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Preliminary risk assessment of the wet landscape option for reclamation of oil sands mine tailings: bioassays with mature fine tailings porewater

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Abstract

Chemical and biological assays have been carried out on the "pore water" that results from settling of the tailings that are a byproduct of bitumen recovery from the Athabasca oil sands. Examination of the non-acidic extracts of pore water by GC-MS allowed the identification of numerous 2-3 ring polycyclic aromatic compounds (PACs), to a total concentration of 2.6 μg per liter of pore water. The PACs were biodegraded by microflora naturally present in the pore water. Acute toxicity was associated principally with the acidic fraction (naphthenic acids) of pore water extracts according to the Microtox™ assay; other work has shown that acute toxicity dissipates fairly rapidly. Both individual PACs and concentrated porewater extracts showed minimal levels of binding to the rat Ah receptor, and induced minimal EROD (ethoxyresorufin-*O*-deethylase) activity in primary rat hepatocytes, showing insignificant risk of inducing monooxygenase activity. Taken together with previous work showing negligible mutagenic activity of these extracts, we conclude that it should be possible to develop tailing slurries into biologically productive artificial lakes.

Résumé

On a effectué des essais chimiques et biologiques avec des « eaux interstitielles » provenant de la décantation de stériles de sables bitumineux d'Athabasca, un sous-produit de la l'extraction du bitume. L'examen par CG-SM d'extraits non acides d'eaux interstitielles a permis l'identification de nombreux composés aromatiques polycycliques à 2 ou 3 anneaux (CAP), jusqu'à une concentration totale de 2,6 µg par litre d'eau interstitielle. Les CAP étaient biodégradés par la microflore naturelle des eaux interstitielles. Selon l'essai Microtox™, la toxicité aiguë était surtout associée à la fraction acide (acides naphténiques) des extraits d'eaux interstitielles. D'autres travaux ont montré que la toxicité aiguë se dissipait assez rapidement. Avec chacun des CAP et des extraits d'eaux interstitielles concentrés, on n'a mis en évidence que de très faibles taux de liaison au récepteur Ah du rat et une très faible induction de l'activité de l'EROD (éthoxyrésorufine-*o*-déséthylase) dans les hépatocytes primaires des rats, ce qui indique que les risques d'induction de l'activité de la monooxygénase ne sont pas significatifs. Compte tenu de l'ensemble des travaux antérieurs selon lesquels l'activité mutagène de ces extraits est négligeable, nous concluons qu'il devrait être possible de transformer les bassins contenant les boues de stériles en lacs artificiels sains et productifs.

Évaluation préliminaire des risques de l'option des écopaysages humides pour l'assainissement des résidus d'exploitation de sables bitumineux : épreuves biologiques effectuées avec des eaux interstitielles de stériles fins à maturité

SOMMAIRE À L'INTENTION DE LA DIRECTION

Ces recherches effectuées à l'Université de Guelph étaient financées par le CRSNG et Syncrude Canada, en collaboration avec Environnement Canada et le PRDE, dans le cadre du secteur d'activité Un environnement sain (substances toxiques).

Il s'agit de la deuxième publication d'une série portant sur les stériles produits par l'exploitation de sables bitumineux et sur l'option des « écopaysages humides » pour la gestion des stériles fins et l'assainissement des sites. La première publication portait sur la mutagénicité des composés aromatiques polycycliques (CAP) dans les eaux interstitielles de stériles fins à maturité de sables bitumineux de l'Athabasca. Cette publication présente les résultats d'essais de CAP types et d'extraits d'eaux interstitielles de stériles fins à maturité, visant à déterminer leurs effets sur le récepteur Ah du rat et sur l'induction de l'activité de l'EROD dans les hépatocytes primaires du rat. Lors de ces deux essais, l'activité des CAP et des extraits était très faible. De même que les études antérieures sur la mutagénicité, nous concluons que la libération lente de CAP dans les eaux de surface de lacs artificiels contenant des stériles fins ne présente que peu de risques de toxicité chronique. La partie expérimentale de ces travaux est terminée.

