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UNIVERSITY OF ALBERTA

A TOXICITY ASSESSMENT OF OIL SANDS WASTEWATER

BY

ALLEN G. VERBEEK ©

A Thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

DEPARTMENT OF ZOOLOGY

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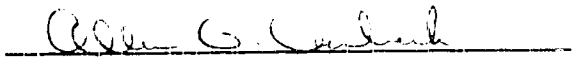

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled A TOXICITY ASSESSMENT OF OIL SANDS WASTEWATER submitted by ALLEN G. VERBEEK in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

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October 31, 1994

Abstract

Toxic industrial wastewaters, such as those from oil sands extraction, must be characterized and assessed for potential sublethal effects before they can be safely disposed in the environment. The objectives of this thesis were firstly, to characterize the fractions responsible for the acute toxicity of these wastewaters and to compare the relative sensitivity of Microtox, *Daphnia magna* and rainbow trout to the toxic fractions. The second objective was to assess the potential of the mixed-function oxygenase system in rainbow trout as an indicator of exposure to sublethal concentrations of oil sands tailings water.

All and approximately 85% of the acute toxicity of surface tailings water and fine tails interstitial water respectively, as measured using the Microtox assay, was due to organic acidic surfactants. Organic acids accounted for all the acute toxicity of surface tailings water to *Daphnia* and rainbow trout. However, *Daphnia* were 0.4 times less sensitive and rainbow trout were 3 times more sensitive than Microtox to these organic acids. These differences in relative sensitivity demonstrate the importance of using more than one test organism to evaluate acute toxicity.

The mixed-function oxygenase system in rainbow trout responded rapidly following a definable concentration-response relationship; however, it proved to be a relatively insensitive indicator of sublethal exposure to oil sands tailings water. Increased activity and maximal induction, as a result of exposure to 0.3 and 0.6 Toxic Units (TU), occurred rapidly within 24 hours of exposure. When outliers were removed, the linearity of the relationship between the concentration of tailings pond water and ethoxyresorufin-O-deethylase activity had an r^2 value of 0.593. Maximal induction required 0.4-0.8 TU of this wastewater. The absolute level of maximal induction was only one-fifth to one-sixth of the potential induction as found in response to the positive control, 0.5 mg/kg β-naphthoflavone (*i.p.*). The sensitivity of this system was further compromised when the undesirably very high No Observed Effect and Lowest Observed Effect Concentrations of 0.15 TU and 0.2 TU were considered.

The acutely toxic organic acids may have been responsible for the observed mixed-function oxygenase induction and speculation in support of this hypothesis was presented.

Acknowledgments

Special thanks to Drs. Mike MacKinnon and Hans Boerger of Syncrude Canada Ltd. for their assistance in providing samples and logistical support. Mr. Munir Jivraj provided diligent summer assistance in running the bioassays for the Toxicity Identification Evaluation component of this thesis. Bill Mackay, my supervisor was very helpful and instructive throughout this thesis and I thank him for his patience and diligence. I thank all the members of my supervisory committee fc their time and energy. Lastly, I thank my wife Marilyn and son David. Marilyn was a terrific support throughout this thesis and her help with lab work late at night or early in the morning was always appreciated. David, I thank for helping in ways that he does not yet understand. His simple, yet miraculous birth and growth over the past 7-1/2 months have been perhaps the strongest encouragement I could have been given for the push to complete this thesis.

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Chapter I
General Introduction

Before industrial wastewaters can be disposed of safely in the environment, sensitive methods must be used to characterize acutely toxic compounds in the water and to assess the potential for sublethal effects from these acutely toxic compounds and possibly other chronically toxic compounds to which aquatic species may be exposed in the disposal ecosystem.

Large volumes of fluid waste are produced during the extraction of oil from oil sand using the hot water flotation method. This fluid waste consists of process-affected water and a relatively stable suspension of solids and unrecovered bitumen called fine tails (MacKinnon, 1989; Martin and Klym, 1991) which are acutely toxic to aquatic organisms (MacKinnon and Retallack, 1982; MacKinnon and Boerger, 1986; Nix and Martin, 1992). This fluid waste which is stored in large tailings ponds on the lease sites must eventually be reclaimed (Boerger *et al.*, 1992; Nix and Martin, 1992). The safe reclamation of these wastewaters requires, at least in part, sufficient characterization of the acutely toxic fractions so that the wastewater can be detoxified.

The characterization of acutely toxic compounds in a complex industrial wastewater can be readily accomplished by removing various different fractions, each of which contains a 'class' of compounds that are similar on the basis of a general physical or chemical property, and then identifying the proportion of acute toxicity in each fraction. This characterization can be efficiently accomplished through the use of a bioassay-directed **T**oxicity **I**dentification **E**valuation (TIE) protocol as developed by the U.S. **E**nvironmental **P**rotection **A**gency (EPA, 1988; 1991). In the process of this determination, treatment options for the detoxification of the wastewater are identified.

The success of a characterization process such as a TIE is intimately linked to the use of several indicator species for acute toxicity. Since the toxicity of a single compound can differ markedly between species (Munkittrick *et al.*, 1991), one species can be a much more sensitive indicator of a specific toxicant or class of toxicants than another. A direct consequence of this is that any attempt to identify the acutely toxic fractions of a given wastewater using a TIE approach must not be based on the results of one indicator species. Such a procedure could potentially identify fractions that are toxic to the one indicator

species used but not identify other fractions which would be toxic to other species in the eventual disposal ecosystem. Proper use of multiple indicator species in a bioassay-directed characterization procedure such as a TIE also yields valuable information on the comparative toxicity of various wastewater fractions. Chapter II of this thesis addresses the characterization or description of the physical and chemical properties of the acutely toxic fractions of oil sands wastewaters using a modified U.S.E.P.A. TIE (EPA, 1991) with Microtox (*Vibrio fischeri*)[1], *Daphnia magna* and rainbow trout (*Oncorhynchus mykiss*), as the indicator species.

In addition to the need to classify potential acute toxicants, another important aspect of a toxicity assessment can be a biochemical evaluation of the potential for sublethal effects in aquatic species exposed to diluted wastewater. An evaluation at the biochemical level is important whether a wastewater will be detoxified with respect to any identified class(es) of acutely toxic compounds or not. This is because of the potential for toxicants that have a mode of action such that they will affect a biological process other than those measured in any acute bioassays (*i.e.* lethality, immobilization or light emission). One indicator of the potential for sublethal effects is the biochemical capacity to transform and excrete non-polar organic compounds.

Xenobiotics (foreign, biologically active compounds) are taken up by the body via three principal routes; the skin, the respiratory surfaces or the gastrointestinal tract, after which they pass into the interstitial fluid and then into the blood where they are excreted by the kidney, excreted in the bile, stored in fatty tissue or converted from fat-soluble parent compounds to water-soluble metabolites that can be more readily excreted from the body (Zakrzewski, 1991). Once in the body, the action of most xenobiotics ends in either excretion or metabolic conversion to more water-soluble inactive metabolites which are then excreted (Zakrzewski, 1991). Some xenobiotics, however, are actually converted to more toxic forms by the biotransformation systems and these more toxic, biologically active metabolites can be very harmful to the organism. Water-soluble compounds usually do not require metabolic conversion as they

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[1] The strain of bacteria used in the Microtox assay (Microbics Corporation) was formerly classified as a strain of *Phobacterium phosphoreum*. Based on recent identification of the species-specific *lux A* gene in the strain used for the Microtox assay, this strain has been reclassified as *Vibrio fischeri* following the work of Wimpee *et al.*, (1991) (Bulich, Microbics Corporation; pers. comm.).

can be excreted by the kidney in their original form (Zakrzewski, 1991). Some fat-soluble compounds are excreted in the bile without metabolic conversion (transformation or conjugation) (Zakrzewski, 1991). Most fat-soluble compounds, however, require metabolic conversion to water-soluble metabolites that can be excreted from the body (Zakrzewski, 1991).

The conversion or inactivation of non-polar xenobiotics in fish liver is facilitated almost exclusively by two liver detoxification systems, the mixed-function oxygenase (MFO) system and the conjugating system (Chambers and Yarbrough, 1976). Of these two biotransformation systems, the MFO system has been extensively studied for use as an environmental bioindicator of fish exposure to non-polar organic contaminants in an industrial wastewater (Jimenez and Stegeman, 1990). In this respect, MFO enzymes can be used as an indicator of a possible sublethal risk to fish and other aquatic species. In Chapter III, data are presented to assess the potential of MFO activity as an indicator of sublethal exposure of rainbow trout to oil sands tailings pond water.

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Chapter II
Acute Toxicity of Oil Sands Wastewater: A Toxic Balance[1]

Introduction

Toxic industrial wastewaters may contain thousands of compounds, and all or only a few of these may contribute to the acute toxicity of the wastewater, so the acutely toxic compounds of a wastewater must be characterized prior to the initiation of efficient mitigative action. The goals of this work were 1) to characterize and determine the relative importance of the acutely toxic fractions of oil sands extraction wastewater from Syncrude Canada Ltd. using a bioassay-directed Toxicity Identification Evaluation (TIE) and to use these data to construct a toxic balance, and 2) to determine whether the same fractions were acutely toxic to Microtox[2] (*Vibrio fischeri*)[3], *Daphnia magna* and rainbow trout (*Oncorhynchus mykiss*).

Syncrude Canada Ltd., located in northeastern Alberta, Canada, (~400 km north of Edmonton) separates oil from oil sand using hot water flotation, which produces large volumes of fluid wastes (process-affected water and a relatively stable suspension of solids and unrecovered bitumen called fine tails) (MacKinnon, 1989) which are acutely toxic to aquatic organisms (MacKinnon and Retallack, 1982; MacKinnon and Boerger, 1986). This fluid waste, which is currently stored in large tailings ponds on the Syncrude lease site, must eventually be reclaimed (Boerger *et al.*, 1992). While the acute toxicity of oil sands process water has been well documented using a number of bioassays (Microtox, *D. magna* (water fleas) and rainbow trout)(MacKinnon and Retallack, 1982; MacKinnon and Boerger, 1986; MacKinnon and Boerger, 1991; Nix and Martin, 1992), there has not been a thorough evaluation of the potentially toxic fractions of these wastewaters.

An efficient way to characterize and determine the relative importance of

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[1] A version of this chapter has been published. Verbeek, A.G., Mackay, W.C., and MacKinnon, M.D. 1994. Can. Tech. Rep. Fish & Aqu. Sci. 1989: 196-207.

[2] Microtox is a registered trademark of Microbics Corporation.

[3] The strain of bacteria used in the Microtox assay (Microbics Corporation) was formerly classified as a strain of *Phobacterium phosphoreum*. Based on recent identification of the species-specific *lux A* gene in the strain used for the Microtox assay, this strain has been reclassified as *Vibrio fischeri* following the work of Wimpee *et al.*, (1991) (Bulich, Microbics Corporation; pers. comm.).

various classes of **acutely** toxic compounds in a complex mixture is to use a TIE protocol such as developed by the U.S. Environmental Protection Agency (EPA, 1991). The TIE method uses bioassays to characterize the general toxicants in a complex mixture thereby allowing either the focused analytical identification of the specific toxicants or the treatment of the toxic fractions without identifying the specific toxicants. The TIE protocol uses eight different manipulations, each of which removes a different class of compounds from a process water sample on the basis of specific physical or chemical properties. The eight manipulations of the TIE are:

1) the removal of suspended solids at the initial sample pH (pH_i) if required.

2) the removal of compounds that precipitate at pH 3.0 or pH 11.0 by pH adjustment and filtration.

3) the removal of compounds that have a non-polar component as well as the removal of some cationic metals by passage of the sample at pH 3.0, pH_i and pH 9.0 through a reverse phase solid phase (C_{18}) sorbent pre-conditioned with methanol.

4) the removal of compounds that are readily oxidized or purged from solution by sparging (bubbling) the sample with oxygen at pH_i for one hour (oxidizable or volatile compounds).

5) the removal of compounds that are not oxidized, but purged from solution by sparging with nitrogen at pH_i for one hour (volatile compounds).

