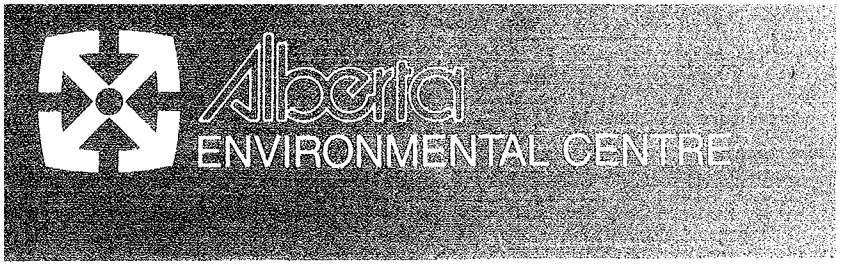
AECV86-R5

Acute and Subacute Toxicity of Different Fractions of Athabasca Bitumen to Fish



ACUTE AND SUBACUTE TOXICITY OF DIFFERENT FRACTIONS OF ATHABASCA BITUMEN TO FISH

ALBERTA ENVIRONMENTAL CENTRE

VEGREVILLE, ALBERTA

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ABSTRACT

The purpose of this study was to determine the acute and subacute toxicity of bitumen to rainbow trout. The bitumen was collected along the banks of the Athabasca River downstream of Fort McMurray during 1984. Prior to experimentation, the bitumen was fractionated in water at 35°C, using an ultrasonic shaker. This procedure yielded three fractions: whole bitumen, extractable bitumen and residual bitumen. In order to assess the potential interaction of the fractions with the receiving waters (Athabasca River), three additional fractions were also generated: whole Athabasca River water, suspended solids (Athabasca River), dissolved fraction (Athabasca River).

Acute toxicity of the six fractions was determined over a 96h period at concentrations of 1, 10, 50, 75 and 100 mg L^{-1}. Subacute toxicity was determined by initially exposing the fish to the various fractions at 100 mg L^{-1} for 96 hours. For the next 24h, the tanks were flushed with dechlorinated municipal water, and the fish were left in this water for another 96h. At the end of the first 96h, and then at the end of the experiment, fish were euthanized and submitted for necropsy.

Based on these studies, it can be concluded that:

1. The 96h LC_{50} of all six fractions was greater than 100 mg L^{-1}. The fractions were considered not acutely toxic to fish.
2. In the acute studies, no significant histopathological changes were seen in fish exposed to the different fractions.
3. No histopathological changes indicative of toxicity were found in fish exposed to sublethal concentrations of the fractions. In addition, blood analysis data (electrolytes, pH, blood gases, enzymes and other biochemical parameters) were similar between principals and controls.
4. Overall, it was concluded that bitumen was not acutely toxic to fish under the dosages and conditions in which this study was conducted.

PROJECT TEAM

M. Albassam*

K. Blumhagen

F. Dieken

E. McGuinness

James W. Moore*

A. Mutalib

S. Ramamoorthy

Kevin Smiley

K. Wilson

*Correspondence

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1. INTRODUCTION

1.1 Background

The Athabasca deposit of tar sands is now estimated to contain at least 869 billion barrels of bitumen (Mossop, 1980). Although most of this material lies well below the surface, the Athabasca River Valley cuts through the deposit downstream of Fort McMurray (Figure 1). Accordingly, some bitumen is naturally exposed, leading to erosion of oily material into the river. The concentration of oil and grease in the Athabasca River often exceeds 100 $\mu g\ L^{-1}$, especially on hot sunny days when erosion from the tar sands is at its maximum (Figure 2). Such levels are among the highest in the world and comparable to those reported after major oil spills (Moore and Ramamoorthy 1984).

Athabasca bitumen resembles the residue left after the microbial degradation of conventional crude oils (Rubenstein *et al*. 1977). The bulk of the material consists of high molecular weight compounds including complex cyclic and branched aliphatics, aromatics and heteroaromatic hydrocarbons (Selucky *et al*. 1977). Low molecular weight compounds, which are often highly toxic to fish, constitute a small fraction of the weathered bitumen.

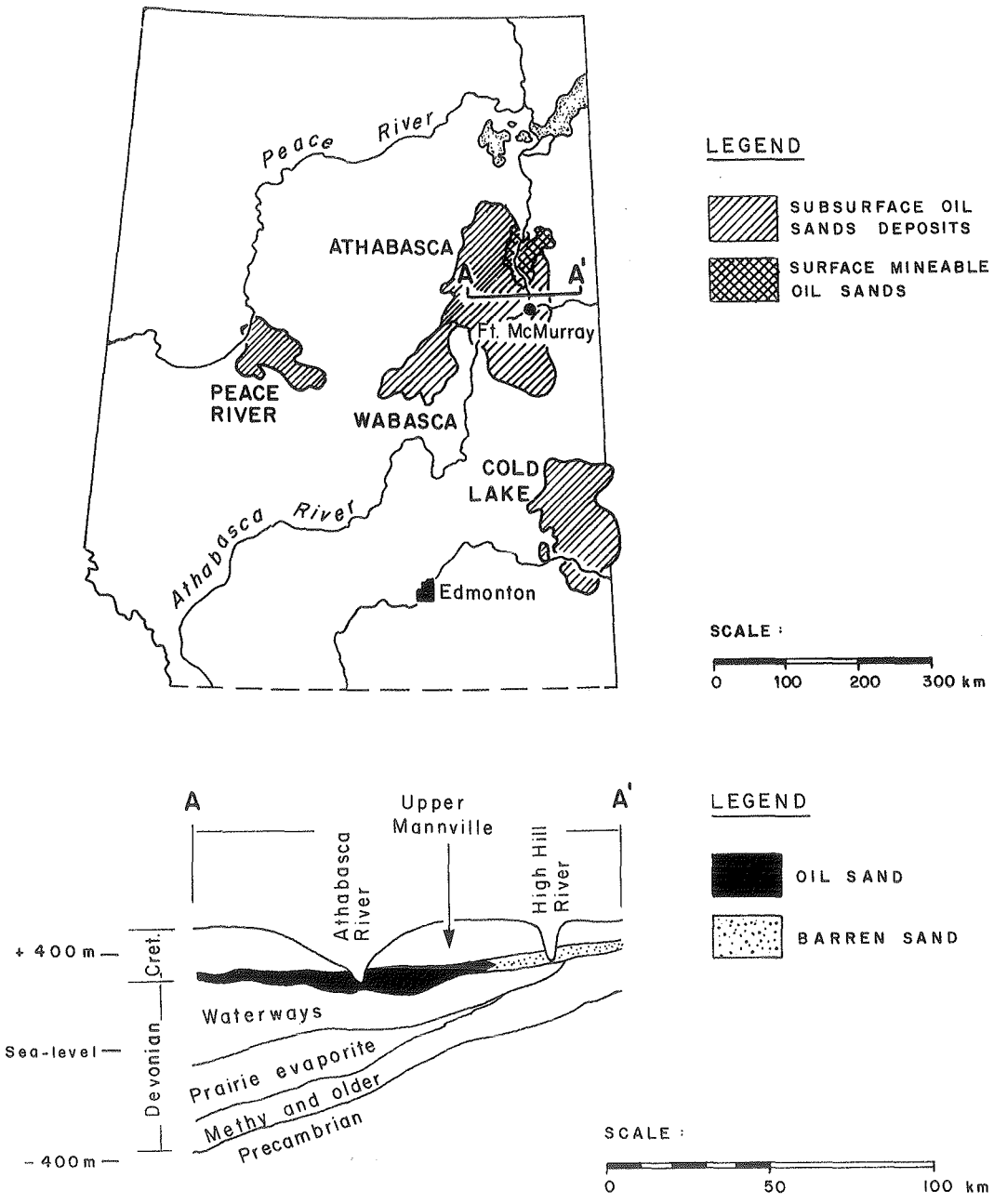

FIGURE 1. ALBERTA OIL SANDS ACCUMULATIONS AND SCHEMATIC CROSS SECTION OF THE ATHABASCA DEPOSIT.