On prépare une troisième publication sur les caractéristiques environnementales dynamiques de CAP représentatifs.

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MANAGEMENT PERSPECTIVE

This research was carried out at the University of Guelph funded by NSERC and Syncrude Canada, in collaboration with Environment Canada and PERD funding under the Clean Environment (Toxic) Issue.

This is the second paper in a series on oil sands mine tailings and the "wet landscape" option for management of fine tailings and site reclamation. The first paper was on the mutagenicity of polycyclic aromatic compounds (PACs) in porewater from Athabasca oil sands mature fine tailings. The present paper presents results of testing of model PACs and mature fine tailings porewater extracts with rat Ah receptor and EROD induction in rat primary hepatocytes. Activity of PACs and extracts was minimal in both tests. Together with the earlier work on mutagenicity, we conclude that slow release of PACs to the capping water in artificial lakes containing fine tailings presents minimal risk of chronic toxicity. The research component of this work is completed.

A third paper on the environmental dynamics of representative PACs is in preparation.

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Preliminary Risk Assessment of the Wet Landscape Option for Reclamation of Oil Sands Mine Tailings: Bioassays with Mature Fine Tailings Pore Water

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ABSTRACT: Chemical and biological assays have been carried out on the "pore water" that results from the settling of the tailings that accompany bitumen recovery from the Athabasca oil sands. Examination of the nonacidic extracts of pore water by gas chromatography–mass spectroscopy allowed the identification of numerous two- to three-ring polycyclic aromatic compounds (PACs), to a total concentration of 2.6 μg/L of pore water. The PACs were biodegraded by microflora naturally present in the pore water. Acute toxicity was associated principally with the acidic fraction (naphthenic acids) of pore water extracts according to the Microtox assay; other work has shown that acute toxicity dissipates fairly rapidly. Both individual PACs and concentrated pore water extracts showed minimal levels of binding to the rat Ah receptor and induced minimal ethoxyresorufin-*O*-deethylase activity in primary rat hepatocytes, showing an insignificant risk of inducing monooxygenase activity. Taken together with previous work showing negligible mutagenic activity of these extracts, we conclude that it should be possible to develop tailing slurries into biologically productive artificial lakes. © 2001 by John Wiley & Sons, Inc. Environ Toxicol 16: 197–208, 2001

Keywords: Athabasca oil sands; oil sands fine tailings, Ah receptor activity

INTRODUCTION

The Athabasca oil sands in northeastern Alberta are an important Canadian fossil fuel resource, comprising over 700 billion barrels of bitumen and covering an area of >42,000 km^2. Hot water extraction of bitumen from the oil sands, which is ~90% efficient, followed by upgrading into a synthetic sweet crude oil, currently provides about 20% of Canada's crude oil. Tailings, comprising of water, fine clay, silt, and unrecovered bitumen, are accumulating at the rate of ~10^5 m^3/day, because ~0.1–0.2 m^3 of tailings are formed per tonne of bitumen extracted. Over the past 30 years, ~3×10^8 m^3 of tailings have accumulated; these are currently stored in tailings ponds (FTFC, 1995a; MacKinnon, 1989).

Bitumen is a highly complex matrix that comprises saturated hydrocarbons containing long chain alkyl groups attached to bi- to tetracyclic cores, polycyclic

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aromatic compounds (PACs), including alkyl, aza- and thia-PACs, and naphthenic acids (CEATAG, 1998; FTFC, 1995b; Mojelsky et al., 1986; Payzant et al., 1985; Thiel, 1988). Naphthenic acids are long chain alicyclic hydrocarbons functionalized with carboxylic acid groups. They have surfactant properties, making them acutely toxic to aquatic life, but are quickly biodegraded; PACs, by contrast, are of concern due to their likely persistence and because many PACs are known mutagens and carcinogens (IARC, 1987).