6) the manipulation of the ionization equilibrium in compounds whose acute toxicity changes with pH ranging from 6 to 8.5 (*eg.*ammonia, cyanide, hydrogen sulphide and some organic compounds such as pentachlorophenol).

7) the removal of compounds that are either a) readily reduced (*eg.* chlorine, bromine and iodine) or b) complexed (*eg.* some cationic metals: Cd^{2+}, Cu^{2+}, Ag^{1+}, and Hg^{2+}) by treatment with sodium thiosulphate.

8) the removal of compounds that form a nontoxic complex by chelation with ethylenediamine-tetraacetate (EDTA) (*eg.* cationic metals: Al^{3+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, Mn^{2+}, Ni^{2+}, Sr^{2+} and Zn^{2+}).

In the TIE, the acute toxicity of the water being tested is determined before and after the removal of each class of compounds (before and after each manipulation) in order to determine which class(es) are responsible for the majority of the acute toxicity of the wastewater (EPA, 1991). In addition to characterizing the class(es) of compounds that are responsible for the acute

toxicity, this characterization will permit the development of treatment options for the detoxification of the wastewater.

The TIE is facilitated through the use of the Microtox assay to screen the acute toxicity of samples. The Microtox assay is a rapid (15 min), bacterial-based bioassay that requires only small volumes of test sample (<5 mL) (Bulich and Isenberg, 1981) and correlates well with *Daphnia* and trout bioassays for the acute toxicity of tailings pond water (MacKinnon and Retallack, 1982; Boerger *et al.*, 1986; MacKinnon, 1985; 1989) and waste drilling fluids (Strosher, 1983). The Microtox assay uses a bacterium, *V. fischeri* which normally emits light in water with a pH range of 6 to 9 (Ribo and Kaiser, 1987) and a temperature range of 5 to 25 °C (Bulich and Isenberg, 1981). The endpoint for the Microtox assay is an IC_{50}[4]. This is analogous to the determination of an LC_{50}, a Lethal Concentration of test sample that causes 50% mortality in exposed test animals within a specific time period (*eg. Daphnia* (48 h) or rainbow trout (96h)).

The entire TIE was used to completely characterize the acutely toxic fractions in what was thought to be a representative oil sands wastewater (surface tailings pond water from Syncrude) using the Microtox assay. Where significant reductions in acute toxicity were found, further acute toxicity tests were carried out using *D. magna* and rainbow trout (*O. mykiss*). In the second phase of this project, the Microtox assay was used to characterize the acutely toxic fractions of fine tails interstitial water from Syncrude. In this phase, the manipulations that had resulted in a decrease in the acute toxicity of Syncrude surface tailings pond water were repeated with interstitial water. In addition, manipulations to remove compounds that are readily oxidized and purged (TIE manipulation #4) and compounds that are not oxidized but purged (TIE manipulation #5) were performed on the fine tails interstitial water.

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[4] The IC_{50} is used in place of the EC_{50} term since the true definition of an EC_{50} does not apply to the Microtox assay. An EC_{50} is the median Effective Concentration, *i.e.*, the concentration of test sample in water that is estimated to cause a specified effect in 50% of the individuals exposed to that concentration. The effect is usually sublethal. EC_{50}, like LC_{50}, refers to a quantal effect since each exposed individual must be categorized as either showing the effect or not showing it. The term does not accurately apply to a percent reduction in some rate or process in a **group** of organisms. For this reason it is more accurate to refer to the endpoint of the Microtox assay as an IC_{50}. An IC_{50} is the median Inhibiting Concentration, *i.e.*, the concentration of material in water that is estimated to cause a 50% impairment in a quantitative biological function such as light production by bacteria or growth of fish, relative to a control (EPS, 1992).

Materials and Methods

Sample collection

Fine tails and surface tailings pond water were obtained from the Syncrude tailings pond during the summers of 1991 and 1992. Fine tails were collected from approximately 10 m below the fine tails/surface tailings pond water interface. Tailings pond water was collected from 15-100 cm below the surface of the pond. The headspace of all sample containers was $\leq$ 0.5% to minimize oxidation of compounds in the sample during transport to Edmonton, where further sample handling took place.

Sample handling and storage

All containers of fine tails and tailings pond water were held at $\leq$ 6° C, during transport, subsequent handling and storage until testing. Since the suspended solids content of surface tailings pond water at Syncrude varies with the time of year (MacKinnon and Retallack, 1982), the suspended solids were removed from all test samples in order to obtain a fluid which yielded reproducible acute toxicity data. The degree to which the suspended solids from surface tailings pond water contributes to the acute toxicity of this water has been previously documented using a number of bioassays (Microtox, *D. magna*, fathead minnow and rainbow trout) (MacKinnon and Retallack, 1982). Within 10 days of sample collection, the fine tails and tailings pond water were centrifuged at 25,000 x g for 30 min while being maintained at 6 °C. The supernatant was collected and centrifuged again if required at this time. When bitumen particles still remained as an emulsion after centrifugation, they were removed by filtration at 0.45 µm. With this sample preparation procedure, the acute toxicities of the supernatants from different samples (hereafter referred to as surface tailings pond water or fine tails interstitial water) were reproducible. The surface tailings pond water and fine tails interstitial water were stored in amber glass bottles with Teflon-lined caps for the duration of all testing. Samples were stored and used for up to a maximum of one month. The headspace in sample storage bottles was purged with nitrogen to minimize oxidation of the sample during storage.

Acute toxicity tests

The Microtox bioassay

The Microtox bioassay was run for each of the eight sample manipulations of the TIE approach. Microtox assays were performed as described by Environment Canada (EPS, 1992) for an exposure period of 15 min. All samples were tested at pH 7.5, except those specifically designed to test the change in acute toxicity with changing pH (TIE manipulation #6).

In addition to the standard control used in the Microtox assay, two other controls were used. Toxicity blanks were used to control for the pH and conductivity of the waters tested as well as controlling for possible toxic effects resulting from the various TIE manipulations. The water used for the toxicity blanks was 0.14 % sea salts (Instant Ocean) in distilled water adjusted to pH 7.5. Phenol standards (100 mg/L) were used as a positive control to assess the relative sensitivity of the bacterial reagent.

For two of the TIE manipulations, those designed to i) remove compounds that can be reduced (TIE manipulation #7) and ii) remove cationic metals (TIE manipulation #8), standard 15 min IC_{50} values were not determined. Each of these two manipulations involves the treatment of sample with multiple concentrations of sodium thiosulphate and EDTA respectively. As a result, determinations of IC_{50} values for these manipulations would be have been very time consuming. Alternatively, the Inhibitory Time required to cause a 50% reduction in the light emission of *V. fischeri* populations was determined. This is known as an IT_{50} and is analogous to an LT_{50} for animal bioassays. For an IT_{50}, bacteria were exposed to multiples of only one concentration of surface tailings pond water (450 mL/L) plus a control. Instead of exposing the bacteria to varying concentrations of surface tailings pond water, the dosages of sodium thiosulphate (to reduce compounds) and EDTA (to bind cationic metals to form nontoxic complexes)(EPA, 1991) were varied. To determine the IT_{50} values, the light output of the bacteria was measured at various times (0, 2, 3.5, 5, 6.5, 8, 10 and 15 min). The IT_{50} values of the test samples were calculated as the time required to reach a 50 % reduction of light output from the maximal values.

The Daphnia bioassay

The 48 h acute toxicity test using *Daphnia* (EPS, 1990a), like the Microtox

test is a static bioassay, in which the same water is held in test containers at a constant temperature (20 ± 2 °C), constant pH (~7.5) and without aeration for the duration of the assay. Ten 1-day-old *Daphnia* were used per concentration of test sample at a loading density of 1 daphnid/12 mL of test water. Controls consisting of 10 *Daphnia* held in 120 mL culture water (0.14 % sea salts in distilled water adjusted to pH 7.5) were run with each test. In all cases, there was no mortality in the controls. The *Daphnia* were not fed during the test. The test containers were observed at 6, 12, 24, 36, and 48 h for mortality. Cessation of heartbeat was the criteria used to assess mortality of *Daphnia*. The test concentrations for the *Daphnia* bioassay varied with the sample being tested (range 700 to 1000 mL/L).

The rainbow trout bioassay

The rainbow trout acute toxicity tests (EPS, 1990b) were run on surface tailings pond water from Syncrude and surface tailings pond water with the organic acids removed (TIE manipulation #2). Static 96 h rainbow trout acute toxicity tests were performed using ten fingerling rainbow trout (0.3-0.8 g) per concentration of test solution, at a loading density of 1.0 ± 0.3 g/L. Controls consisting of ten fingerlings in dechlorinated aquarium water from the University of Alberta were run with each test. In all tests, there were no mortalities in the controls. Tests were performed under constant temperature (15 ± 2 °C) and constant aeration. The fingerlings were not fed 24 h prior to or during the test. Test concentrations for surface tailings pond water from Syncrude were set at 90, 100, 110 and 120 mL/L. Surface tailings pond water without the organic acids was tested at 1000 mL/L. The test containers were observed at 6, 12, 24, 36, 48, 60, 72, 84 and 96 h for mortality. Mortality was defined as the lack of opercular movement for 10 sec.

The Toxicity Identification Evaluation

Surface tailings pond water and fine tails interstitial water were subjected to the manipulations of the TIE as found in the U.S. EPA TIE manual (EPA, 1991) with the following modifications. In this specific TIE, organic acid precipitate[5] was removed with centrifugation, not filtration, as centrifugation was

[5] Throughout this paper, reference is made to the specific classes of compounds being removed by TIE manipulations. As a precautionary note, the reader should be aware that the following specific "labels" (organic acids, organic bases & non-polar organic compounds) **cannot** be

found to be more effective and more reproducible than filtration for the removal of the organic acid precipitate from these wastewaters. Non-polar organic compounds[5] were removed from sample water using a C_{18} column at pH 2.5, pH_i (~8) and 11.0 as opposed to the recommended 3, pH_i, and 9. We found that the integrity of the C_{18} column (Waters Sep Pak), as defined by the lack of column breakthrough as determined by toxicity in the Microtox assay, was maintained at the greater pH range. The relative % removal in acute toxicity for effective TIE treatments is expressed using the following formula:

$$\% \text{ reduction in acute toxicity} = \frac{(\text{post-treatment toxicity}) - (\text{pre-treatment toxicity})}{(1000 - \text{pre-treatment toxicity})} * 100$$

This relative % reduction allows for the focus on the fractions containing the majority of the acute toxicity. It does not provide a measure of the absolute reduction in acute toxicity as with Toxic Equivalency Units (TEU).

Results

Acute toxicity screening with the Microtox assay

The acutely toxic fractions in surface tailings pond water

There was no evidence that any portion of the acute toxicity of Syncrude surface tailings pond water resulted from the presence of the following compounds: 1) organic bases that precipitate at pH 11, 2) oxidizable and purgable compounds, 3) purgable compounds, 4) reducible compounds or 5) cationic metals (Table II-1 & II-2). Specifically, there was no difference in the range of acute toxicity for pre-treatment and post-treatment samples for the first three of the above manipulations (Table II-1). The data relating to the last two negative manipulations are presented in Table II-2. If either reducible compounds or cationic metals were responsible for an appreciable degree of the acute toxicity of surface tailings pond water, then treatment with increasing concentrations of sodium thiosulphate and EDTA respectively, would have resulted in an increase and subsequent decrease in the IT_{50}. This translates into a continuous decrease in the acute toxicity as the treatment would become

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applied in the generic sense to all TIEs. These labels can only be used after interpreting the results of all the sample-specific TIE manipulations within the context of the available chemical data for the specific sample.

Table II-1: Results for the Toxicity Identification Evaluation (TIE) manipulations that did not result in a reduction of the acute toxicity of surface tailings pond water assessed using the Microtox assay. (IC_{50} - median Inhibitory Concentration).