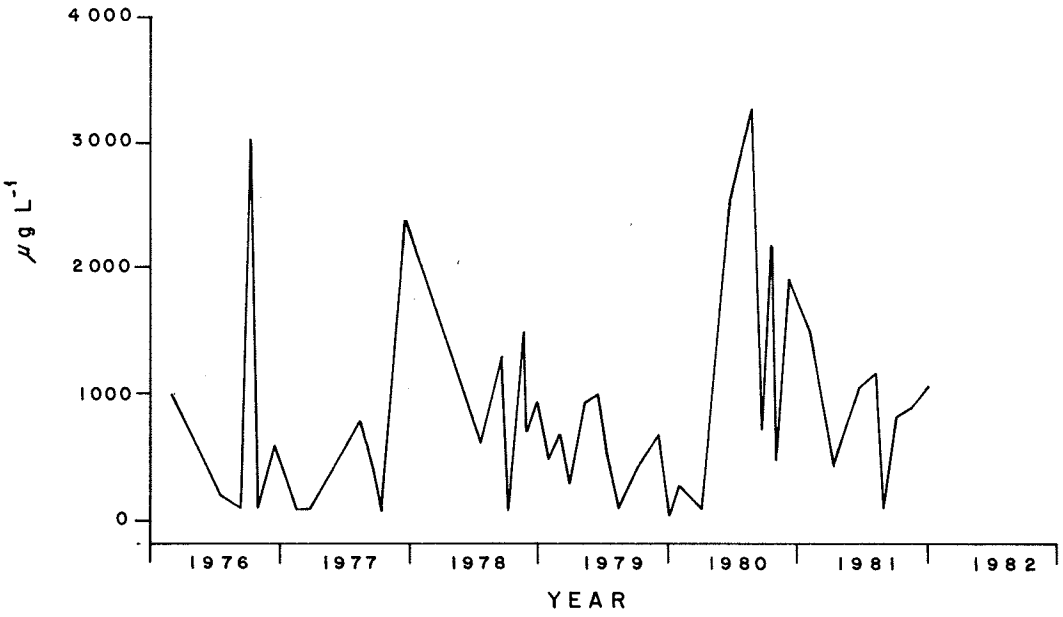

FIGURE 2. CONCENTRATION OF OIL AND GREASE IN THE ATHABASCA RIVER ABOVE FORT McMURRAY (From Naquadat, 1976-81).

Studies on the hydrocarbon-degrading potentials of indigenous micro-organisms from the Athabasca River showed that i) the biodegradation potential exhibited a marked seasonal variation, being greatest in summer, and ii) microbial activity increased with the oil and grease in the substrate (Wyndham and Costerton 1981). It has also been shown that the hydrocarbon-degrading micro-organisms can utilize all fractions of Athabasca bitumen except the asphaltene fraction (Wyndham and Costerton 1981). These micro-organisms are either not present or show only low activity in rivers in southern Alberta.

Although high concentrations of hydrocarbons are often reported from the Athabasca River, there are few accounts of fish kills. Apparently the volatilization and microbial metabolism of the various fractions of bitumen reduces toxicity to fish. Craddock (1977), in his review of the effects of petroleum on fish, reported that the lethal concentration of fresh crude oil to adults varies from 90 to 18,000 mg L^{-1}, whereas the corresponding range for eggs and larvae is 0.1-100 mg L^{-1}. Woodward et al. (1981) found that mortality could be induced in adult fish at concentrations as low as 0.5 mg L^{-1} following long term exposure. At present, the Athabasca River and Lake Athabasca support substantial fisheries for walleye, pike, goldeye, whitefish and other species.

1.2 Purpose of Study

The erosion of bitumen into the Athabasca River introduces the possibility of naturally induced toxic effects in native fish due to the presence of oil and grease in the water column. The river is also periodically turbid, with suspended solid levels averaging more than 20 mg L^{-1} and occasionally much more. Accordingly, oil, grease and suspended solids may interact, leading to either antagonistic or synergistic effects in fish. As a prelude to more detailed investigations of the toxic effects of tar sands plant holding pond effluent, it was necessary to assess the toxicity of bitumen and suspended solids to fish.

2. MATERIALS AND METHODS

2.1 Collection Site and Materials

Athabasca River water was collected from one site near the confluence with the Clearwater River (Figure 3). Samples were taken on three separate dates: 23 May 1984, 5 July 1984 and 20 September 1984.

A total of 4,660 L (1025 Imp. gallons) was collected using a 12 volt 1/3 hp Ruypp submersible pump held in the river approximately 15 cm off the bottom in approximately 60 cm of water.

The water was pumped through a rinsed 1 1/2" reinforced vinyl hose into a rinsed 1365 L (300 Imp. gallon) fiberglass tank.

After collection, the water was transported by truck to the laboratory. The water was then transferred into a 2730 L (600 Imp. gallon) covered reservoir, which is housed in a cooler with an ambient temperature of 4°C.

On the 5 July 1984 collection, an additional six 115 L (25 Imp. gallon) barrels of water were also collected. The plastic barrel liners were rinsed prior to water collection with dechlorinated city water. This water was used in studies of the uptake of PAH by fish (described later).

The samples of naturally occurring bitumen needed for this study were collected from two sites on an exposed and slumping bank of limestone and tar sand located approximately 11.5 km downstream of Fort McMurray on 4 July 1984.

A total of five 76 cm x 91 cm plastic bags of bitumen were collected using a small spade to scrape or cut through the layer of often weathered bitumen. The samples were collected from 0-4 meters above the river level. The sample used predominantly for this study was collected from a bitumen seam located 1 metre above the surface of the river. The sampling containers were tied shut and transported to the laboratory where they were stored in a cooler at 4°C.