The Alberta Environmental Protection and Enhancement Act (AEPEA) prohibits the release of the potentially toxic fine tailings into the Athabasca River and requires the oil sands companies to remediate their leases to a state approximating the environment present before mining operations began (AEPEA, Section 32, 1993). The "wet landscape" option for complying with the AEPEA involves transferring the fluid tailings into a mined-out pit and capping them with clean water, to yield artificial lakes with appearance and biological productivity like those of natural lakes in the region (FTFC, 1995c). Tailings management is complicated by the extremely slow settling rate (decades to centuries) of the fine particles in the tailings, which release "pore water" into the capping layer. Transfer of toxic levels of contaminants from residual bitumen in the tailings into the water column could compromise the biological productivity of the wet landscape ecosystem for several decades, making this scenario environmentally unacceptable. Although we previously showed that the PACs present in pore water have low mutagenic potential (Madill et al., 1999), Barron et al. (1999) recently demonstrated that heteroatom-containing PACs may be a significant source of acute toxicity in weathered petroleum. The objectives of the present work were to further characterize compounds present in the PAC fraction of pore water and to carry out bioassays, in order to assess the likely toxicity of mature fine tailings in the context of the wet landscape option.

EXPERIMENTAL METHODS

Chemicals

Naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]pyrene, benzo[*a*]anthracene and dibenzo[*a,h*]anthracene were obtained from Supelco Canada (Mississauga, ON); 1-methylnaphthalene, 2-methylnaphthalene, 3,6-dimethylphenanthrene, benzo[*e*]pyrene, carbazole, acridine, 3,6-dimethylphenanthrene, 9-phenanthrol, and phenanthrenequinone were obtained from Aldrich Chemical (Milwaukee, WI); indeno[1,2,3-*cd*]pyrene, benzo[*g,h,i*]perylene, dibenzothiophene, 2-methylanthracene, 9-methylanthracene, 2,7-dimethylnaphthalene, 1,3-dimethylnaphthalene, 1,5-dimethylnaphthalene, 1,2-dimethylnaphthalene, 1,8-dimethylnaphthalene, 2,3,5-trimethylnaphthalene, and 1-methylphenanthrene were acquired from Chem Service (West Chester, PA); 1- and 2-methylphenanthrene, 3,6-dimethylphenanthrene, 4- and 6-methylchrysene, 2-methylfluoranthene, and 5,6-benzoquinoline were supplied by Accustandard and were purchased from Chromatographic Specialties (Brockville, ON); retene and 7,8-benzoquinoline were supplied by ICN Biomedicals Inc. (Aurora, OH); biphenyl-d_{10}, phenanthrene-d_{10}, and pyrene-d_{10} were purchased from C/D/N Isotopes (formerly MSD Isotopes) (Pointe Claire, QC); 2,8-dimethyldibenzothiophene and 1-, 2-, 3-, and 4-methyldibezothiophene were purchased as toluene solutions from ASTEC (Münster, Germany); and pure 4-methyldibenzothiophene was a gift from Dr. J. Andersson, University of Ulm, Germany, provided by Dr. P. Fedorak, University of Alberta, Edmonton, AB.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was a generous gift from Wellington Laboratories (Guelph, ON); dimethyl sulfoxide (DMSO), $MgSO_4$ and tris-(hydroxymethyl)-aminomethane were purchased from Fisher Scientific Co. (Toronto, ON); resorufin was purchased from ICN Biomedicals Inc.; sodium pentobarbital (Somnitol) was obtained from MTC Pharmaceuticals (Cambridge, ON); Percoll, Hank's balanced salt solution, Dulbelco's phosphate buffered saline (PBS), and 7-ethoxyresorufin (7-ER) were purchased from Sigma Chemical Co. (St. Louis, MO); and hepatocyte qualified collagenase, minimal essential medium, and all components of attachment medium and serum free medium were purchased from GIBCO BRL (Burlington, ON). Stock solutions of PACs were prepared in DMSO and diluted into buffer; solutions were inspected visually for signs of precipitation of the PACs.