			IC_{50} (mL/L) **mean** (replicates)	
#	TIE manipulations*	pH	Pre-treatment	Post-treatment
1	BASELINE (without suspended solids)	~8	--	**265** (260,270)
2	without organic bases	11	**265** (200,330)	**265** (240,290)
4	without oxidizable & purgable compounds	~8	**290** (260,320)	**290** (290,290)
5	without purgable compounds	~8	**290** (260,320)	**285** (300,270)

* Throughout this paper, reference is made to the specific classes of compounds being removed by TIE manipulations. As a precautionary note, the reader should be aware that the following specific "labels" (organic acids, organic bases, non-polar organic compounds) **cannot** be applied in the generic sense to all TIEs. These labels can only be used after interpreting the results of all the sample-specific TIE manipulations within the context of the available chemical data for the specific sample.

Table II-2: The change in acute toxicity as measured by Microtox for surface tailings water subjected to two manipulations of the TIE. An increase and subsequent decrease in the IT_{50} with increasing sodium thiosulphate or EDTA concentrations would implicate reducible compounds or cationic metals respectively as contributing to the acute toxicity of surface tailings pond water. (IT_{50} - median Inhibitory Time, ↑ - increase, ↓ - decrease)

#	TIE manipulations	Dose (g/L) of $Na_2S_2O_3$ or EDTA added to 450 µL surface water/mL test sample	Post-treatment IT_{50} (min) **mean** (replicates)	Relative change in toxicity	Expected trend if punitive compounds present
7	varying degrees of reducible compounds (treatment with $Na_2S_2O_3$)	0.0	**11.9** (11.3,12.4)	--	--
		0.53	**12.4** (10.5,14.2)	0	↓
		1.05	**9.4** (8.8,10.0)	↑	↓
		1.58	**7.8** (7.7,7.9)	↑	↑
8	varying degrees of complexed (less toxic) cationic metals (treatment with EDTA)	0.0	**10.7** (12.6,8.7)	--	--
		0.04	**10.1** (12.2,8.0)	0	↓
		0.08	**7.6** (7.2,7.9)	↑	↓
		0.16	**9.7** (7.3,9.7)	0	↓
		0.32	**8.9** (9.6,8.1)	0	↑
		0.64	**8.4** (8.3,8.5)	↑	↑

more effective until such a point as the treatment itself begins to cause acute toxicity and the acute toxicity would then continue to increase with increasing concentration of sodium thiosulphate or EDTA. This trend or "positive result" was not observed, but instead the acute toxicity remained the same and then increased as the treatment presumably began to cause toxicity (Table II-2).

All of the acute toxicity of surface tailings pond water can be removed by either one of two treatments. The removal of organic compounds that have a non-polar component, by reverse phase solid phase extraction using C_{18} sorbent at pH 2.5, ~8 and 11, eliminated the acute toxicity of surface tailings pond water (Table II-3). In this manipulation the acute toxicity of the surface tailings pond water decreased from pre-treatment IC_{50} values of 205 mL/L, 265 mL/L, and 265 mL/L to IC_{50} values >1000 mL/L at pH 2.5, ~8 and 11 respectively (Table II-3). The removal of organic compounds that precipitate at pH 2.5 (organic acids) also eliminated the acute toxicity of surface tailings pond water (Pre-treatment IC_{50}: 205 mL/L vs. Post-treatment IC_{50}: >1000 mL/L) (Table II-3).

The acute toxicity of surface tailings pond water also changed with the degree of ionized/unionized compounds at various pH. For this manipulation of the TIE, the data indicate that the acute toxicity of the surface tailings pond water increases as the relative degree of unionized acids increases within the tolerable physiological range of the *V. fischeri* (Table II-3).

The acutely toxic fractions in fine tails interstitial waters

The acute toxicity of fine tails interstitial water can be completely removed by the same two treatments that removed all the acute toxicity of surface tailings pond water. The removal of organic compounds that have a non-polar component by C_{18} column extraction at pH 8 removed all the acute toxicity of fine tails interstitial water (Pre-treatment IC_{50}: 290 mL/L vs. Post-treatment IC_{50}: >1000 mL/L) (Table II-4). Similarly, the removal of organic compounds that precipitate at pH 2.5 (organic acids) from fine tails interstitial water eliminated the acute toxicity of the fine tails interstitial water (Pre-treatment IC_{50}: 265 mL/L vs. Post-treatment IC_{50}: >1000 mL/L) (Table II-4).

A portion of the acute toxicity of fine tails interstitial water was removed by sparging the sample with oxygen and nitrogen at pH ~8 (Table II-4). The

Table II-3: Results for the TIE manipulations that produced a reduction of the acute toxicity of surface tailings pond water assessed using the Microtox assay. A change in the acute toxicity, relative to the IC_{50} at the initial sample pH (~8), with pH ranging from 6 to 8.5 (TIE manipulation # 6) would indicate that compounds whose relative ionization changes within this pH range contribute to the acute toxicity of surface tailings pond water.

			IC_{50} (mL/L) **mean** (replicates)		% Change or (↓/0/↑)
#	TIE manipulations	pH	Pre-treatment	Post-treatment	
1	BASELINE (without suspended solids)	~8	--	**265** (260,270)	--
2	without organic acids	2.5	**205** (190,220)	**>1000** (>1000,>1000)	↓ 100
3	without non-polar organic compounds	2.5	**205** (190,220)	**>1000** (>1000,>1000)	↓ 100
		~8	**265** (260,270)	**>1000** (>1000,>1000)	↓ 100
		11	**265** (240,290)	**>1000** (>1000,>1000)	↓ 100
6	graduated pH tests	6	--	**250** (250)	↑
		7	--	**310** (310)	↑
		~8	--	**380** (380)	0
		8.5	--	**380** (380)	0

Table II-4: Acute toxicity of fine tails interstitial water assessed using the Microtox assay for six of the manipulations of the TIE. A ↓ indicates a decrease in acute toxicity with treatment.

			IC_{50} (mL/L) **mean** (replicates)		% Change
#	TIE manipulations	pH	Pre-treatment	Post-treatment	
1	BASELINE (without suspended solids)	~8	--	**290** (300,280)	--
2	without organic acids	2.5	**265** (250,280)	**>1000** (>1000,>1000)	↓ 100
3	without non-polar organic compounds	~8	**290** (300,280)	**>1000** (>1000,>1000)	↓ 100
4	without oxidizable & purgable compounds	~8	**290** (300,280)	**340** (440,240)	↓ 10
5	without purgable compounds	~8	**290** (300,280)	**390** (420,360)	↓ 15

acute toxicity of fine tails interstitial water decreased 10% by sparging the sample with oxygen to remove oxidizable and purgable compounds from solution (Table 4). Similarly, the acute toxicity of fine tails interstitial water was decreased 15% by sparging the sample with nitrogen to remove purgable compounds from solution (Table 4).

Validation of the Microtox results with *Daphnia* and rainbow trout tests

The reductions in acute toxicity with sample treatment, as measured with *Daphnia* and rainbow trout bioassays, parallel those obtained with Microtox. The acute toxicity of surface tailings pond water, as determined with *Daphnia* was eliminated with the removal of organic compounds that have a non-polar component by passage of the sample through a C_{18} column (Table II-5) (Pre-treatment LC_{50}: 760 mL/L vs Post-treatment LC_{50}: >1000 mL/L). Similiarly, the acute toxicity of surface tailings pond water as determined with *Daphnia* and rainbow trout, was eliminated with the removal of compounds that precipitate at pH 2.5 (Table II-5) (*Daphnia* Pre-treatment LC_{50}: 760 mL/L vs Post-treatment LC_{50}: >1000 mL/L) (Rainbow trout Pre-treatment LC_{50}: 110 mL/L vs Post-treatment LC_{50}: >1000 mL/L). As with the Microtox assay, the acute toxicity of Syncrude surface tailings pond water to rainbow trout increases as the pH decreases within the tolerable physiological range of rainbow trout (Table II-5).

Although the reductions in acute toxicity with sample treatment, as measured with the various organisms parallel each other, it is evident that major differences exist in the relative sensitivity of *V. fischeri*, *Daphnia* and rainbow trout to the toxic components of surface tailings pond water. Rainbow trout were much more sensitive (~3 times) than *V. fischeri* to surface tailings pond water (LC_{50} of 125 mL/L vs IC_{50} of 355 mL/L for *V. fischeri*) (Table II-6). Conversely, *Daphnia* were less sensitive (~0.4 times) to surface tailings pond water than *V. fischeri* (LC_{50} of 760 mL/L vs IC_{50} of 290 mL/L for *V. fischeri*)(Table II-6). Rainbow trout were approximately seven times more sensitive to surface tailings pond water than *Daphnia* when both LC_{50} values were standardized for a Microtox IC_{50} of 290 mL/L (LC_{50} of 105 mL/L for rainbow trout vs an LC_{50} of 760 mL/L for *Daphnia*).

Discussion

The acute toxicity of surface tailings pond water and fine tails interstitial

Table II-5: Acute toxicity as measured with *D. magna* and rainbow trout for surface tailings pond water subjected to three of the TIE manipulations. A dash (--) indicates tests that were not performed on these samples. A change in the acute toxicity, with pH ranging from 6.5 to 8.5 (TIE manipulation # 6) would indicate that compounds whose relative ionization changes within this pH range contribute to the acute toxicity of surface tailings pond water.

			D. magna			Rainbow trout		
			LC_{50} (mL/L) **mean** (replicates)		% Change	LC_{50} (mL/L) or LT_{50} (h) **mean** (replicates)		% Change or relative change
#	TIE manipulations	pH	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment	
1	BASELINE (without suspended solids)	~8	-	**760** (800,710,770)	--	--	**125** (120,130)	--
2	without organic acids	2.5	**800** (800)	**>1000** (>1000,>1000,>1000)	↓ 100	**110** (110)	**>1000** (>1000,>1000)	↓ 100
3	without non-polar organic compounds	~8	**760** (800,710,770)	**>1000** (>1000,>1000,>1000)	↓ 100	--	--	--
6	graduated pH tests	6.5	--	--	--	--	2.0 h	↑
		7.5	--	--	--	--	7.2 h	0
		8.5	--	--	--	--	168 h	↓

Table II-6: The comparative acute toxicity of surface tailings pond water to *V. fischeri*, *D. magna* and rainbow trout.

	Microtox vs. *D. magna*			Microtox vs Rainbow trout		
	IC_{50} or LC_{50} (mL/L) **mean** (replicates)		Microtox / *Daphnia*	IC_{50} or LC_{50} (mL/L) **mean** (replicates)		Microtox / Trout
Sample	Microtox	*D. magna*		Microtox	Rainbow trout	
BASELINE (without suspended solids)	**290** (270,250,345)	**760** (800,710,770)	0.38	**355** (355)	**125** (120,130)	2.8

water from Syncrude Canada Ltd., as determined by Microtox, can be eliminated in whole or in part by three treatments. Analysis of the physical and chemical characteristics that these treatments have in common yields useful information pertaining to the types of compounds responsible for the acute toxicity of oil sands wastewaters.

The majority of the acute toxicity of oil sands wastewaters can be explained by a class of compounds that have a non-polar component and an acidic polar component that precipitates at pH 2.5. The removal of organic compounds that have a non-polar component by C_{18} column extraction, regardless of the pH, eliminated the acute toxicity to *V. fischeri* and *D. magna* in surface tailings pond water and fine tails interstitial water (Tables 3-5). The removal of compounds that precipitate at pH 2.5 (organic acids) eliminated the acute toxicity of both wastewaters to *V. fischeri*, *D. magna* and rainbow trout (Tables 3-5). This overlap in the removal of toxicity between these two treatments indicates that the organic acids that precipitate at pH 2.5 must also have a non-polar component. The C_{18} column extraction removes simple non-polar organic compounds as well as surfactants, which have a molecular structure that includes both a polar end and a relatively large non-polar end (EPA, 1991). This evidence is consistent with the finding that the acid fraction that precipitates at pH 3 is composed of approximately 95% carboxylic acids (Zenon, 1986) with characteristics similar to naphthenic acids (Zenon, 1986; Morales *et al.*, 1993) and these naphthenic acids are naturally occurring surfactants that are leached from the oil sand when it is treated with sodium hydroxide in the hot water flotation method for extraction (Schramm *et al.*, 1984).