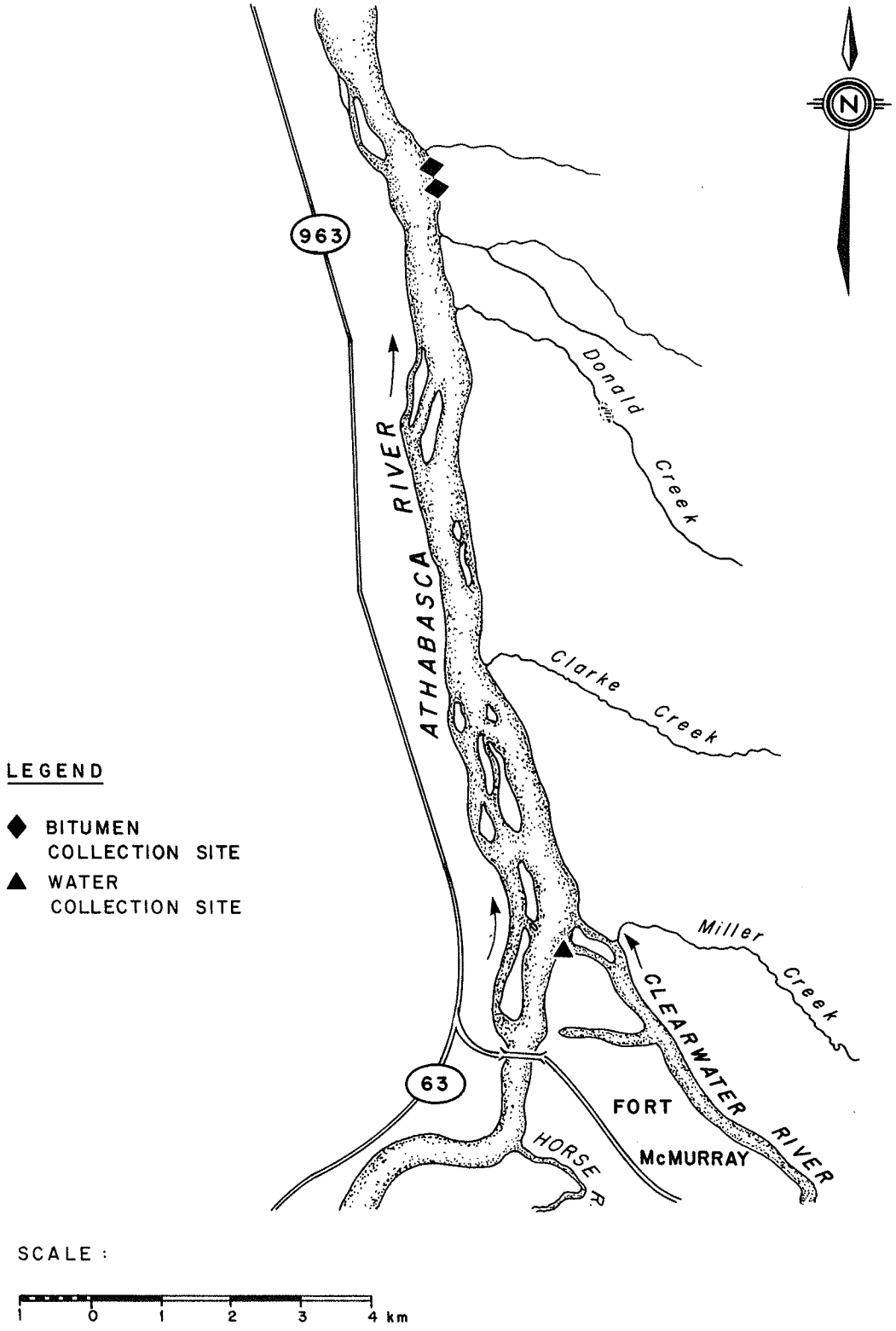

FIGURE 3. ATHABASCA RIVER SHOWING COLLECTION SITES.

2.2 Fractionation of Bitumen and River Water

A Branson ultrasonic shaker (480 watts) was used to fractionate whole bitumen into two components (extractable, residual). Whole bitumen is also considered a fraction. Approximately 0.5 kg of bitumen and 0.5 L of water were used during each extraction procedure. In order to improve the effectiveness of the method, the bitumen was stirred lightly with a glass rod. Temperature of the overlying water was kept at approximately 35°C. The stirring procedure caused an oily liquid substance, released from the bitumen, to appear on the surface of the water. This substance is considered the extractable bitumen fraction and may represent the type of material that would erode into the Athabasca River. After collection of the extractable fraction, the solid material remaining in the ultrasonic shaker (residual bitumen fraction) was retrieved and stored in a glass jar.

The use of the shaker in fractionation was designed to parallel natural erosion of bitumen deposits during the summer into the Athabasca River. No attempt was made to collect and retain volatile hydrocarbons since, under natural conditions, these substances would dissipate into the atmosphere. Although organic solvents could have been used to extract oil and grease from the bitumen, thereby obtaining a more specific extractable component, this would not have been representative of erosion processes in nature.

2.3 Chemical Analysis

Elemental and hydrocarbon analyses were conducted on the three bitumen fractions. Since only one sample from each fraction could be analyzed, the data are intended to give only a gross indication of composition. Following the fractionation procedure, the material was stored at 4°C in the dark. Elemental analysis was conducted by Chemex Laboratories (Calgary) using an indirectively coupled plasma technique. Hydrocarbon analysis was also conducted on the three fractions by Chemex Laboratories (Figure 4). The asphaltene fraction was precipitated by n-pentane and determined gravimetrically. The n-pentane soluble components were chromatographically separated into saturates, aromatics, resins I and resins II, using clay, alumina and silica gel as absorbants and n-pentane, benzene, methyl ethyl ketone, and tetrahydrofuran-water as eluents. The fractions were then determined gravimetrically.

2.4 Fish

Small rainbow trout, measuring 2.7 to 6 cm in fork length and 0.2 to 2 g live weight at the time of experimentation, were used for the acute toxicity tests. The fish were obtained as eggs from the Mount Lassen Farm (California) and reared in the laboratories of the Environmental Protection Service, Edmonton. Table 1 gives the chemistry of water used in egg rearing. All parameters fall well