Solvents

Methanol, pentane, hexane, isopropanol, and isooctane (distilled in glass) were obtained from Caledon Laboratories Inc. (Georgetown, ON); dichloromethane (DCM) (distilled in glass) was obtained from Burdick and Jackson (Muskegon, MI). Organic-free water was prepared by a Milli-Q system (Millipore SA, Molsheim, France).

Animals

Sprague–Dawley rats (100 g) were obtained from Charles River Breeding Laboratories (Canada). Treatment of animals was in accordance with the University of Guelph Animal Care Policy C5.1.

Chromatography

Whatman glass fiber filters were supplied by Whatman (Clifton, NJ), Superclean silica gel was supplied by Supelco Canada (Oakville, ON), and the Burdick and Jackson inert solid phase system silica diol chromatography columns were purchased from Baxter Ltd. (Toronto, ON). Normal-phase high-performance liquid chromatography (HPLC) was performed with a Waters 600 pump controller with a Rheodyne manual injector and Waters 490E programmable multi-wavelength ultraviolet detector set at 254 nm. The detector output was recorded on a Hewlett-Packard 3396A integrator. Separation was effected with a Whatman Partisil PAC column, 10 μm particle size, 9.5 mm inside diameter (i.d.) × 500 mm (Mandel Scientific, Guelph, ON) protected by a Waters Guard-Pak precolumn.

Gas chromatography–mass spectrometry (GC–MS) was done on a Hewlett-Packard model 5890 series II gas chromatograph interfaced to a model 5971 mass selective detector operating in electron impact mode at 70 eV. The column was 30 m × 0.25 mm HP-5MS, film thickness 0.25 μm (Hewlett-Packard, Mississauga, ON). The gas chromatograph was programmed at 3°C/min from 80–245°C and at 4°C/min from 245–280°C for 10 min, using He carrier gas at a linear velocity of ~35 cm/s. A 1-μL sample was injected in the splitless mode using a Hewlett-Packard 7673 autosampler. Data were acquired in scan and single ion monitoring (SIM) modes. PACs were identified by comparing spectra and retention times with components of a standard containing more than 30 PACs and alkyl-PACs, prepared in our laboratory from authentic reference materials.

Preparation of Solid-Free Pore Water and Acid–Base Extraction

Mature oil sands fine tailings (MFTs), collected by Syncrude Canada Ltd. at a depth of 14 m from the south end of the Mildred Lake Settling Basin, were centrifuged at 30,000 × g for 1 h and then filtered through a combusted (450°C) glass fiber filter. The filtrate was stored in a sealed 1-L amber bottle at 3°C in the dark, for no longer than 1 month before use. The yield of pore water from the MFTs was approximately 50% (v/v).

Pore water (1–3 L) was adjusted to pH 12 with isooctane-washed 10 M NaOH and extracted with 4 × 50 mL of DCM to isolate the base/neutral organic fraction (I). The remaining aqueous solution was adjusted to pH 2 with 12 M H_2SO_4 (DCM-washed) and extracted with DCM (4 × 50 mL) to produce the acid organic fraction (II). Clean separations were impossible due to emulsion formation, and so the acid organic fraction was back-extracted with DCM to recover additional base/neutral material, which was combined with fraction (I). Extracts were dried by passage through anhydrous Na_2SO_4 (combusted at 450°C) into a clean round-bottom flask and evaporated on a rotary evaporator at ~35°C to ~15 mL; the residue was transferred to a clean test tube and further evaporated to 2 mL under argon. Samples for GC–MS were solvent exchanged into isooctane at this stage. Control samples used organic-free water (Milli-Q) in place of MFTs pore water.