Moreover, these organic acids/surfactants must have a pKa such that a major shift toward their unionized (free) form occurs within the physiologically tolerable pH range (6-8.5) of *V. fischeri* and rainbow trout as demonstrated by a dramatic increase in the acute toxicity of surface tailings pond water as the pH is lowered (Tables 3 & 5). A similar relationship between the influence of pH (approaching the pKa) and the acute toxicity of two organic acids (oleic and linoleic acid) to rainbow trout has been demonstrated by Hrudey and Tookwinas (1982).

A portion of the acute toxicity was also removed from fine tails interstitial water with the removal of volatile organic compounds by sparging the samples with oxygen or nitrogen. Since approximately equal reductions in acute toxicity

occurred whether samples were sparged with oxygen or nitrogen it can be concluded that both manipulations were removing volatiles from solution rather than oxidizable compounds. Approximately 15% of the acute toxicity of fine tails interstitial water can be attributed to volatile compounds (Table II-4). The volatiles removed by these manipulations must also be non-polar organic compounds as they were removed by the C_{18} column. This is consistent with the effluent discharge to the pond. The tailings pond at Syncrude receives low molecular weight hydrocarbons (volatile non-polar organic compounds) that are used as diluent in the bitumen froth treatment and not recovered by the diluent recovery system (MacKinnon and Sethi, 1993). These light hydrocarbons enter the pond as a slurry, so that some is lost to the atmosphere and the remainder is trapped in the fine tails (MacKinnon and Sethi, 1993). This is supportive of our result that acutely toxic volatile non-polar organic compounds were present in fine tails interstitial water but not in surface tailings pond water. However, there is also an apparent contradiction in these data. All the acute toxicity of the interstitial water was removed with the precipitation of organic acids at pH 2.5 and some (~15%) was removed with the removal of volatiles, yet most organic acids are not known for their volatility. It is possible that the removal of volatile compounds in the treatment to remove organic acids was an artifact of centrifugation.

While the bioassay results with *Daphnia* and rainbow trout paralleled those for Microtox for the reductions in acute toxicity with sample treatment, the sensitivity of each species to surface tailings pond water differed dramatically. Rainbow trout were approximately three times more sensitive than *V. fischeri* and approximately seven times more sensitive than *Daphnia* to the acutely toxic compounds found in surface tailings pond water. Conversely, *Daphnia* were approximately 0.4 times less sensitive than *V. fischeri* to the acute toxicity of surface tailings pond water. These results demonstrate that the acute toxicity of wastewaters should not be tested with Microtox alone. At the very minimum, Microtox results indicating no acute toxicity should be validated with bioassays using other test organisms such as *Daphnia* and rainbow trout.

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Chapter III

Induction of ethoxyresorufin-O-deethylase activity in rainbow trout (*Oncorhynchus mykiss*) as a result of exposure to diluted wastewater from oil sands extraction

Introduction

The safe disposal of diluted industrial wastewaters requires the assessment of potential physiological effects as a result of chronic exposure to sublethal concentrations of organic compounds.

Syncrude Canada Ltd., located in northeastern Alberta, Canada, (~400 km north of Edmonton), separates oil from oil sand using the hot water flotation method. This results in the production of large volumes of fluid wastes (process-affected water and a relatively stable suspension of solids and unrecovered bitumen called fine tails) (MacKinnon, 1989) which must eventually be reclaimed (Boerger *et al.*, 1992). These fluid wastes, which are initially acutely toxic to aquatic organisms (MacKinnon and Retallack, 1982; MacKinnon and Boerger, 1986) will be diluted in the final disposal habitat (Boerger *et al.*, 1992). The majority (85-100%) of the acute toxicity of these fluid wastes is due to a group of organic acidic surfactants (Verbeek *et al.*, 1994) that have characteristics similar to naphthenic acids (Zenon, 1986; Morales *et al.*, 1993). To monitor the safe disposal of these fluid wastes, a sensitive method for the assessment of the effects of chronic exposure to sublethal concentrations of these organic acidic surfactants or other possible organic compounds in oil sands wastewater is required.

The most useful method of assessing the potential response due to chronic exposure to sublethal concentrations of a wide variety of compounds is probably through the use of the cytochrome P450 mixed-function oxygenase (MFO) system. The primary function of the MFO system is the conversion of mostly lipophilic (fat-loving), charged or non-charged (Porter and Coon, 1991), xenobiotics (foreign, biologically active compounds) as well as endogenous compounds (found naturally in the body) to more polar metabolites that are readily excreted from the body (Brattsten *et al.*, 1977). Most of the more than 200,000 anthropogenically generated "environmental chemicals" are thought to be potential substrates for the cytochrome P450 MFO system (Porter and Coon, 1991). In this capacity, cytochrome P450 has been called the most versatile

biological catalyst known (Porter and Coon, 1991).

The cytochrome P450 gene superfamily encodes over 150 isoforms of the enzyme, of which there can be >30 in a single organism (Guengerich, 1991) that catalyze many chemical reactions which attack tens of thousands of substrates (Porter and Coon, 1991). The cytochrome P450 system catalyzes aromatic and aliphatic hydoxylations and epoxidations, peroxygenations, N-, and O-dealkylations, deaminations, desulfurations, dehalogenations, nitrogen and thioether oxidations, as well as reductions (Brattshen *et al.*, 1977; Nebert and Gonzalez, 1987; Porter and Coon, 1991). The substrates for these cytochrome P450 mediated reactions include a host of xenobiotics including various natural toxins (drugs including antibiotics, carcinogens, antioxidants, solvents, anesthetics, dyes, pesticides, petroleum products, alcohols, odorants and secondary plant metabolites such as aldehydes, terpenoids, steroids, N-heterocyclics and alkaloids) as well as numerous endogenous compounds (steroids, fatty acids, prostaglandins, eicosanoids, leukotrines, biogenic amines, pheromones, lipid hydroperoxides, retinoids, acetone, and acetol) (Brattsten *et al.*, 1977; Guengerich, 1991; Nebert and Gonzalez, 1987; Porter and Coon, 1991). The substrates range in size from that of ethylene (MW 28) to that of cyclosporin A (MW 1201) (Guengerich, 1991). Given this versatility it is understandable that the cytochrome P450 MFO system of fish has been extensively studied for use as an environmental indicator of water pollution (Jimenez and Stegeman, 1990).

The cytochrome P450 gene superfamily is comprised of at least 27 gene families of isoforms, 10 of which are believed to exist in all mammals and are classified using a system based on similarity of their amino acid sequence (Nebert *et al.*, 1991; Nebert and Gonzalez, 1987). The numbering of the gene families using this classification system starts with the gene families that function to metabolize drugs and ends with the gene families that are specifically responsible for the metabolism of endogenous compounds (Nebert and Gonzalez, 1987). The gene families cytochrome P450-11(CYP11), CYP17, CYP19 and CYP21, which are found in two cellular locations, the mitochondria and the microsomes of the endoplasmic reticulum (Nelson and Strobel, 1987), produce P450 isoforms that carry out functions that are critical to the survival of an organism such as the anabolism of steroids (Guengerich, 1991; Nebert and Gonzalez, 1987; Nebert *et al.*, 1991). Both the mitochondrial and microsomal

P450 isoforms in the gene families 11, 17, 19 and 21 are fairly selective in their choice of substrates (Porter and Coon, 1991; Nebert *et al.*, 1991). The gene families CYP1 through CYP4 produce microsomal P450s that metabolize complicated secondary plant metabolites such as flavones, coumarin derivatives and terpenes as well as numerous drugs and synthetic and endogenous steroids and fatty acids (Nebert and Gonzalez, 1987). In addition, the CYP4A1 gene (Nebert *et al.*, 1991), previously called the P450LM2 gene, appears to be normally responsible for the ω-hydroxylation of fatty acids in mammals (Nebert and Gonzalez, 1987) and fish (Williams *et al.*, 1984). Unlike the P450 enzymes in the gene families 11, 17, 19 and 21, the enzymes in the CYP1 through 4 gene families have unusually broad and overlapping substrate specificity (Porter and Coon, 1991). Furthermore, the individual isoforms in liver microsomes can catalyze all types of reactions depending on the substrate presented (Guengerich, 1991; Nebert *et al.*, 1991; Porter and Coon, 1991). Among this second group of gene families are the isoforms involved in enzymatic assays commonly used as environmental indicators of chronic exposure of fish to water pollution (Jimenez and Stegeman, 1990).

The major xenobiotic metabolizing P450 gene families (1 & 2) have been further classified based on their specificity of induction by two types of substrates, exemplified by phenobarbitol (PB) and the 3-methylcholanthrene (3-MC) a polycyclic aromatic hydrocarbon (Nelson and Strobel, 1987). P450 isoforms that exhibit PB-type induction (2B subfamily and several in the 2C subfamily) (Nebert and Gonzalez, 1987) are able to metabolize a large variety of substrates (Lech *et al.*, 1982). PB-type induction does not appear to be very common in fish yet its presence has been suggested in some species (Pohl *et al.*, 1974; Burns, 1976). P450 isoforms that exhibit a preference for potent 3-MC -type induction (1A1, 1A2 isoforms & 2A subfamily) metabolize only a limited number of compounds, mostly polycyclic aromatic hydrocarbons (Lech *et al.*, 1982). In the "fish" literature, the CYP1A1 gene, previously called the P450LM4 gene (Williams and Buhler, 1983), is also said to exhibit β-naphthoflavone (βNF) -type induction as this gene was first purified in fish in rainbow trout that had been treated with βNF (Williams and Buhler, 1983). It is this 3-MC or βNF-type MFO induction that has been the subject of widespread investigation in fish in response to chronic exposure to sublethal concentrations of industrial wastewaters.

Cytochrome P450 MFO activity can be measured using several different substrate assays, one of which is the ethoxyresorufin-O-deethylase (EROD) assay. The EROD assay is one of the most commonly used assays for MFO activity as the product of the reaction, resorufin, is known so the reaction can be followed easily and reliably (Klotz *et al.*, 1984). EROD activity is considered indicative of 3-MC (Lech *et al.*, 1982) or βNF-type induction specifically by the CYP1A1 isoform (Hodson *et al.*, 1991). However EROD activity has also been demonstrated by the CYP4A1 isoform (old name - P450LM2), after induction by βNF (Williams and Buhler, 1983).

In this paper, the cytochrome P450 MFO system was assessed for its potential as an indicator of sublethal exposure of rainbow trout to oil sands tailings pond water. Specifically, the laboratory concentration-response relationship and time course to induction were determined using EROD activity as the catalytic measure of MFO activity. In addition, evidence is presented here that suggests that while EROD has been considered indicative of 3-MC or βNF-type induction by the CYP1A1 and the CYP4A1 isoforms, EROD may also serve as a substrate in response to induction by organic acidic surfactants.

Materials and Methods

Sample collection and storage

Surface tailings pond water was obtained from the edge of the Syncrude tailings pond, approximately 150 m from the nearest area of fresh discharge, on Jan 27, 1993. Tailings pond water was collected from 15-100 cm below the surface of the pond in large (~20 L) polyethylene jugs. The chemical characteristics of the water at this sampling station are consistent with those of water found anywhere else in the pond except in the immediate vicinity of discharge (MacKinnon, pers. comm.). Sample jugs of tailings water (~5°C) were held at approximately 10°C until they could be shipped. They were shipped the same day at ≤ 6°C to Edmonton. Upon arrival samples were stored at ≤ 6 °C until used. Samples were stored for a maximum of four months.

Test fish: source and maintenance

Immature rainbow trout (*O. mykiss*) (Total weight = 134.5 ± 3.4 g, Gonadosomatic Index (GSI)[1] = 0.71 ± 0.23 %)(Mean ± 1SE) were purchased

[1] GSI = gonad weight / gutted weight * 100. A small GSI, (<1 %) is indicative of sexual

from Circle M Trout Farm (St. Paul, AB) and transferred to a holding tank in the aquatic facilities of the Department of Zoology, University of Alberta. Fish were held at 14 ± 2°C in a 1000 L fiberglass aquarium at the University of Alberta. The holding tank received a continuous flow of municipal tap water that had been dechlorinated with sodium thiosulphate (hardness = 100-140 mg/L as $CaCO_3$, conductivity = 200-300 μS/cm, pH = 6.0-8.0). Holding tanks were illuminated with fluorescent lighting under ambient photoperiod for Edmonton between January and May. Fish were fed trout chow (Ranken Starter No. 1) once daily in the morning at a rate of approximately 2% total body weight in the tank. Approximately 5% mortality occurred following transfer of the fish to the University, but after this, fish appeared to be in good health and <1% mortality occurred. Holding tanks were flushed and disinfected with dilute potassium permanganate monthly. Fish were allowed to acclimate to the holding conditions for at least 2 weeks prior to exposure to tailings pond water.