SRA ANALYSIS/ELUTION CHROMATOGRAPHY FLOW CHART

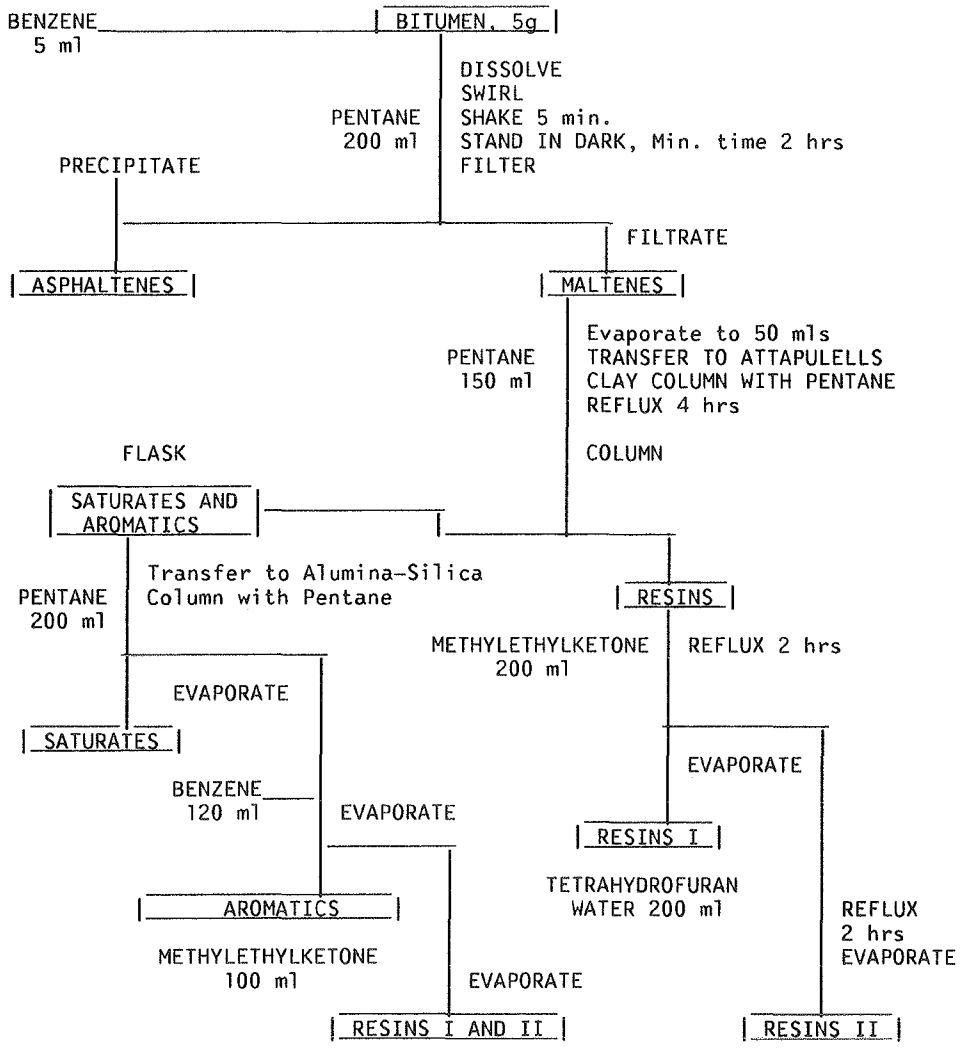

Figure 4.

within levels that are suitable for rearing eggs. Fry were transported to the Alberta Environmental Centre and allowed to acclimate at least 2 weeks prior to experimentation. Physico-chemical conditions in the maintenance water were suitable for rearing fish (Table 2). Large fish measuring 25-30 cm in fork length and 220-350 g live weight at the time of experimentation, were used for the subacute toxicity tests.

Table 1. Physico-chemical characteristics of water used to rear rainbow trout eggs. Concentrations expressed as averages (mg L^{-1}) during rearing period.*

Temperature (°C)	11.5-12.5	Calcium	26
Dissolved Oxygen	8.4- 8.9	Chromium	<0.05
pH	7.6- 7.9	Copper	<0.02
Chloride	3.4	Iron	<0.02
Total hardness	99	Lead	<0.0005
Calcium hardness	66	Magnesium	81
Magnesium hardness	33	Nickel	<0.008
Total alkalinity	34	Potassium	0.8
Sulfate	68	Sodium	3.9
Conductivity	210	Zinc	0.03

*Chemical parameters based on a single determination provided by Environmental Protection Service (Edmonton).

Table 2. Physico-chemical conditions of water used to rear rainbow trout fry in treated municipal water. Concentrations expressed as mg L^{-1}.

Temperature (°C)	12.5-15.0	Calcium	12-15
Dissolved Oxygen	8.0- 9.5	Iron	<0.02
pH	7.0- 7.9	Magnesium	5
Chloride	2	Potassium	0.3-0.4
Total Hardness	51-58	Sodium	3-13
Total Alkalinity	42-49	Silica	1.4-1.5
Sulfate	26-28	Nitrite	<0.05
Conductivity	136-154		

2.5 Acute Toxicity

Acute toxicity was measured using 96h LC_{50} rainbow trout bioassays.

Six groups of five fish each were exposed for 96h to the following fractions at a concentration of 1, 10, 50, 75 and 100 mg L^{-1} adjusted in treated municipal water. Another six groups of five fish each served as control groups.

I. Whole bitumen

II. Extractable bitumen fraction

III. Residual (Extracted) bitumen fraction

IV. Suspended solids (Athabasca River)

V. Dissolved fraction (Athabasca River)

VI. Whole Athabasca River water

All acute toxicity tests were conducted in recycle exposure chambers illustrated in Figure 5. The exposure chambers are specifically designed to permit the continuous suspension of solid

material. Particles that settle are drawn by a pump from the bottom of the chambers to the surface of the water. Each chamber had a dilution volume of 20 L.

Additional procedures are listed below:

1. The temperature, pH, conductivity and dissolved oxygen level of each chamber were determined.
2. Five fish were used in each test chamber; these fish were not fed throughout the experiment.
3. Observations for potential mortality were made at the start of the test and at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 24 hours, 48 hours, 72 hours and 96 hours.
4. Fish were considered dead when there was no respiratory or other movement, and no response to gentle prodding.
5. Control chambers, using only the dilution water, were also maintained throughout the 96h experimental period.
6. All moribund and dead fish as well as all fish remaining at the end of the study were submitted for histopathological examination.

2.6 Subacute Toxicity

Subacute toxicity tests were carried out to study the possible effect of bitumen. Three groups of eight to ten fish each were used to test the subacute toxicity of 100 mg L^{-1} of whole bitumen, extractable bitumen or residual (extracted) bitumen in treated municipal water. Another two groups of twelve fish each were used as controls.