The base/neutral fraction was further separated by silica diol chromatography. The column was wetted and activated with 10 mL of DCM, before 0.5 mL of concentrated extract of fraction (I) was added. Fractions eluted were A (10 mL of DCM), B (15 mL of DCM–MeOH, 1:1 v/v), and C (15 mL of MeOH), of which A contained almost all the PACs (shown by GC–MS). Fraction A was further separated by silica gel column chromatography (1.1 cm i.d. × 5 cm length; 5.0 g of Superclean silica gel, activated at 130°C for 8 h, with a 1 cm layer of anhydrous Na_2SO_4 at the top of the column). To clean the column it was rinsed twice with 2 × 10 mL of DCM and then with 25 mL of pentane and allowed to drain. Fractions A1 eluted with 15 mL of pentane, A2 with 15 mL of DCM–pentane (1:1 v/v), A3 with 15 mL of DCM, and A4 with 15 mL of isopropanol–DCM (1:9 v/v). Fraction A2, which was shown to contain almost all the PACs, was further separated by normal-phase HPLC, eluting with a hexane–DCM gradient, with flow rate 1.0 mL/min. Thirty-five fractions were collected and evaporated under argon to 1 mL and solvent exchanged into isooctane for GC–MS analysis.

Biodegradation of PACs in the MFTs Pore Water

Three sterile [DCM-washed (4 × 50 mL)] 500-mL glass amber bottles were filled to the 500-mL mark with pore water that had been stored in the dark at 3°C for a maximum of 2 weeks. One sample was not sterilized, one was sterilized using a 240 Ci Gammacell cobalt 60 source (AECI Corp., Folsom CA) for 6 h, and the third was sterilized with 0.05% (w/v) sodium azide (Fisher Scientific). Duplicates of the above were set up with sufficient glass beads (sterilized by washing in warm soapy water and then soaked in DCM for 24 h) to double the surface area of the 500-mL bottles. All samples were stored at room temperature in the dark for 129 days. On each sampling day, exactly 25 mL of pore water was decanted from each 500-mL bottle into sterile 25-mL amber glass bottles and 25 μL of biphenyl-d_{10} and phenanthrene-d_{10} (1 mg/mL) and pyrene-d_{10} (0.2 mg/mL) were added as internal standards. PAC standards (5 mg/L of naphthalene, 1 mg/L

each of biphenyl-d_{10}, fluorene, dibenzothiophene, phenanthrene-d_{10} and phenanthrene, 0.45 mg/L of 4-methyldibenzothiophene, 0.5 mg/L of 1-methylphenanthrene, and 0.2 mg/L each of fluoranthene, pyrene-d_{10}, and pyrene) were also added on the day of testing to 25 mL of organic-free water in sterile amber glass bottles.

Samples were extracted using solid phase microextraction (Potter and Pawliszyn, 1994; Louch et al., 1992): the 100-μm polydimethylsiloxane-coated fiber (Supelco Inc.) was fully immersed in the pore water sample for exactly 1 h with stirring. For analysis by GC-MS, injections were made in the splitless mode at 250°C for 1 min before the injector valve was opened. The fiber was then cleaned for 10 min by placing it in a second injection port at 250°C. All samples and standards were extracted and analyzed by GC–MS on the same day, using SIM mode with the retention window and m/z ratios set up to detect specific PACs (Table I).

