	10.6										

Table 77. Ammonia (NH_3), nitrite (NO_2^-), and nitrate (NO_3^-) concentrations (µg/L) in test waters recorded at t = 48 and 96 h in the rainbow trout embryological bioassay.

Conc. (mg/L)	t = 48 pH	$NH_3 + NH_4^+$	NH_3	NO_2^-	NO_3^-	t = 96 pH	NH_3	$NH_3 + NH_4^+$	NO_2^-	NO_3^-
COPPER										
Control	7.40	650	3.06	19	42	7.23	1130	3.28	13	19
0.03	7.30	590	2.18	8	5	7.36	780	3.67	12	
0.08	7.46	510	3.01	8	5	7.28	870	3.22	16	
0.24	7.39	490	2.30	8	5	7.43				
0.86	7.36	450	2.12	13	5	7.37	230	1.08	16	
1.76	7.63	540	4.00	8	5					
4.78	7.63	750	5.55							
VANADIUM										
Control	7.26					7.16	1220	3.54		
25	7.38	560	2.63			7.36	720	3.38		
44	7.42	440	2.07			7.39	700	3.29		
86	7.52	410	2.42							
181	7.64	530	3.92			7.63	530	3.92		
334	7.89	210	3.07			7.66	110	1.01		
595	8.16	170	4.86							

Table 78. Analyzed toxicant concentrations in mg/L recorded in the studies of copper and vanadium effects on rainbow trout embryology.

Sample Time (h)	Nominal Copper Concentrations						
	Control	0.03	0.08	0.24	0.86	1.76	4.78
00	0.002	0.03	0.07	0.22	0.80	2.07	5.31
48	0.01	0.02	0.06	0.04	0.79	1.45	4.26
72	0.006	0.04	0.09	0.30	0.96		
96	0.02	0.04	0.11	0.39	0.91		
Mean	0.010	0.032	0.082	0.238	0.865	1.76	4.78

Sample Time (h)	Nominal Vanadium Concentrations						
	Control	25	44	86	181	334	595
00	0.5	20	38	72	148	293	579
48	2.0	22	43	85	177	319	611
72	2.0	29	49	92	200	360	
96	2.0	28	48	96	200	365	
Mean		24.8	44.5	86.2	181.2	334.2	595.0

Table 79. Water chemistry records of post-exposure holding vessels for the eyed eggs of rainbow trout. Dissolved oxygen is presented as mg/L; all other values as µg/L.

Treatment Metal Conc. (mg/L)	Time (days from termination of exposure) 5 pH	5 DO	7 pH	7 DO	9 pH	9 DO	11 pH	11 DO	11 NH_3	11 $NH_3 + NH_4^+$	11 NO_2^-	11 NO_3^-
COPPER												
Control	7.08	8.4	7.00	8.2	6.98	7.8	6.92	6.9	950	1.42	4	5
0.03	7.06	8.6	7.02	8.1	6.98	8.0	6.95	6.8	940	1.79	4	5
0.08	7.11	8.6	7.08	8.4	6.99	8.0	6.96	6.6	910	1.73	4	5
0.24	7.06	8.4	7.04	8.0	6.98	7.8	6.94	6.2	990	1.48	6	5
VANADIUM												
Control	7.05	8.4	6.90	7.8	6.86	7.5	7.03	7.2	1220	2.32	6	5
25	7.07	8.4	6.98	7.9	6.89	7.6	6.94	7.1	1060	1.59	4	5
44	7.06	8.4	7.00	8.0	7.96	8.0	6.92	7.6	1050	1.58	4	5

Table 80. LC50 values of copper and vanadium for eyed rainbow trout eggs.