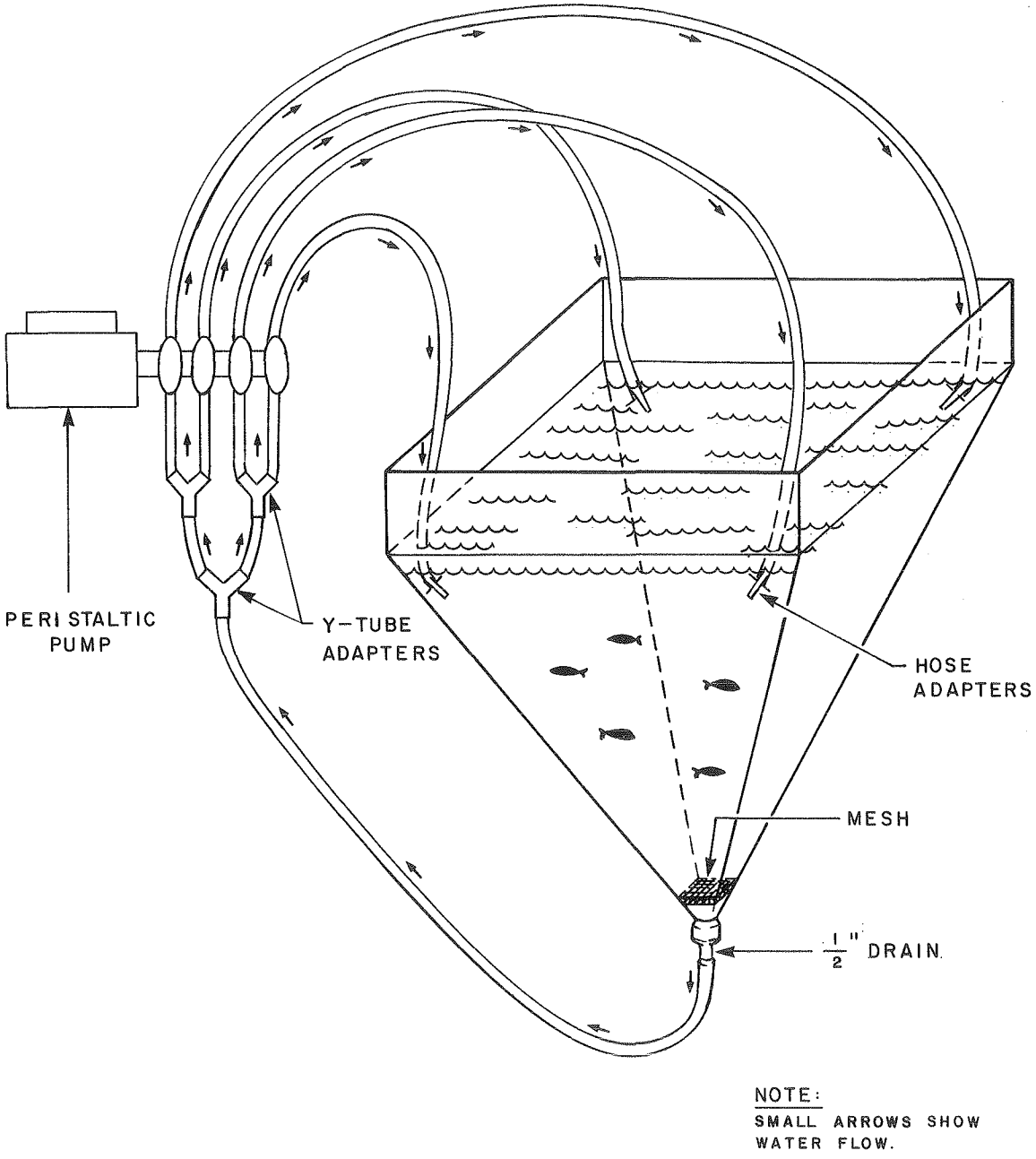

FIGURE 4. CHAMBERS USED FOR ACUTE TOXICITY BIOASSAY.

Groups I. Whole bitumen

II. Extractable bitumen

III. Residual (extracted) bitumen

IV. Athabasca Water (control)

V. Treated municipal water (control)

Exposures were conducted in tanks with a volume of 935 L at 15°C. The exposure period was initially for 96h followed by a 24h period in which each tank was flushed with treated municipal water and half of the fish were then left in this water for another 96h period.

At the end of each time period, three fish were netted from the tank and submitted for blood analysis and histopathology. Moribund and dead fish were submitted for histopathology.

2.7 Histopathology

Histopathology was carried out in both acute and subacute studies. Small fish were first slit open at their abdomen and immediately placed in the fixative. Large fish were routinely necropsied and pieces of different tissues were collected and placed in the fixative. Bouin's solution was used as a fixative for the first 24 hours, tissues were then preserved in 70% alcohol until trimming.

Tissues were routinely processed and sections of five to six microns in thickness were prepared and stained by hematoxylin and eosin. Gills, heart, oral cavity, eye, brain, spinal cord, liver,

stomach, pyloric ceca, intestine, pancreas, spleen, anterior and posterior kidneys, gonads, skin and muscle were examined in each fish.

2.8 Blood Analysis

This was carried out in the subacute study only. Fish were stunned with a blow on the head and immediately after this 1-3 ml of blood was collected by cardiac puncture using a 22-gauge needle attached to a 3 ml syringe containing traces of heparin. The blood was immediately analyzed for a number of parameters as outlined below:

1. Hematocrit
2. Blood oxygen using a Clark-type electrode
3. Carbon dioxide using a Severinghaus-stow type electrode
4. pH by flow-through glass capillary and reference assembly
5. Total carbon dioxide bicarbonate and base excess were calculated by using the Henderson-Hasselbach equation.

The above parameters were measured using a Corning pH/blood gas 178 analyzer.

All measurements and calculations of the analyzer were corrected according to body temperature of the fish (12°C) and hemoglobin values (9.0 gm/dl.) using the Kelman and Nunn formula (Davidsohn and Henry, 1974).

6. The remaining blood was then centrifuged and the plasma was harvested for chemical analysis. A KDA biochemistry instrument and packed reagents (American Monitor Corporation,

Mississauga, Ontario) were used for determination of the following variables:

a. Plasma sodium (Na^+) and potassium (K^+) by flame photometry.

b. Chloride (Cl^-) by modified Schoenfeld and Lowellen method.

c. Glucose using glucose oxidase reaction.

d. Total protein by biuret method.

e. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity correlated with the disappearance of NADH as double enzymatic reactions.

3. RESULTS

3.1 Chemical Analysis of Bitumen

Whole bitumen was characterized by a high level of aluminum (5530 mg kg^{-1}), potassium (2970 mg kg^{-1}), titanium (1030 mg kg^{-1}) and iron (1000 mg kg^{-1}) (Table 3). Toxic metals such as copper, mercury, lead and zinc were present in the samples, but at low levels. In the residual bitumen fraction, the concentration of aluminum declined to 4240 mg/kg, a trend also observed for potassium, titanium and iron (Table 3). Toxic metal levels also remained low. In the extractable liquid fraction, calcium, sodium and magnesium were

recorded at 41.1, 37.1 and 20.8 mg L^{-1} respectively. Most of the metals fell below the level of detection and would not be acutely toxic to fish.

Asphaltenes, which are largely insoluble in water, accounted for 11-12% by dry weight of the whole bitumen fraction (Table 4). This value increased to 34.6% in the residual fraction, reflecting the loss of soluble components to the extractable fraction. Asphaltenes were low in this latter fraction, representing 3.2% of the material. Aromatic hydrocarbons and saturated hydrocarbons were prevalent in the residual fraction, declining substantially in the extractable and whole fractions. The same trends were observed for Resins I and Resins II.

3.2 Acute Toxicity

Mortality did not occur in any of the acute tests. Therefore neither bitumen fractions nor Athabasca water solids were considered acutely toxic to fish. The 96h LC_{50} was thus greater than 100 mg L^{-1}, the highest concentration used.

No histopathological lesion indicative of toxicity was seen in fish exposed to any fraction of bitumen. Mild gill lesions were seen in a number of exposed fish as well as in the control groups. These lesions were characterized by slight shortening, hypercellularity and fusion of the secondary lamellae. Mild to severe hepatic fatty changes were seen in fish in all groups. Table 5 gives a summary of these findings.

Table 3. Elemental composition of three fractions of bitumen.