Chemicals

The 7-ethoxyresorufin (7-ER) was obtained from Molecular Probes Inc., Eugene, Oregon. βNF, glycerol, NADPH, dithiothreitol, bicinchoninic acid (BCA) sodium salt solution, copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) and bovine serum albumin (BSA) were obtained from Sigma.

Rainbow trout LC_{50}

A 96 h static rainbow trout acute toxicity test (EPS, 1990) was determined for surface tailings pond water in order that the exposure concentrations for subsequent MFO tests might be expressed as a fraction of the LC_{50}, that is in terms of Toxic Units (TU). This procedure was used to normalize exposure concentrations for any detoxification over the 5 month period in which the MFO experiments were run. This procedure was based on the assumption that the compounds that induced EROD would detoxify at the same rate as the acute toxicants. Its use was not, however, based on the assumption that the inducers of EROD should have an acute effect. For the LC_{50}, a preliminary range finding test was run with one fish/concentration using the following test concentrations (15, 30, 60, 125 and 250 mL/L). Subsequently, a definitive test was run with the following five concentrations (33.4, 40, 43.3, 50 and 60 mL/L) of surface tailings

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imaturity.

pond water were tested in addition to a control consisting of dilution water (dechlorinated tap water from the University of Alberta). Immature rainbow trout (Total weight = 107.3 ± 5.5 g) of either sex were equally and randomly distributed into the six 150 L test tanks (8 fish per tank). Three times daily, 100 L of the water was changed for a loading density of 2.75 ± 0.14 g/L/day. There were no mortalities in the controls. The test was performed under constant temperature (14 ± 2 °C) and constant aeration (0.33 mL/min/L). The fish were fed trout chow (Ranken Starter No. 1) once daily in the morning at approximately 2% total body weight/tank. Excess food and solid waste was removed from the test tanks approximately 8 h after the fish were fed. Test tanks were observed at 6, 12, 24, 36, 48, 60, 72, 84 and 96 h for mortality. Mortality was defined as the lack of opercular movement for 10 sec and the lack of movement in response to gentle prodding. Dead fish were removed from the test tanks, and the time removed, weight and length of each fish was recorded. At the end of the test, all surviving fish were sacrificed and the weight and length of each was recorded. The 96 h median lethal concentration (LC_{50}) and its 95 percent confidence interval was calculated using the probit method on a PC BASIC program developed by Stephan (1977).

The Microtox assay

The Microtox[2] assay was run in conjunction with the rainbow trout LC_{50} and prior to each exposure of rainbow trout for MFO analysis. With this procedure, the exposure concentrations for the MFO tests could be set in terms of Toxic Units without the necessity of repeating a rainbow trout LC_{50} prior to each MFO experiment. Microtox assays were performed as described in Ribo and Kaiser (1987) at a pH of 7.5. In the Microtox assay, equal concentrations of *V. fischeri*, a light emitting bacterium, were held in each of five 3 mL test tubes filled with 500 µL of control water (2% NaCl in distilled water) at 15 ± 1°C for 15 min. After 15 min, the maximal light output of the bacterial population in each test tube was measured. The bacteria were then exposed for 15 min to 450.0, 225.0, 112.5 and 56.25 mL/L of the test sample in addition to a dilution control. After the 15 min of exposure, the bacterial light output was measured again and the IC_{50} of the test sample was calculated using the Microbics Corporation PC BASIC computer program. This program calculates the median inhibitory

[2] Microtox is a registered trademark of Microbics Corporation.

concentration and its 95 percent confidence interval using interpolation based on simple linear regression of the log concentration vs the percent light reduction.

In addition to the standard control used in the Microtox assay, phenol standards (100 mg/L) were used as a positive control to assess the relative sensitivity of each lot of bacterial reagent.

Dose response for MFO induction using a 48 h exposure

Immature rainbow trout (Total weight = 142.1 ± 5.0 g, GSI = 0.99 ± 0.42 %) of either sex were equally and randomly distributed into seven 150 L test tanks (2 fish per tank). Once daily, 50% of the water was changed for a loading density of 2.53 ± 0.09 g/L/day. Fish were exposed to 0.8, 0.6, 0.4, 0.3, 0.2 and 0.15 TU, of surface tailings pond water. Dechlorinated tap water was used as the dilution water and control water. Control fish were held under the same loading density and handled in the same manner as test fish. The test was performed under constant temperature (14 ± 2 °C) and constant aeration (0.33 mL/min/L). Fish were fed trout chow (Ranken Starter No. 1) once daily in the morning at approximately 2% total body weight/tank. Fish were sacrificed in a random order after 48 h of exposure. The experiment was repeated until an n value of 6-8 was achieved for each dosage. Each time the experiment was repeated the tank used for each dosage was randomized.

Time course for MFO induction at 0.6 TU

Immature rainbow trout (Total weight = 115.9 ± 4.6 g, GSI = 0.43 ± 0.30 %) of either sex were equally and randomly distributed into four 150 L test tanks (2 fish per tank). Once daily, 50% of the water was changed for a loading density of 2.06 ± 0.08 g/L/day. Fish were exposed to 0.6 TU of surface tailings pond water. Dechlorinated tap water was used as the dilution water. In addition, two control fish were sampled prior to the exposures. The test was performed under constant temperature (14 ± 2 °C) and constant aeration (0.33 mL/min/L). Fish were fed trout chow (Ranken Starter No. 1) once daily in the morning at approximately 2% total body weight/tank. Fish were sacrificed, one from each tank, at 9, 24, 48 and 72 h. The experiment was repeated until an n value of 5-6 was achieved for each exposure time.

Time course for MFO induction at 0.3 TU

Immature rainbow trout (Total weight = 144.1 ± 7.7 g, GSI = 0.52 ± 0.34 %) of either sex were equally and randomly distributed into four 150 L test tanks (4 fish per tank). Twice daily, 50% of the water was changed for a loading density of 3.71 ± 0.20 g/L/day. Fish were exposed to 0.3 TU of surface tailings pond water. Dechlorinated tap water was used as the dilution water. In addition, four control fish were sampled prior to the exposures. The test was performed under constant temperature (14 ± 2 °C) and constant aeration (0.33 mL/min/L). Fish were fed trout chow (Ranken Starter No. 1) once daily in the morning at approximately 2% total body weight/tank. Fish were sacrificed, one from each tank, at 9, 24 and 48 h as well as at 14 days.

Preparation of βNF positive control fish

Four fish were injected with 0.5 mg/kg βNF (*i.p.*) dissolved in corn oil as described by Miller *et al.* (1989). After 48 h, the fish were sacrificed, and their tissues and microsomes were prepared as a batch (Hodson *et al.*, 1991). Microsomes were frozen in cryovials in liquid nitrogen and they were thawed as needed to analyze alongside each set of samples in the subsequent assays.

Tissue preparation

Fish were sacrificed by cutting their spinal cord. Wet weights were determined. Livers were excised, rinsed in 0.15 M KCl (Hodson *et al.*, 1991), weighed, placed in plastic bags, labeled and frozen in liquid nitrogen. The sex of the fish was determined if possible and the gonads were removed and weighed. The GSI was calculated as in Hodson *et al.*, 1991.

Microsomal preparation

Microsomes were prepared as described by Hodson *et al.* (1991). Livers were weighed, minced in a cold room at 4 °C, and homogenized while on ice in 4 volumes KCl-HEPES buffer (0.15 M KCl, 0.02 M HEPES, pH 7.5) (HEPES = N-2-Hydroxethyylpiperazine-N'-2-ethanesulphonic acid) using a Dounce hand held glass-glass tissue grinder. The microsomal preparation was isolated from a 9000 x *g* supernatant fraction (30 min, 1 °C) by further centrifugation at 100,000 x *g* for 75 min at 1 °C. Microsomal pellets were resuspended in 1 volume tris-glycerol buffer (50 mM Tris-HCl, pH 7.4, 1 mM dithiothreitol, 1 mM EDTA, 20%

glycerol v/v) /g of liver. Glycogen sediment, which precipitates as a separate layer under the microsomal pellet was not included in the resuspensions.

Spectrophotometric EROD assay for catalytic activity

EROD activity was determined using the method of Klotz *et al.* (1984) as described in Hodson *et al.* (1991). Briefly, 20 μL of microsomal sample was added to 960 μL of 7-ER/EROD buffer solution, which consisted of 2.0 ± 0.1 μM 7-ER in EROD buffer (0.1 M Tris-HCl, pH 8.0, 0.1 M NaCl). This mixture was incubated for 2.5 min at 25 °C. Following this incubation, 20 μL of a 25 mg/L NADPH solution was added and the change in absorbance, due to resorufin formation, was determined at 572 nm. Sample absorbance was monitored continuously for 3.5 min at 25 °C. The change in sample absorbance over time was used to calculate enzyme activity as described by Hodson *et al.* (1991). The spectrophotometric reference was a sample with EROD buffer, without microsomal extract or NADPH. In addition to samples, the positive control (0.5 mg/kg βNF), prepared as described by Miller *et al.* (1989), with known high enzyme levels was run with each set of samples.

Protein determination

Protein concentration was determined using the bicinchoninic acid (BCA) method of Smith *et al.* (1985). Briefly, 2.5 μL of microsomal extract, 2.5 μL tris-glycerol buffer, and 45 μL distilled water was added to 1.0 mL of standard assay reagent. The standard assay reagent consisted of 100 volumes reagent A (1% BCA-Na_2, 2% $Na_2CO_3 \cdot H_2O$, 0.16% Na_2 tartrate, 0.4% NaOH, and 0.95% $NaHCO_3$ in distilled water) and 2 volumes of reagent B (4% $CuSO_4 \cdot 5H_2O$ in distilled water). The microsomal extract/standard assay reagent mixture was incubated for 2.0 h at room temperature. Following this incubation, the sample absorbance was determined at 562 nm. The spectrophotometric reference was a sample with standard assay reagent, but without microsomal extract, tris-glycerol buffer or distilled water added. In addition to this reference, a reagent blank was run with samples to determine the interference by the tris-glycerol buffer. The reagent blank consisted of 5.0 μL tris-glycerol buffer and 45 μL distilled water added to 1.0 mL standard assay reagent. Protein standards of bovine serum albumin (BSA) prepared in a tris-glycerol buffer/distilled water solution (3-36 mg/mL) were run with each set of samples.

Statistical analysis

For each of the three experiments, EROD activity vs concentration or time was plotted to visually assess the concentration-response and time-courses respectively. Significant differences between concentrations or times were assessed using one-way ANOVAs. The point of maximal induction was determined using the Tukey's test for multiple comparisons. Significant differences between the control and treatment means were determined using the Bonferroni's test at $\alpha = 0.05$. The no observed effect concentration (NOEC) or time (NOET) and the lowest observed effect concentration (LOEC) or time (LOET) were assigned on the basis of these results.

Results

Rainbow trout LC_{50}

The 96h rainbow trout LC_{50} for surface tailings pond water was 52 mL/L (95% Confidence Interval: 45-84; slope: 6.36) at the start of the 5 month period of sample usage. This LC_{50} value was based on the following mortalities after 96 h (33.4 mL/L - 1, 40 mL/L - 2, 43.3 mL/L - 3, 50 mL/L - 3 and 60 mL/L - 6). In the preliminary range finding test, all concentrations above 60 mL/L had 100% mortality and all concentrations below 33.4 mL/L had 0% mortality. At the end of the 5 month period the estimated LC_{50}, based on Microtox results was 70 mL/L.