	Exposure Time (h)	LC50 (mg/L)	Standard Deviation
Copper	24	1.076	1.639
	36	0.730	1.740
	48	0.550	1.627
	72	0.490	1.539
	96	0.40	
	96[a]	0.38	
Vanadium	24	261.4	2.3
	36	197.6	2.1
	48	159.4	2.2
	72	134.2	2.1
	96	118.2	2.0
	96[a]	95.8	2.3

[a] Post-exposure mortality three days after transfer from toxicant to uncontaminated dilution water.

Table 81. Concentrations, range, mean, and standard deviations of dissolved oxygen, temperature, and pH in vanadium and copper continuous flow bioassays with whitefish (*Coregonus clupeaformis*).

Vanadium	Dissolved Oxygen mg/L			Temperature °C			pH		
(mg/L)	Range	Mean	St. Dev.	Range	Mean	St. Dev.	Range	Mean	St. Dev.
40.0	10.4-11.0	10.70	0.194	10.2-11.0	10.84	0.250	7.70-8.02	7.87	0.041
30.0	10.6-11.2	10.93	0.204	10.2-11.1	10.67	0.265	7.94-8.03	7.99	0.028
22.5	10.5-11.2	10.90	0.214	10.2-11.0	10.54	0.268	7.80-8.02	7.92	0.047
16.8	10.4-11.3	10.83	0.262	10.2-11.0	10.56	0.258	7.85-7.91	7.91	0.023
12.5	10.4-11.0	10.80	0.167	10.2-11.0	10.55	0.273	7.85-8.00	7.92	0.017
9.5	10.5-11.0	10.84	0.130	10.2-11.0	10.60	0.245	7.71-7.96	7.89	0.050
7.1	10.5-11.1	10.82	0.176	10.2-11.2	10.68	0.277	7.88-7.99	7.93	0.034
5.3	10.4-11.1	10.86	0.185	10.3-11.1	10.62	0.288	7.86-8.01	7.90	0.021
4.0	10.6-11.0	10.88	0.140	10.2-11.0	10.47	0.308	7.98-8.03	8.01	0.020
3.0	10.6-11.2	10.90	0.186	10.2-11.1	10.53	0.322	7.94-7.98	7.97	0.012
Control	10.7-11.1	10.89	0.125	10.2-11.1	10.57	0.334	7.98-8.03	8.00	0.014
Copper (mg/L)									
2.00	11.4-11.6	11.51	0.105	10.0-10.1	10.03	0.046	7.31-7.56	7.42	0.087
1.70	11.3-11.6	11.46	0.142	9.7-10.0	9.91	0.093	7.27-7.47	7.39	0.063
1.45	11.2-11.6	11.47	0.150	10.0-10.2	10.04	0.070	7.39-7.52	7.45	0.051
1.23	11.3-11.6	11.44	0.097	10.0-10.2	10.04	0.070	7.36-7.56	7.44	0.061
1.04	11.2-11.5	11.37	0.095	10.0-10.2	10.05	0.071	7.40-7.69	7.52	0.085
Control	11.4-11.6	11.56	0.089	10.0-10.1	10.02	0.045	7.45-7.77	7.59	0.146

10. 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
10.	HE 2.1	Development of a Research Design Related to Archaeological Studies in the Athabasca Oil Sands Area
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
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
18.	HY 1.1	Interim Compilation of Stream Gauging Data to December 1976 for the Alberta Oil Sands Environmental Research Program
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	Air System Winter Field Study in the AOSERP Study Area, February 1977.
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
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45.	VE 3.3	Interim Report on Physiology and Mechanisms of Air-Borne Pollutant Injury to Vegetation, 1975 to 1978

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59.	TF 3.1	Self-Aquatic Mammals. Annotated Bibliography
60.	WS 1.1.1	Synthesis of Surface Water Hydrology
61.	AF 4.5.2	An Intensive Study of the Fish Fauna of the Steepbank River Watershed of Northeastern Alberta.
62.	TF 5.1	Amphibians and Reptiles in the AOSERP Study Area
63.		An Overview Assessment of <u>In Situ</u> Development in the Athabasca Deposit

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