Microtox Acute Toxicity Assay

This assay was carried out using a Microbics model 500 Analyzer (Azure Environmental Corp., Carlsbad, CA), following the manufacturer's protocol. Briefly, the test samples and standards were first diluted with an osmotic adjusting solution (22% NaCl) to 2% NaCl. Clean cuvettes were then set up in the incubator and reagent wells of the instrument. The reagent well cuvette was filled with "reconstitution solution" (1.0 mL; Azure Environmental Corp.) and allowed to warm to 15°C. "Diluent solution" (0.5 or 1.5 mL, Azure Environmental Corp.) was added to the other cuvette wells in the incubator. The sample (2.5 mL) was added to one cuvette well and 1:2 serial dilutions were performed in each series of seven cuvettes. A separate set of cuvettes was set up for the addition of the Microtox reagent. After preparation of the sample dilution series, the pellet of freeze-dried bacterium (*Vibrio fischeri*) was dissolved in reconstitution solution (from the reagent well) and placed in the reagent well. After mixing, 10 mL of reagent was transferred into each sample cuvette in the incubator. Each sample was then mixed gently on a Vortex mixer for 10 s. After 15 min the initial light reading (I_0) was taken for all reagent solutions. The solutions containing the reagents were then added to those containing the diluted samples and the light levels were read after 15 min (I_{15}). All tests were performed in triplicate for each sample dilution. The parameter $I' = [(B_{15}/B_0) \times I_0 - I_{15}]/I_{15}$ was calculated using the Microbics software: B_0 and B_{15} represent bioluminescence of the control at $t = 0$ and $t = 15$ min, and I_0 and I_{15} are the corresponding values for the sample. Linear regression of I' versus concentration gives the concentration (EC_{50}) corresponding to $I' = 1$, at which light emission is reduced by 50%.

TABLE I. Identification and concentrations of low molecular weight PACs from the base/neutral organic fraction (I) of mature oil sands fine tailings pore water using GC–MS SIM

Compound/Group[a]	Concentrations of PACs or Groups of PAC Congeners (ng/L of pore water)
2- and 3-Ring PACs	
Naphthalene	101 ± 30
2-Methylnaphthalene	28 ± 8
1-Methylnaphthalene	33 ± 9
C2-naphthalene	61 ± 16
C3-naphthalene	203 ± 55
Acenaphthylene	91 ± 25
Acenaphthene	206 ± 56
Fluorene	117 ± 32
C1-fluorene	232 ± 63
Phenanthrene	330 ± 89
C1-phenanthrene/anthracene	389 ± 105
1-Methylphenanthrene	88 ± 24
2-Methylphenanthrene	134 ± 36
C2-phenanthrene/anthracene	200 ± 54
3,6-Dimethylphenanthrene	45 ± 12
4-Ring PACs	
Fluoranthene	10 ± 2.7
Pyrene	14.5 ± 4
C1-fluoranthene/pyrene	5.7 ± 1.4
Chrysene	12 ± 3
S-PACs	
Dibenzothiophene	37 ± 10
4-Methyldibenzothiophene	77 ± 21
2/3-Methyldibenzothiophene[b]	56 ± 15
1-Methyldibenzothiophene	59 ± 16
C2-dibenzothiophene	152 ± 41
2,8-Dimethyldibenzothiophene	24 ± 7
C3-dibenzothiophene	98 ± 26
N-PACs	
2,4-Dimethylquinoline	24 ± 6
7,8-Benzoquinoline	63 ± 17
5,6-Benzoquinoline	123 ± 33
Acridine	31 ± 8

[a]C1 = methyl, C2 = dimethyl/ethyl, C3 = trimethyl(methyl + ethyl)/propyl/isopropyl).
[b]2- and 3-Methyldibenzothiophene were not separated under the GC conditions used.

Preparation of Hepatic Cytosol

Rodent hepatic cytosol was prepared as previously described (Schneider et al., 1995). Briefly, immature male animals were euthanized by exposure to carbon dioxide

followed by cervical dislocation. Livers were immediately perfused in situ with ice-cold HEGD buffer via the hepatic portal vein and then excised. Tissue was homogenized using three strokes of a Potter Elvehjem Teflon tissue homogenizer. The homogenate was spun at 9000 × g for 20 min in a Sorvall Superspeed RC2-B centrifuge at 4°C and then spun at 100 000 × g for 68 min in a Beckman L7-65 ultracentrifuge at 4°C. The cytosol was stored in 1-mL aliquots at −70°C until required. The protein content of the cytosol was determined by the method of Bradford (1976) using Bio-Rad protein assay dye reagent with bovine serum albumin as a standard.