Dose response for MFO induction

While there was an obvious concentration-response (Figure III-1), the theoretically linear ln EROD-concentration relationship was poor with the inclusion of all data (r^2=0.396). The ln EROD transformation was also required in order to validate one of the more important assumptions of regression analysis, homogeneity of the variance. This transformation did not change the r^2 value significantly (untransformed = 0.391, transformed = 0.396). When outlying EROD values[3] were removed (Table III-1), data which corresponded to fish that were observed to be under gross acute stress such as heavy scale loss,

[3] Two types of outying data existed. The first corresponded to individual fish that died at the 0.6 and 0.8 TU exposures and their EROD activities were not determined. The second type of outlying EROD data corresponded to fish that were observed as stressed in a gross acute manner at the time of sampling. All of these living yet grossly stressed fish had low EROD activities.

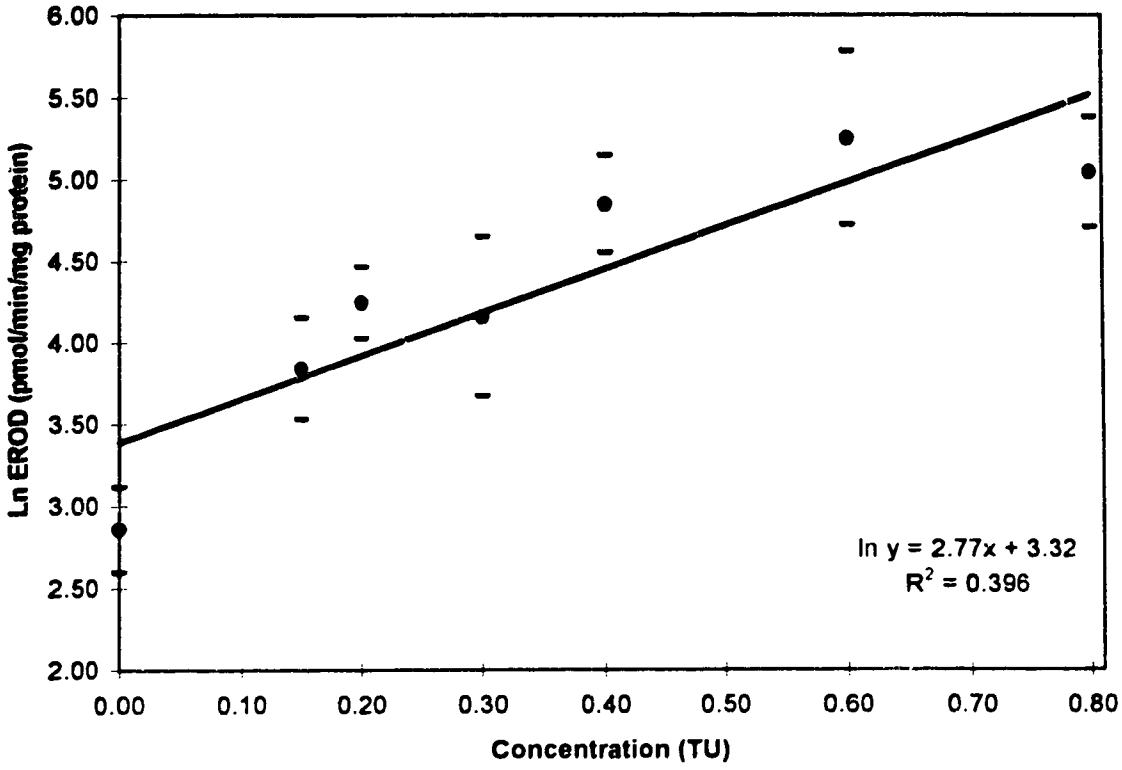

Figure III-1: LnEROD activity in rainbow trout in response to various concentrations of oil sands tailings water. Data shown are means ± 1SE, before the rejection of outliers.

Table III-1: Ethoxyresorufin-O-deethylase (EROD) activity in response to various concentrations of oil sands tailings water as compared to EROD activity in response to 0.5 mg/kg β-naphthoflavone (βNF)(*i.p.*) as the positive control.

Conc (TU)	EROD activity (pmol/min/mg protein)								n	mean	SD	±1SE
0	12	24	27	5.7	42	26	6.5	27	8	21	12	17-25
0.15	35	11	67	76	74	70			6	56	26	45-66
0.2	131	76	31	106	76	46			6	78	40	63-93
0.3	9.4	81	76	183	28	230			6	101	87	66-137
0.4	70[b]	389	64[b]	161	119				5	161	134	101-220
0.6	14[a]	354	368	266	440	224			6	278	150	216-339
0.8	62[b]	385	61[b]	115	290	283			6	199	138	143-256
βNF	1710	1780	1740	1670	1720	1790			6	1735	47	1716-1754

a This test individual displayed gross acute stress, defined as moderate to heavy scale loss and fin fray.
b These test individuals displayed the above characteristics in addition to disoriented/upside down swimming behavior.

fin fray, and disoriented upside-down swimming behaviour, the relationship improved dramatically (Figure III-2). After the rejection of EROD data corresponding to acutely stressed fish, the relationship (ln EROD = 3.68conc. + 3.20) had an r^2 value of 0.593 (Figure III-2).

Visually, maximal induction was reached with exposure to 0.6 TU of oil sands tailings water (Figure III-3) at the 48 h exposure period, for all data at 278 ± 31 pmol/min/mg protein (± 1SE)(Table III-1), and for data exclusive of outliers at 330 ± 38 pmol/min/mg (Figure III-4). The maximal induction for the positive control, 0.5 mg/kg βNF (*i.p.*), was 1735 ± 19 pmol/min/mg protein (Table III-1). EROD values for maximal induction by exposure to oil sands tailing water, determined visually, were one sixth (all data) to one fifth (excluding outliers) those of 0.5 mg/kg βNF (*i.p.*).

Statistically, there was no significant difference between the EROD activity at 0.4, 0.6 and 0.8 TU either before (ANOVA - Tukey's test, $\alpha = 0.05$, 7,36) or after ($\alpha = 0.05$, 7,31) the rejection of outliers. Maximal induction was reached with exposure to 0.4 TU at a grand mean and SE of 216 ± 34 pmol/min/mg before the rejection of outliers and 283 ± 32 pmol/min/mg after the rejection of outliers. EROD values for maximal induction by exposure to oil sands tailings water, determined statistically, were one eighth (all data) to one sixth (excluding outliers) those of 0.5 mg/kg βNF (*i.p.*).

Visually, induction appeared slightly lower at 0.8 TU than at 0.6 TU before (Figure III-3) and after (Figure III-4) the rejection of outliers, but this difference was not statistically significant either before (ANOVA - Tukey's test, $\alpha = 0.05$, 7,36) or after ($\alpha = 0.05$, 7,31) the rejection of outliers.

The LOEC and the NOEC did not differ before and after the rejection of outliers. The LOEC that induced EROD activity was 0.2 TU (ANOVA - Bonferroni's one-tailed test, $\alpha = 0.05$, 6,36 and $\alpha = 0.05$, 6,31). The highest NOEC that did not induce EROD activity was 0.15 TU (ANOVA - Bonferroni's one-tailed test, $\alpha = 0.05$, 6,36 and $\alpha = 0.05$, 6,31).

EROD induction in the Short time course study

Visually, maximal EROD induction to 0.6 TU of oil sands tailings water was reached after 48 h of exposure (Figure III-5) for all data at 278 ± 31 pmol/min/mg (Table III-2) and for data exclusive of outliers at 330 ± 38 pmol/min/mg (Figure III-6).

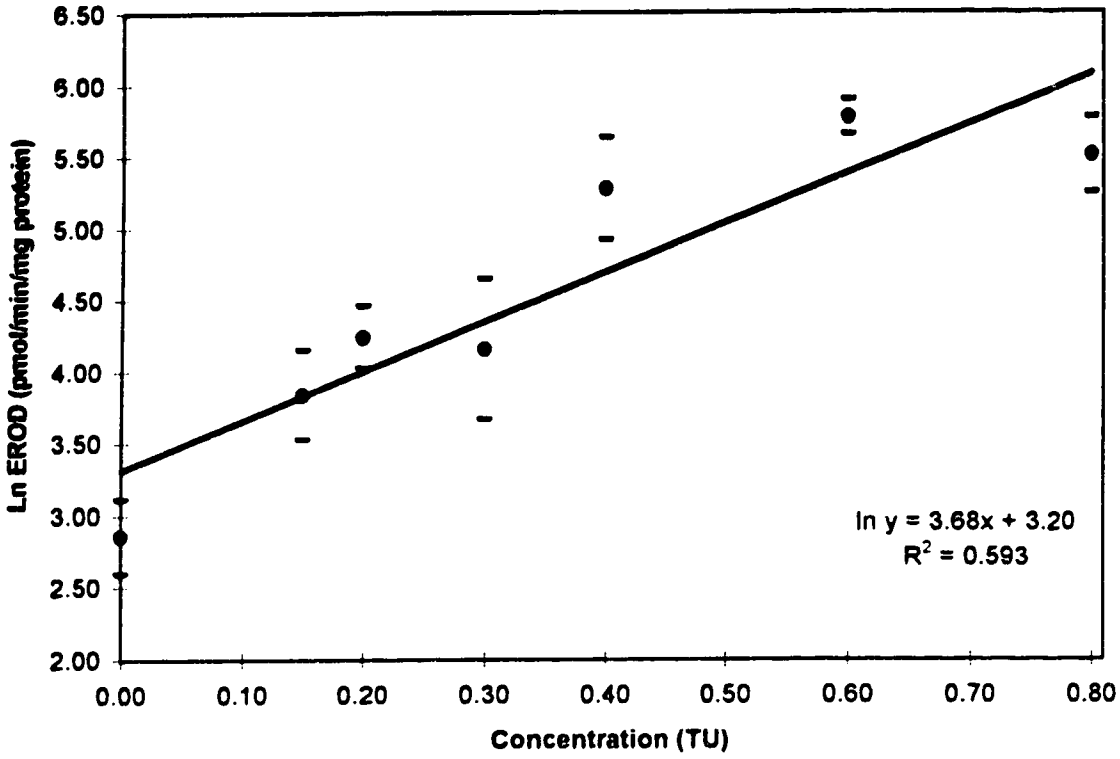

Figure III-2: LnEROD activity in rainbow trout in response to various concentrations of oil sands tailings water. Data shown are means ± 1SE, after the rejection of outliers.

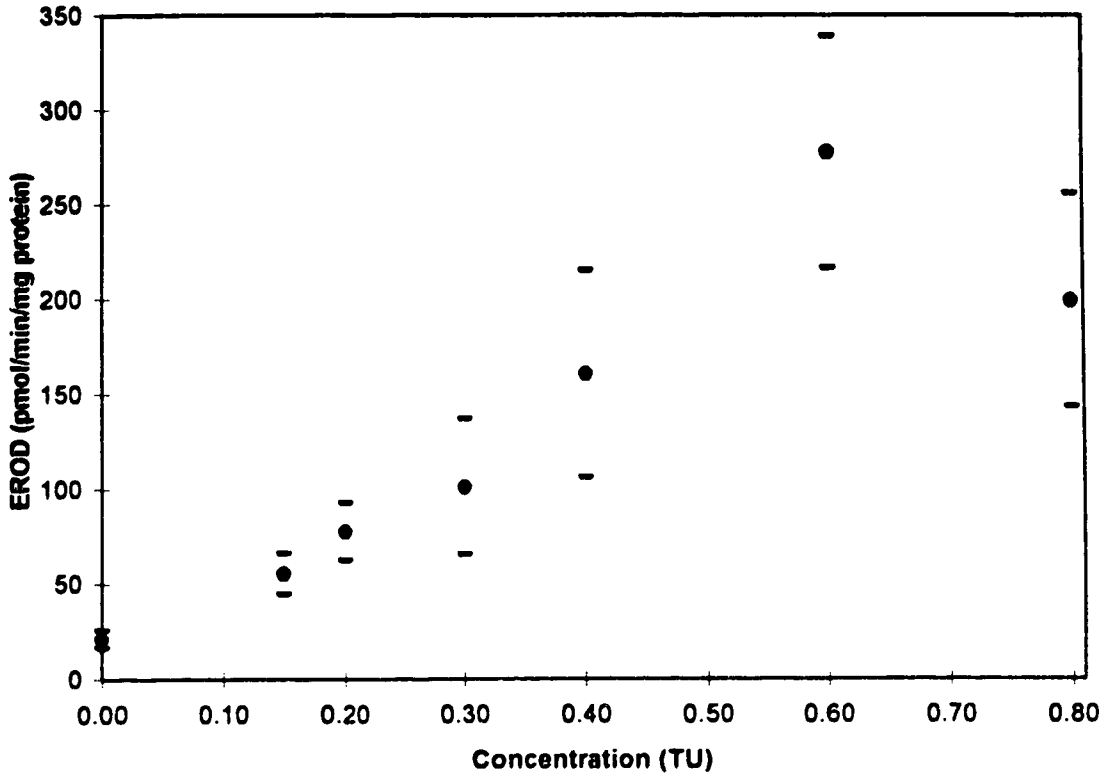

Figure III-3: EROD activity in rainbow trout in response to various concentrations of oil sands tailings water. Data shown are means ± 1SE, before the rejection of outliers.