Element	Whole Fraction (mg kg^{-1})	Residual Fraction (mg kg^{-1})	Extractable Fraction (mg L^{-1})
Aluminum	5530	4240	1.91
Antimony	<10	<9	<0.1
Arsenic	<30	<30	<0.3
Barium	55.3	41.4	0.089
Beryllium	<0.10	<0.09	<0.001
Bismuth	<20	<20	<0.2
Boron	2.	0.9	0.31
Cadmium	<0.5	<0.4	<0.005
Calcium	140	92	41.1
Chromium	6.9	5.0	<0.005
Cobalt	2.	1.	<0.01
Copper	3.	2.	<0.01
Iron	1000	770	3.65
Lead	<5	<4	<0.05
Lithium	<10	<9	<0.1
Magnesium	170	110	20.8
Manganese	19.8	10.1	0.547
Mercury	<10	<9	<0.1
Molybdenum	5.	<3.	<0.03
Nickel	14.	12.	0.03
Phosphorus	30	20	<0.2
Potassium	2970	2230	6.9
Selenium	<10	<9	<0.1
Silicon	20	40	7.4
Sodium	240	170	37.1
Strontium	16.7	11.9	0.452
Thorium	<5	<4	<0.3
Titanium	1030	760	<0.005
Uranium	<30	<30	<0.3
Vanadium	39.3	32.4	0.021
Zinc	2.	2.	0.05
Zirconium	31	24	<0.01

Table 4. Percent hydrocarbon composition of three bitumen fractions.

	Whole Fraction*	Residual Fraction	Extractable Fraction*
Asphaltenes	11.0-12.0	34.6	3.2
Saturates	3.2- 3.7	15.7	4.5-4.7
Aromatics	6.2- 6.3	14.8	7.0-7.1
Resins I	5.4- 6.9	36.6	3.3-3.4
Resins II	1.6- 2.4	6.4	2.2
Asphaltene and toluene insoluble	81.3-83.2	75.1-76.4	40.2
Toluene insoluble	70.3-71.2	71.9-73.2	5.6

*Duplicate analyses.

Table 5. Histopathological findings in small fish exposed to different bitumen fractions.

Treatment	Gill Lesions	Hepatic fatty changes MI	MO	SR
Whole bitumen	0/5	1/5	3/5	0
Control	2/5	0	1/5	2/5
Extractable fraction (bitumen)	0/5	0	0	2/5
Control	0/5	0	0	2/5
Extracted fraction (bitumen)	0/5	1/5	0	1/5
Control	0/5	1/5	1/5	0
Suspended Solids (Athabasca River)	1/4	0	1/4	0
Control	1/4	1/4	1/4	1/4
Dissolved solids (Athabasca River)	0/5	3/5	1/5	0
Control	0/5	0	3/5	0
Whole Athabasca River water	0/5	1/5	0	0
Control	0/5	1/5	2/5	0

MI=Mild; MO=Moderate; SR=Severe

3.3 Subacute Toxicity

3.3.1 Histopathology

No histopathological lesion indicative of toxicity was seen in fish exposed to any of the three bitumen fractions (Table 6). Mild to moderate gill lesions were seen in 8/28 fish and characterized by hypercellularity, shortening and occasionally fusion of the secondary lamellae. Other incidental findings include: hepatitis 3/28, hepatic fatty changes 5/28, perineuritis 1/28, necrosis and erosion of the oral mucosa 1/28 and skin necrosis 1/28. Similar lesions were seen in the control fish.

3.3.2 Blood Analysis

No significant clinicopathological findings were seen in fish exposed to any of bitumen fractions (Table 7). The blood analysis results were compared with the control groups as well as with those in the historical controls (Table 8).

Table 6. Histopathological findings in large fish exposed to subacute levels of different bitumen fractions.

	100 mg L^{-1} Whole Bitumen	100 mg L^{-1} Extractable Bitumen	100 mg L^{-1} Extracted Bitumen (Residual)	Control Athabasca water	Treated water
Gills					
mild	4/8	0	4/10	7/12	4/12
moderate	1/8	0	0	1/12	0
Liver					
Hepatitis	2/8	0	1/10	0	1/12
Fatty changes	1/8	1/10	3/10	2/12	2/12
Heart					
Necrotic myocarditis	3/8	0	1/10	3/12	0
Pericarditis	1/8	0	1/10	4/12	4/12
Stomach					
Gastritis	1/8	0	1/10	0	0
Perineuritis	0	1/10	0	0	0
Oral mucosa					
Necrosis and erosion	0	1/10	0	3/12	2/12
Skin necrosis	0	0	1/10	0	0

Table 7. Blood analysis findings in rainbow trout exposed to bitumen fractions.