Hydroxylapatite (HAP) Ligand Binding Assay

The hydroxylapatite assay followed the method of Gasiewicz and Neal (1982). Aliquots (1 mL) of tissue cytosol were diluted with HEGD buffer to give a final protein concentration of 2 mg/mL. Cytosol was incubated with 10 nM [^{3}H]benzo[*a*]pyrene and a range of concentrations of an unlabeled PAC. The protein components of the cytosol were then adsorbed onto hydroxylapatite [$Ca_5(PO_4)_3OH$] and rinsed with a mild detergent (1.5% v/v Triton X-100) to remove free and loosely bound radioligand, and the bound radioactivity was quantitated by liquid scintillation counting. Nonspecific binding was determined in a parallel incubation of 10 nM [^{3}H]-benzo[*a*]pyrene plus 2 μM unlabeled benzo[*a*]pyrene and was assumed to be independent of the concentration of the unlabeled competitor (Schneider et al., 1995). Data were elaborated by plotting "probit of percent specific binding" versus log[PAC] and obtaining the EC_{50} from the log[PAC] corresponding to probit = 5.000; the value 100% specific binding was obtained when no unlabeled PAC was present.

Preparation of Primary Rat Hepatocytes

Primary cultures of rat hepatocytes were prepared by a modified protocol of Kreamer (1986). An immature male Sprague–Dawley rat was anesthetized with sodium pentobarbital, and the liver was perfused with collagenase, excised, rinsed, and desegregated in a sterile 150-mm Petri dish. The cells were filtered and resuspended in 25 mL of attachment media (William's E medium supplemented with 10 mM HEPES, 2 mM L-glutamine, and 10% fetal bovine serum) combined with 24 mL of Percoll in Hank's balanced salt solution. The cells were then spun at 50 × g, for 10 min, and the pellet was washed with 50 mL of attachment media, spun for 3 min at 50 × g, and resuspended in 30–40 mL of attachment media. The cells were then counted using a hemocytometer. Cells were plated in sterile 48-well collagen-coated culture plates at a density of 50,000 cells/well in 0.5 mL of attachment media. After 2 h, the medium was aspirated away and 0.5 mL of serum-free media (William's E medium supplemented with 10 mM HEPES, 2 mM L-glutamine, 10 mM pyruvate, and 0.35 mM proline) was added to each well. The cells were then incubated for 24 h at 37°C (95% air, 5% CO_2).

7-Ethoxyresorufin-O-Deethylase (EROD) Assay of Primary Rat Hepatocytes

The EROD assay was performed directly in whole cell cultures of primary rat hepatocytes (Kennedy et al., 1993); 24 h after plating, hepatocytes were treated with various PACs in 2.5 μL of DMSO, incubated for a further 24 h, then rinsed with 250 μL of PBS (pH 7.6). To each well was then added 250 μL of minimal essential medium containing 0.2 μg/mL of 7-ER. Fluorescence measurements were obtained with a Bio-Rad Fluoromark fluorescence multiwell plate reader (excitation at 530 nm/25 nm bandwidth and emission at 590 nm/35 nm bandwidth). Individual experiments involved three to five replicates, each using a new hepatocyte preparation; reported results are the means of the replicates of at least two independent experiments with different hepatocyte preparations. Data elaboration involved plotting "probit of percent EROD activity" versus log[PAC]; the EC_{50} was obtained from the log[PAC] corresponding to probit = 5.000; percent EROD activity was referenced to a 1.0 nM TCDD solution = 100%.

RESULTS AND DISCUSSION

Characterization of PACs in Pore Water

Acid–base extraction (Fig. 1) of raw pore water was used to isolate the base-insoluble fraction (the "base/neutral fraction") from the base-soluble naphthenic acid fraction, to give a 10^4-fold concentrated extract (e.g., 300 μL from 3 L of pore water). Spiking experiments with isotopically labeled PACs (pyrene-d_{10}, phenanthrene-d_{10}, and biphenyl-d_{10}) indicated recoveries of 82, 68, and 74% of these three analytes. Losses are attributed to volatilization during the various evaporation steps.