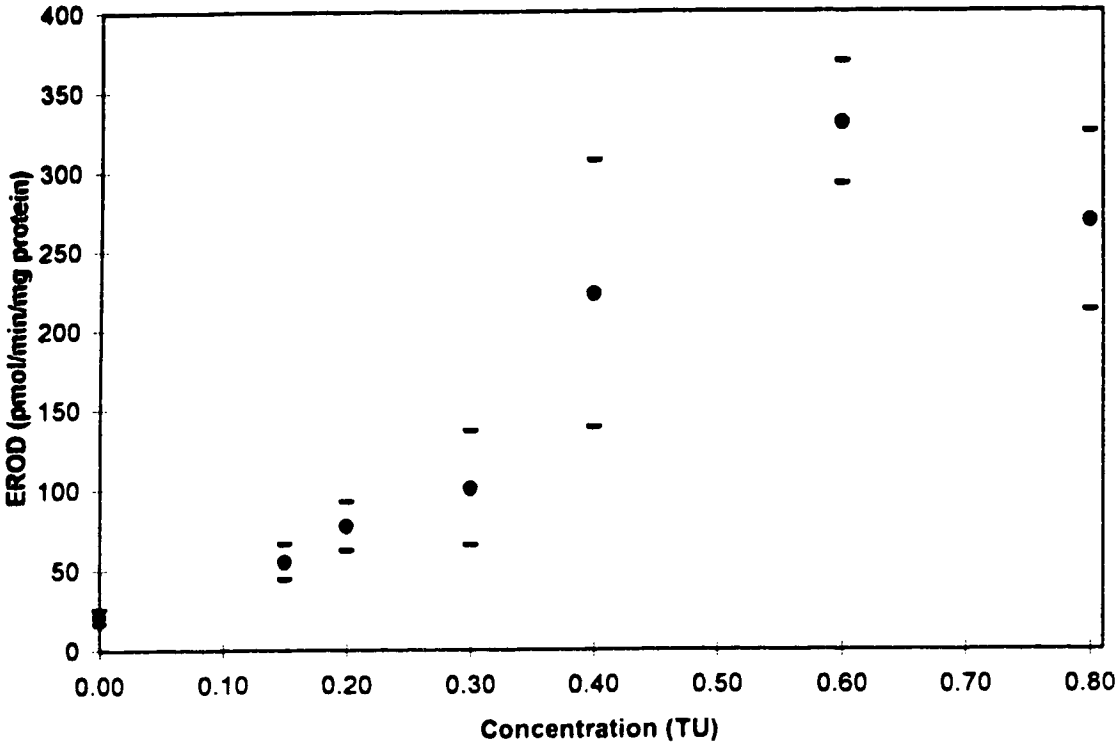

Figure III-4: EROD activity in rainbow trout in response to various concentrations of oil sands tailings water. Data shown are means ± 1 SE, after the rejection of outliers.

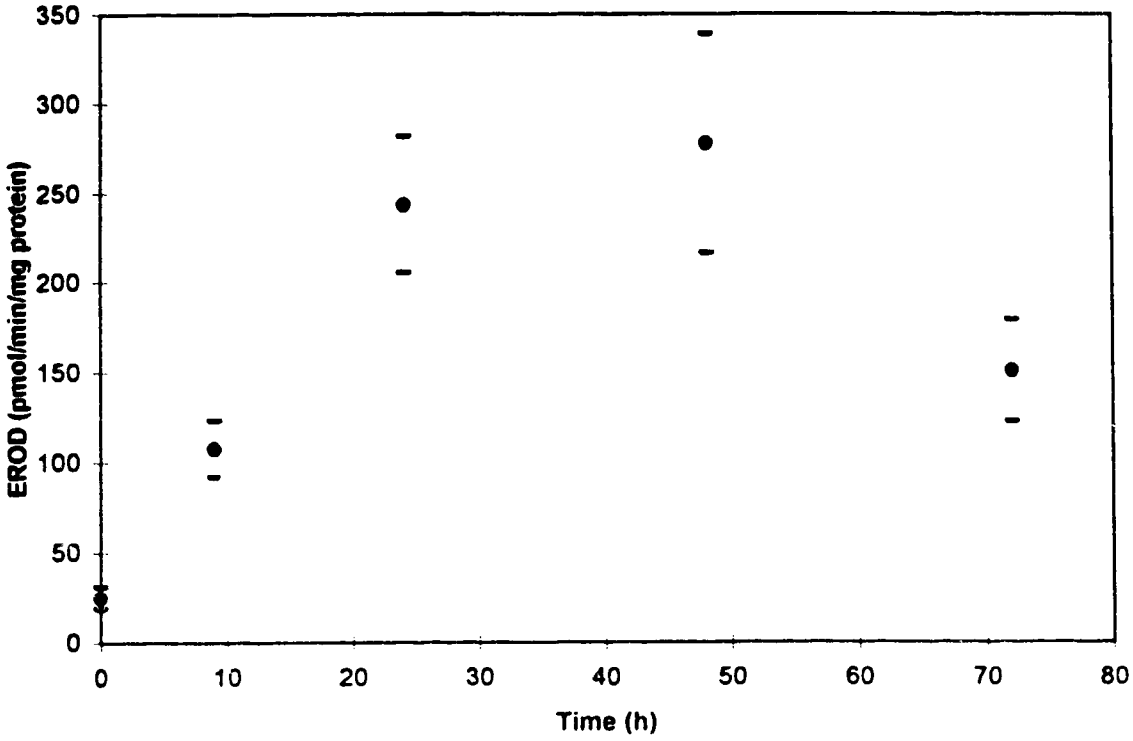

Figure III-5: EROD activity in rainbow trout exposed for various times to 0.6 TU of oil sands tailings water. Data shown are means ± 1 SE, before the rejection of outliers.

Table III-2: EROD activity in rainbow trout exposed to 0.6 T U of oil sands tailings water for various times as compared to EROD activity in response to 0.5 mg/kg βNF (*i.p.*) after 72 h.

Time (h)	EROD activity (pmol/min/mg protein)						n	mean	SD	±1SE
0	20	30	39	5.3	14	42	6	25	14	19-31
9	106	81	68	154	130		5	108	35	92-124
24	142	202	236	373	264		5	243	86	205-282
48	14[a]	354	368	266	440	224	6	278	150	216-339
72	23[a]	150	228	187	153	164	6	151	69	123-179
βNF							6	1735	47	1716-1754

a These test individuals displayed gross acute stress, defined as moderate to heavy scale loss and fin fray.

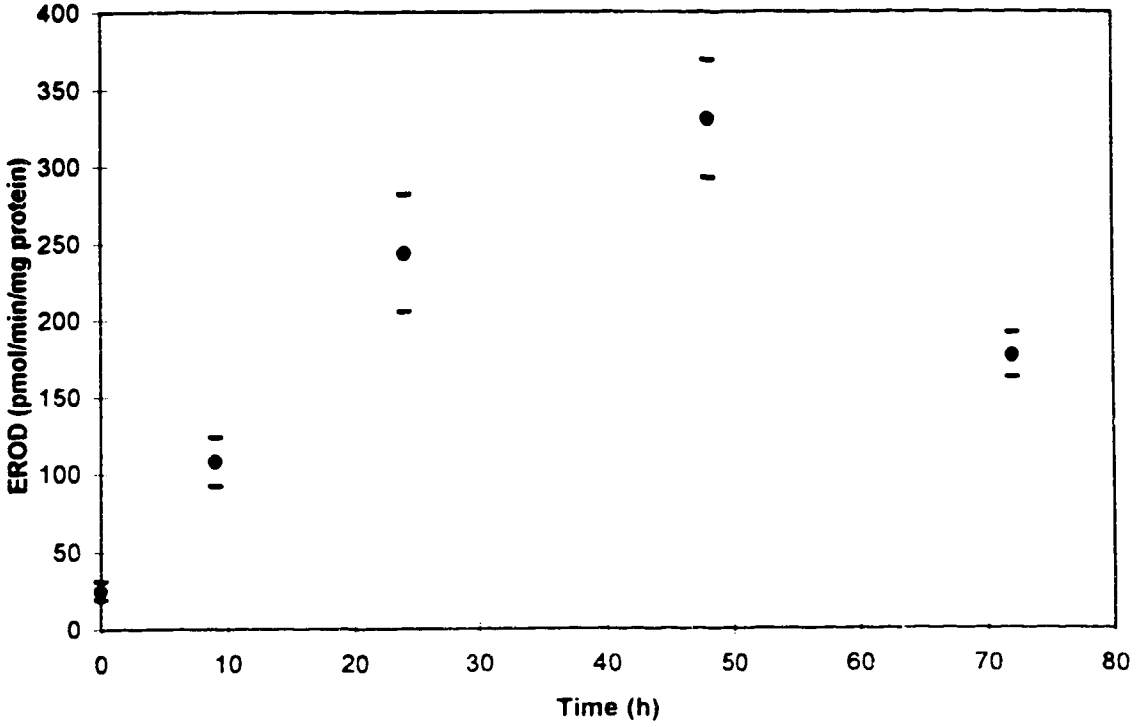

Figure III-6: EROD activity in rainbow trout exposed for various times to 0.6 TU of oil sands tailings water. Data shown are means ± 1 SE , after the rejection of outliers.

Statistically, there was no significant difference between the ln EROD activity after 24, 48 and 72 h before the rejection of outliers (ANOVA - Tukey's test, α = 0.2, 2,14), but there was a significant difference between the maximal induction after 48 and 72 h after the rejection of outliers (ANOVA - Tukey's test, α = 0.05, 2,12). Statistically, maximal induction was reached with exposure to 0.6 TU after 24 h at a grand mean and SE of 262 ± 36 pmol/min/mg before the rejection of outliers and 287 ± 29 pmol/min/mg after the rejection of outliers using 24 and 48 h EROD values.

The LOET and the NOET did not differ before and after the rejection of outliers. The LOET that induced EROD activity at 0.6 TU was 9 h (ANOVA - Bonferroni's one-tailed test, α = 0.05, 4,23 and α = 0.05, 4,21). The highest NOET that did not significantly induce EROD activity at 0.6 TU was not determined but it is <9 h.

EROD induction in the Long time course study

Visually, maximal induction to 0.3 TU of oil sands tailings water was reached after 1 day of exposure (Figure III-7) at 260 ± 50 pmol/min/mg (Table III-3). Induction appeared lower after 2 and 14 days of exposure (Figure III-7), but this difference was not statistically significant (ANOVA -Tukey's test, α = 0.1, 2,9). Statistically, maximal induction was reached with exposure to 0.3 TU after 1 day at a grand mean and SE of 166 ± 31 pmol/min/mg.

In contrast to the short time course experiment at 0.6 TU, the LOET and the NOET were both determined for the long time course experiment at 0.3 TU. The LOET that induced EROD activity at 0.3 TU was 24 h (Bonferroni's one-tailed test, α = 0.05, 4,15). The highest NOET that did not significantly induce EROD activity at 0.3 TU was 9 h.