Parameter	Whole Bitumen 100 ppm 96h		Whole Bitumen t.m. water 96h		Extractable Bitumen 100 ppm 96h		Extractable Bitumen tt.m. water 96h		Extracted (residual) bitumen 100 ppm 96h		Extracted (residual) bitumen t.m.water 96h		Control Athabasca water 96h		Control t.m. water 96h		Control t.m. water 96h		Control t.m. water 96h	
	n	x±SD	n	x±SD	n	x±SD	n	x±SD	n	x±SD	n	x±SD	n	x±SD	n	x±SD	n	x±SD	n	x±Std
HCT	4	0.32 ± 0.01	4	0.38 ± 0.02	5	0.39± 0.09	4	0.51 ± 0.07	5	0.38 ± 0.07	2	0.42 ± 0.04	5	0.33 ± 0.04	5	0.31 ± 0.12	5	0.27 ± 0.04	5	0.35 ± 0.06
Total protein g/L	4	36 ± 3	4	36 ± 3	5	37 ± 8	5	36 ± 3	5	34 ± 8	2	32 ± 5	5	32 ± 2	4	40 ± 5	5	28 ± 5	5	40 ± 7
Glucose mmol/L	4	4.4 ± 0.3	4	7.0 ± 3.9	5	3 6 ± 1.6	5	5.3 ± 1.1	5	5.6 ± 1.4	2	6.0 ± 0.2	5	3.8 ± 0.2	5	4.6 ± 0.7	5	4.0 ± 0.2	5	4.2 ± 0.8
Na^+mmol/L	4	152 ± 1	4	151 ± 7	5	156 ± 3	5	154 ± 2	5	158 ± 11	2	155 ± 4	5	148 ± 9	5	149 ± 2	5	149 ± 4	5	153 ± 5
K^+mmol/L	4	1.6 ± 1.1	4	1.6 ± 1.4	5	1.8 ± 1.0	5	1.1 ± 0.4	5	2.0 ± 0.6	2	1.4 ± 0.3	5	0.6 ± 0.2	5	2.6 ± 1.0	5	1.6 ± 0.5	5	0.9 ± 0.4
Cl^-mmol/L	4	134 ± 1	4	131 ± 6	5	125 ± 6	5	132 ± 5	5	131 ± 5	2	132 ± 2	5	129 ± 8	5	130 ± 3	5	131 ± 3	5	132 ± 3
SGOT AST u/L	4	515 ±97	4	757 ±177	5	722 ±313	5	550 ±175	5	648 ±208	2	614 ±173	5	782 ± 96	4	915 ±170	5	702 ±281	5	640 ±129
SGPT ALT u/L	4	9 ± 3	4	15 ± 3	5	15 ± 6	5	13 ± 4	5	20 ± 8	2	14 ± 2	5	13 ± 4	4	30 ± 19	5	17 ± 10	5	11 ± 2
pH nmol/L	4	7.89 ± 0.04	4	7.79 ± 0.05	5	7.88± 0.08	4	7.63 ± 0.05	5	7.81 ± 0.09	2	7.72 ± 0.06	5	7.89 ± 0.03	5	7.76 ± 0.04	5	7.85 ± 0.04	5	7.75 ± 0.15
pCO_2 mmHg	4	3.9 ± 0.7	4	3.9 ± 0.7	5	5.2 ± 0.2	4	7.6 ± 1.0	5	6.1 ± 2.4	2	6.9 ± 1.8	5	2.80 ± 0.5	5	4.9 ± 0.8	5	3.4 ± 0.3	5	5.4 ± 3.2
pO_2 mmHg	4	42.5 ±32.8	3	3.3 ± 0.9	5	6.1 ± 2.1	4	2.2 ± 2.0	5	8.8 ± 8.0	2	2.1 ± 1.1	5	67.95 ± 21.1	5	6.9 ± 7.8	5	20.5 ± 17.2	5	3.1 ± 3.0
HCO_3mmol/L	4	8.3 ± 0.8	4	7.6 ± 1.5	5	12.4 ± 2.0	4	10.0 ± 1.4	5	11.7 ± 2.2	2	11.1 ± 1.5	5	6.7 ± 1.4	5	8.7 ± 0.5	5	7.5 ± 1.0	5	8.6 ± 1.8
O_2 SAT mol/mol	4	83.2 ± 31.0	3	33.7 ± 15.7	5	72.3 ± 17.1	4	17.2 ± 23.0	5	67.6 ± 38.5	2	13.8 ± 10.9	5	98.89 ± 0.4	5	50.2 ± 37.8	5	94.0 ± 2.9	5	41.8 ± 38.7
B.E. mmol/L	4	-10.4 ± 0.6	4	-13.9 ± 2.0	5	-7.7 ± 3.6	4	-15.1 ± 1.9	5	-9.6 ± 1.7	2	-12.2 ± 0.1	5	-12.8 ± 1.5	5	-13.7 ± 2.2	5	-12.7 ± 1.5	5	-14.0 ± 2.6
TCO_2 mmol/L	4	9.4 ± 0.8	4	7.8 ± 1.5	5	12.6 ± 2.0	4	10.2 ± 1.4	5	11.9 ± 2.3	2	11.3 ± 1.6	5	6.8 ± 1.4	5	8.9 ± 0.5	5	7.6 ± 1.0	5	8.8 ± 1.9

n = number of fish tested

x±SD = mean ± Standard deviation

t.m. water = treated municipal water

Table 8. Summary of clinicopathological findings in the historical control.

Acc.No.	pH	pCO_2	pO_2	HCO_3	O_2SAT	B.E.	TCO_2	HCT	TP	Glucose	Na^+	K^+	Cl	SGOT	SGPT
200-84	7.75	6.52	3.30	11.6	.35	-10.3	13.6	.48	-	-	153	1	136	1443	27
201.84	7.62	6.48	.97	9.0	.03	-16	11	.46	-	-	155	1	126	-	-
202.84	7.80	7.42	.45	14.7	.01	- 6.6	17		-	-	-	1	129	474	90
203.84	7.73	4.42	3.6	7.4	.40	-14.2	8.7		-	-	143	-	-	-	-
204.84	7.78	6.37	.75	12.1	.03	- 9.1	14.07	.35	-	-	156	4	-	-	-
205.84	7.82	3.97	9.90	8.2	.91	-11.2	9.43		-	-	149	2	131	-	14
206.84	7.76	5.70	2.85	10.3	.28	-11.2	12.06	.45	-	-	153	3	128	-	-
217.84	7.71	4.80	2.32	7.8	.18	-14.2	9.28		-	-	149	3	131	903	13
210.84	7.66	8.02	1.2	11.4	.05	-13.1	13.88	.41	-	-	155	4	128	-	-
211.84	7.71	7.65	1.72	12.3	.10	-10.8	14.67		-	-	-	1	130	664	21
212.84	7.73	6.52	2.32	11.0	.18	-11.3	13.02		-	-	157	-	-	-	-
213.84	7.67	8.32	.67	12.1	.02	-12.1	14.6	.49	-	-	152	1	130	1840	31
214.84	7.7	8.40	1.20	13.7	.05	- 9.6	16.3				158	4	130	1481	-
249.84	7.79	5.70	3.45	10.9	.39	- 9.9	12.66	.38							
251.84	7.72	6.97	2.02	11.4	.14	-11.3	13.56	.30							
252.84	7.81	5.25	7.502	10.6	.86	- 9.4	12.11	.37							
253.84	7.64	6.52	2.85	8.8	.23	-15.6	10.82	.35							
459.84	7.72	6.15	3.45	10.1	.35	-13	12.00	-	46		148	5.5	127	899	34.9
616.84	7.74	5.32	3.75	9.1	.42	-13.4	9.3	-	44	2.8	154	0.7	126	924	26
617.84	7.58	8.34	1.12	9.8	.04	-16.4	10	-	39	3.6	159	0.7	133	1510	41
618.84	7.73	5.10	7.80	8.5	.84	-14.2	8.7	37	46	4.6	151	0.5	127	1130	25
619.84	-	-	-	-	-	-	-	-	38	3.8	155	0.8	132	870	22
620-84	7.78	4.57	3.75	8.6	.45	-12.8	8.7	-	43	4.4	153	0.7	130	1150	31
621-84		3.60	42.9	8.2	.98	-11.1	8.3	28	38	3.8	152	1.3	130	1260	30
622-84	7.73	4.65	3.90	7.7	.44	-14.8	7.8	-	-	3.3	148	1.9	124	-	-
623.84		2.92	59.19	7.3	.99	-11.2	7.4	-	39	4.0	156	1.8	133	620	14
624.84	7.84	4.12	-	8.9	-	-11.0	9.0	36	36	4.5	151	2.2	129	820	15
625.84	7.78	4.20	6.22	8.0	.77	-13.3	8.1	-	39	5.5	153	3.2	132	1920	39

	pH	pCO_2 mmHg	pO_2 mmHg	HCO_3 mmol/L	O_2 SAT %	B.E. mmol/L	TCO_2 mmol/L	HCT L/L	TP g/L	Glucose mmol/L	Na^+ mmol/L	K^+ mmol/L	Cl mmol/L	SGOT U/L	SGPT U/L
n	25	27	26	27	26	27	27	13	10	10	22	22	21	16	16
$\bar{x}$	7.74	5.85	6.89	9.98	0.36	-12.11	11.4	0.38	40.5	4.0	152.7	2.0	129.6	1119.3	29.6
SD	0.07	±1.56	±13.40	±1.98	±0.32	±2.32	±2.32	±0.06	±3.51	±0.8	±3.8	±1.4	±2.8	±426.4	±18.3

n = number of fish tested
$\bar{x}$ = mean
SD = standard deviation

4. DISCUSSION

Based on the elemental analysis, the concentration of heavy metals in all three bitumen fractions would not have been acutely toxic to fish such as rainbow trout (Alabaster and Lloyd, 1980; American Public Health Association, 1980). Similarly, because of their stability and insolubility, the asphaltenes and resins would have also been non-toxic. The aromatic and saturated hydrocarbon groups are comprised of many compounds which would probably exhibit various physico-chemical properties. Since a more detailed analysis was not conducted on these groups, it is not possible to identify specific toxic components.