Discussion

The cytochrome P450 MFO system, of rainbow trout that were exposed to oil sands tailings water, was rapidly induced following a definable concentration-response, as assessed using the EROD assay. However, this system did not prove to be an overly sensitive indicator of sublethal toxicity of this wastewater.

The cytochrome P450 MFO system in rainbow trout was activated rapidly and induced within 48 h by exposure to oil sands tailings water and thus at first glance this system appeared to provide promise as a useful indicator of

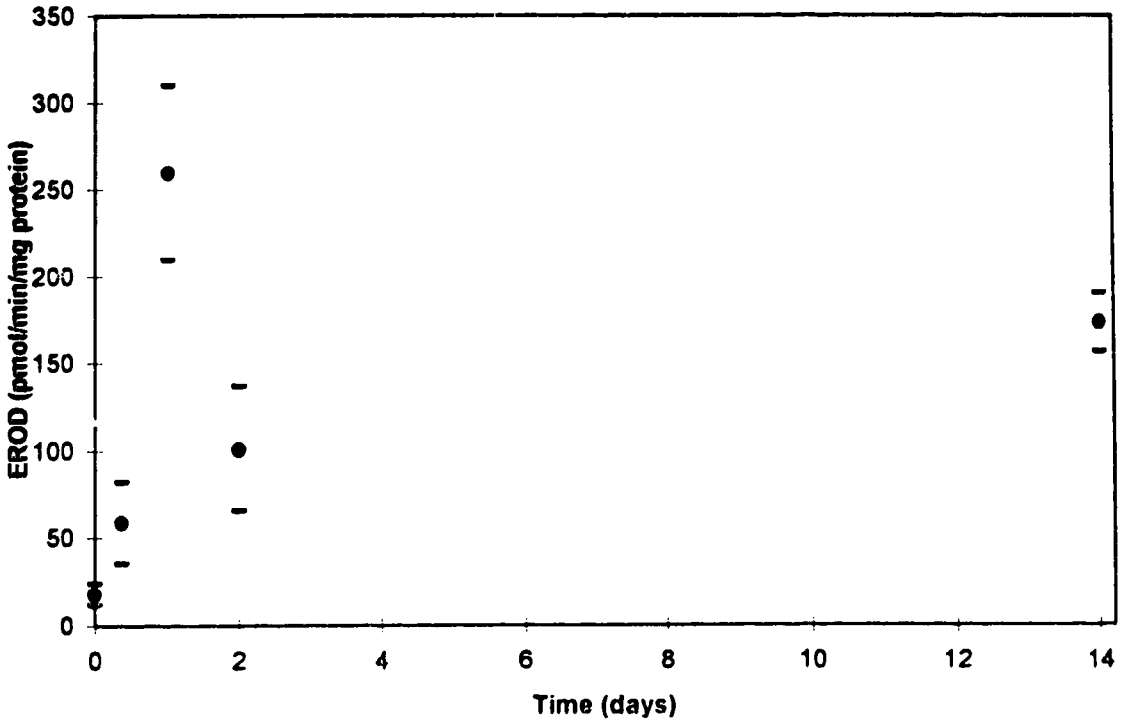

Figure III-7: EROD activity in rainbow trout exposed for various times to 0.3 TU of oil sands tailings water.

Table III-3: EROD activity in rainbow trout exposed to 0.3 TU of oil sands tailings water for various times as compared to EROD activity in response to 0.5 mg/kg βNF (*i.p.*).

Time (days)	EROD activity (pmol/min/mg protein)						n	mean	SD	±1SE
0	24	32	9.1	7.2			4	18	12	12-24
0.375	58	38	124	15			4	59	47	36-82
1	283	173	392	191			4	260	100	210-310
2	9.4	81	76	83	28	230	6	101	87	66-137
14	156	190					2	173	24	156-190
βNF							6	1735	47	1716-1754

sublethal exposure to this wastewater. Increased activity, as a result of exposure to 0.3 and 0.6 TU, occurred rapidly after only 9 and 24 h of exposure respectively. Maximal induction of the cytochrome P450 MFO system was also reached rapidly; within 24 h of exposure to 0.3 and 0.6 TU. Induction in trout exposed to 0.3 TU appeared lower after 2 days of exposure but this apparent difference was not significantly different (α = 0.1) from the induction after 1 day of exposure. The apparent lower induction at day 2 may be related to small sample sizes and a result of a few low EROD activities (Table III-3) in a few fish at day 2 that may have been under subtle acute stress that was not noted at the time of dissection. The rapid activation and induction of the cytochrome P450 MFO system in rainbow trout exposed to oil sands tailings water appeared to provide promise for its use as a simple and rapid indicator of sublethal exposure to this wastewater.

In order to be a useful indicator of sublethal exposure to oil sands tailings water, the EROD activity of rainbow trout exposed to various concentrations of this wastewater had to fit a definable concentration-response. The concentration-response relationship following ln transformation of the EROD data in order to stabilize the variance and rejection of outlying data points, was strong with an r^2 = 0.593. Thus, 59 % of the variation in the ln EROD activity is accounted for by the variation in the concentration of oil sands tailings water associated with the definable ln EROD = 3.68conc. + 3.20 relationship. The basis for rejection of outlying data points was that these fish were observed to be under gross acute stress (Table III-1) and they appeared to be dying. These fish appeared to succumb to the stressors, probably the acutely toxic organic acidic surfactants (Verbeek *et al.*, 1994) in oil sands tailings water, and hence they were incapable of mounting a physiological resistance to the stressors.

Both the maximal inductive response and the determinations of the NOEC and the LOEC for the MFO system in rainbow trout exposed to oil sands tailings water lead to the conclusion that, the sensitivity of the MFO system is poor as an indicator of sublethal exposure to this wastewater. Maximal induction of the MFO system, in rainbow trout exposed to oil sands tailings water, required 0.4-0.8 TU of this wastewater. This is close to what is acutely toxic. Thus, while the MFO system was able to respond to the stressors in oil sands tailings water, it is unlikely that the MFO system in rainbow trout evolved to cope with these types of compounds. The absolute level of maximal induction was at best only one-

fifth to one-sixth of the potential induction as found in response tc the positive control, 0.5 mg/kg βNF (*i.p.*). This relatively small window of potential inductive signal contributes to a much reduced sensitivity of this system as an indicator of sublethal concentrations of oil sands tailings water. The sensitivity of this system as an indicator of sublethal exposure to oil sands tailings water is further compromised when the NOEC and LOEC are considered. The NOEC for MFO induction by exposure to oil sands tailings water was 0.15 TU and the LOEC was 0.2 TU. As these concentrations are determined using hypothesis testing we can say that the true minimum inducing concentration of oil sands tailings water is between 0.15 and 0.2 TU. This minimum inducing concentration is very high. A level approaching 0.015 - 0.02 TU would have been desirable for a sensitive indicator.

The differential in the response to oil sands tailings water and βNF suggests that each response is either related to a different compound specific activity by the same P450 isoform, as suggested by Hollebone (1986), or the activities are indicative of induction by different MFO isoforms responding to different types of compounds. There is little evidence in support of the former argument for different maximal inductive responses by the same P450 isoform in fish. In contrast, MFO activity, has been demonstrated in more than one isoform in rainbow trout.

The relatively small maximal induction that was observed may have been a result of the induction of the CYP4A1 isoform (old name - P450LM2), which acts normally in the hydroxylation of fatty acids (Williams *et al.*, 1984). While xenobiotic transformation is not a major role of CYP4A1 (Nebert and Gonzalez, 1987), its purified form does have the capacity to metabolize βNF, as measured by the EROD assay, at approximately one third the level of the purified form of CYP1A1 (old name - P450LM4) (Williams and Buhler, 1983; 1984). The maximal induction of the MFO system in response to the stressors in oil sands tailings pond water, probably the acutely toxic organic acidic surfactants (Verbeek *et al.*, 1994), was also a fraction, approximately one fifth, of the induction in response to 0.5 mg/kg βNF (*i.p.*). These different levels of induction, as measured by the EROD assay may be indicative of two different P450 isoforms, CYP4A1 responding to the organic acidic surfactants in oil sands tailings pond water and CYP1A1, the isoform generally associated with most xenobiotic transformation in fish, responding to βNF. If this was the case, then it

is possible that the CYP4A1 isoform in some individual fish, could have been incapable of metabolizing the acutely toxic organic acidic surfactants before these same fish succumbed to the acute stress.

Further evidence from an independent study suggests that at the very least, the majority of the EROD induction observed here was likely due to induction of CYP4A1 by the organic acids in oil sands tailings pond water from Syncrude Canada Ltd. Similar levels of induction were found in rainbow trout exposed to oil sands tailings pond water from Syncrude Canada Ltd., the isolated organic acid fraction from an equal volume of this wastewater, and commercially available naphthenic acids (Enviro-Test and HydroQual Labs, 1994). These results are consistent with the hypothesis that the induction observed here was in response to the organic acids. Enviro-Test and HydroQual (1994) also found no statistically significant benzo[a]pyrene hydroxylase (AHH) activity in rainbow trout exposed to either the isolated organic acid fraction or the commercially available naphthenic acids. This evidence is consistent with the finding that purified CYP4A1, isolated from βNF treated rainbow trout has a measurable EROD activity but is not able to metabolize Benzo[a]pyrene as measured by the AHH assay (Williams and Buhler, 1984). The evidence presented in this discussion is not proof, but it suggests that, the majority of the EROD activity observed here was a result of induction of CYP4A1 by the organic acidic surfactants in oil sands tailings water from Syncrude Canada Ltd. Ultimately, the true test of this hypothesis would require the use of either catalytic inhibitors or secondary antibodies that are specific for CYP4A1 (Williams *et al.*, 1984).

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Chapter IV
General Discussion

The data presented here represents a thorough characterization of the potential acutely toxic fractions of oil sands extraction wastewaters as well as the first functional assessment of the potential of the cytochrome P450 MFO system as a possible indicator to sublethal exposure to oil sands tailings water.

This study represents the most thorough published fractionation and characterization of the potential acutely toxic fractions of surface tailings water and fine tails interstitial water, two waste products of oil sands extraction at Syncrude Canada Ltd. In so doing, this study is the first to confirm, that the previously suspect organic acids, are responsible for the majority of the acute toxicity of oil sands surface tailings water and fine tails interstitial water. Approximately 15% of the acute toxicity of fine tails interstitial water was attributable to non-polar volatile organic compounds. Previously (MacKinnon and Retallack, 1982; MacKinnon and Boerger, 1986; Zenon, 1986), the majority of the acute toxicity of oil sands tailings water from Syncrude Canada Ltd. has been removed by the acidification (at pH 2-3) of whole tailings pond water and the precipitation of the organic acids as well as any compounds that might adsorb to the acids as they begin to precipitate. In addition, these studies did not employ a thorough systematic characterization such as the U.S. E.P.A. TIE (EPA, 1991) procedure which is designed to identify most, if not all of the potential acute toxicants in a complex mixture.

This same data set also represents the first comparative toxicological data set for Microtox (*V. fischeri*), *D. magna* and rainbow trout (*O. mykiss*) in response to oil sands tailings pond water after the removal of the organic acidic surfactants. By inference, *Daphnia* were 0.4 times less sensitive and rainbow trout were 3 times more sensitive than *V. fischeri* to these organic acidic surfactants. These differences in relative sensitivity of test organisms to the toxic fraction demonstrate the importance of using more than one test organism to evaluate acute toxicity.

The cytochrome P450 mixed-function oxygenase system as measured by the EROD assay in rainbow trout liver microsomes, responded rapidly, following a definable concentration-response relationship; however, it proved to be a relatively insensitive indicator of sublethal exposure to oil sands tailings water.

Differential maximal EROD induction in rainbow trout exposed to oil sands tailings water as opposed to those injected with βNF (*i.p.*) suggests that each response, may have been a result of induction of two different P450 isoforms. This hypothesis could be investigated using either catalytic inhibitors or secondary antibodies that are specific for each of the two proposed isoforms CYP4A1, and CYP1A1 (Williams *et al.*, 1984).

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