Under natural conditions, there would probably be appreciable loss of volatile substances from surface deposits of bitumen. Such compounds are often moderately or highly toxic to fish (Moore and Ramamoorthy, 1984). The fractionation procedure in this study would have enchanced the volatilization of hydrocarbons, thereby reducing exposure to the fish. In addition, since the exposure chambers in the acute and subacute trials were left uncovered, there would have been further loss of these compounds.

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Appendix I

HISTOPATHOLOGY OF LARGE FISH (SUBACUTE STUDY)

Fish exposed to 100 ppm whole Bitumen in Athabasca water for 96h→Flush→96h treated municipal water.

Acc.# 1717-84

Heart: Subacute moderate focal myocarditis.

Acc.#1718-84

Gills: Mild focal hypercellularity of the secondary lamellae.

Liver: Mild focal lymphocytic hepatitis.

Acc.#1719-84

Gills: Mild hypercellularity, shortening and fusion of the secondary lamellae.

Heart: Subacute focal necrotic myocarditis.

Acc.#1720-84

Gills: Mild hypercellularity of the secondary lamellae.

Liver: Mild diffuse hepatic fatty changes.

Heart: Acute marked myocardial necrosis.

Acc.#1721-84

Gills: Moderate hypercellularity of the secondary lamellae.

Acc.#1722-84

Heart: Mild diffuse pericarditis.

Acc.#1723-84

Stomach: Subacute necrotic gastritis.

Acc.#1724-84

Gills: Mild hypercellularity of the secondary lamellae.

Liver: Mild focal lymphocytic hepatitis.

Fish exposed to 100 ppm extractable Bitumen in Athabasca water →Flush→96h treated municipal water.

Acc.#1998-84

No significant lesion was seen in the examined tissues.

Acc.#1999-84

Stomach: Moderate perineuritis.

Acc.#2000-84

No significant lesion was seen in the examined tissues.

Acc.#2001-84

No significant lesion was seen in the examined tissues.

Acc.#2002-84

Liver: Marked diffuse hepatic fatty changes.

Acc.#2003-84

No significant lesion was seen in the examined tissues.

Acc.#2004-84

No significant lesion was seen in the examined tissues.

Acc.#2005-84

No significant lesion was seen in the examined tissues.

Acc.#2006-84

No significant lesion was seen in the examined tissues.

Acc.#2007-84

Oral mucosa: Mild diffuse superficial erosion of the mucosal epithelium.

Fish exposed to 100 ppm extracted (residual) Bitumen in Athabasca water →flush→96h treated municipal water.

Acc.#1825-84

No significant lesion was seen in the examined tissues.

Acc.#1826-84

Liver: Mild diffuse hepatic fatty changes.

Acc.#1827-84

No significant lesion was seen in the examined tissues.

Acc.#1828-84

No significant lesion was seen in the examined tissues.

Acc.#1829-84

Mild hypercellularity and shortening of the secondary lamellae.

Acc.#1830-84

Liver: Mild multifocal hepatocellular degeneration and necrosis.

Acc.#1831-84

Heart: Acute multifocal necrotic myocarditis..

Liver: Mild diffuse hepatic fatty changes.

Stomach: Acute marked necrotic gastritis.

Acc.#1832-84

Gills: Mild hypercellularity and shortening of the secondary lamellae.

Liver: Moderate diffuse hepatic fatty changes.

Heart: Moderate focal pericarditis.

Acc.#1833-84

Fin: Chronic necrotic dermatitis.

Gills: Mild hypercellularity of the secondary lamellae.

Acc.#1834-84

Gills: Mild hypercellularity of the secondary lamellae.

Fish kept in Athabasca water for 96h→Flush→treated municipal water for 96h.

Acc.#1094-84

No significant histologic lesion was seen in the examined tissues.

Acc.#1095-84

Heart: Moderate focal pericarditis.

Acc.#1096-84

Gills: Mild hypercellularity of the secondary lamellae.

Acc.#1097-84

Heart: Mild focal myocarditis

Liver: Mild diffuse hepatic fatty changes.

Acc.#1098-84

Gills: Mild hypercellularity of the secondary lamellae.

Acc.#1099-84

Gills: Mild hypercellularity and shortening of the secondary lamellae.

Acc.#1100-84

Heart: Moderate focal pericarditis.

Acc.#1101-84

Gills: Moderate hypercellularity and fusion of the secondary lamellae.

Heart: Moderate focal pericarditis.

Oral mucosa:

Moderate multifocal superficial necrosis and erosion of the mucosal epithelium.

Acc.#1102-84

Oral mucosa:

Marked superficial erosion and necrosis of the mucosal epithelium.

Gills: Mild hypercellularity of the secondary lamellae.

Acc.#1103-84

Gills: Mild hypercellularity of the secondary lamellae.

Oral mucosa:

Moderate superifical erosion and necrosis of the mucosal epithelium.

Heart: Moderate focal myocarditis.

Acc.#1104-84

Gills: Mild hypercellularity of the secondary lamellae.

Liver: Moderate diffuse hepatic fatty changes.

Heart: Moderate focal myocarditis.

Acc.#1105-84

Gills: Mild hypercellularity of the secondary lamellae.

Heart: Mild focal pericarditis.

Control fish - kept in treated municipal water for 192h.

Acc.#1106-84

Oral mucosa:

Mild superficial erosion of the mucosal epithelium.

Heart: Moderate focal pericarditis.

Acc.#1107-84

Gills: Mild hypercellularity and shortening of the secondary lamellae.

Acc.#1108-84

Liver: Mild focal lymphocytic hepatitis.

Acc.#1109-84

Gills: Mild hypercellularity of the secondary lamellae.

Acc.#1110-84

No significant lesion was seen in the examined tissues.

Acc.#1111-84

Gills: Mild hypercellularity of the secondary lamellae.

Acc.#1112-84

No significant lesion was seen in the examined tissues.

Acc.#1113-84

Heart: Mild focal pericarditis.

Acc.#1114-84

Oral mucosa:

Marked diffuse superficial necrosis and sloughing of the mucosal epithelium (probably due to rough handling of tissues).

Liver: Moderate diffuse hepatic fatty changes.

Acc.#1115-84

Gills: Mild hypercellularity of the secondary lamellae.

Heart: Marked multifocal pericarditis.

Acc.#1116-84

No significant histologic lesion was seen in the examined tissues.

Acc.#1117-84

Heart: Moderate focal pericarditis.