The GC–MS total ion chromatogram scan of fraction I showed an unresolved complex mixture "hump" that represented over 60% of the total peak area. This type of chromatogram is especially pronounced in biodegraded petroleum (Gough and Rowland, 1990; Barron et al., 1999) and has been deduced to represent mostly aliphatic hydrocarbons (Payzant et al., 1985;

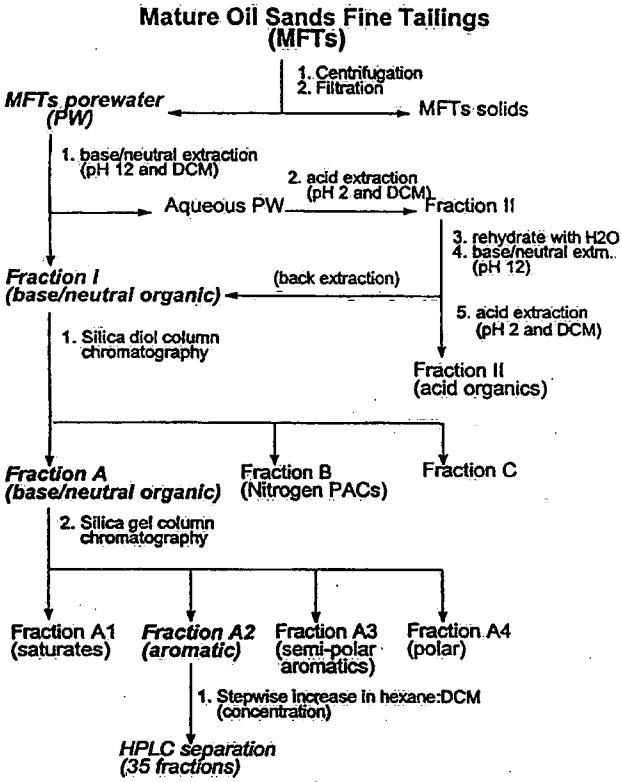


Fig. 1. Flowchart scheme for the modified extraction of base/neutral PACs from the MFTs pore water.

Wong et al., 1994; CEATAG, 1998). Analysis using SIM indicated the presence of the parent and alkyl derivatives of naphthalene, acenaphthylene, fluorene, dibenzothiophene, phenanthrenes/anthracenes, fluoranthene, and pyrene (Table I). SIM increases the sensitivity of the MS for a specific ion; when combined with a strictly defined retention time window for an authentic PAC congener, SIM allows a reasonably confident identification of the PAC. The concentrations of individual compounds were all <500 ng/L (back-calculated to the original pore water) with a total concentration of identified PACs of 2.6 μg/L. The phenanthrene derivatives were most abundant, followed by dibenzothiophenes and naphthalenes. Identification of additional aza- and thia-PACs was hindered by the lack of authentic standards. The high abundance of alkyl PACs relative to the parent compounds is consistent with previous work on the MFT "solids" (Brownlee et al., 1996). Other than small quantities of fluoranthene, pyrene, and chrysene, no high molecular weight PACs were detected in the MFTs pore water, probably because of their low water solubilities. This finding is significant, because the four- to six-ring PACs are of greatest mutagenic concern.

Subfractions obtained by solid-phase extraction diol and silica gel chromatographies gave results similar to those for the whole base/neutral extract, with additional clean-up being largely offset by further loss of material. The cleanest samples were obtained by normal-phase HPLC separation of fraction A2 into 35 subfractions with a hexane–DCM gradient, and GC–MS analysis of the evaporated fractions. PACs were found principally in sub-fractions 24 and 25, and identified by running full-scan MS for comparison with authentic samples (Table II).

Biodegradation

In aquatic systems, PACs mostly sorb to bottom sediments or suspended particles in the water column, where a concentration-dependent equilibrium exists between the sorbed and dissolved states. High molecular weight PACs adsorb more strongly (K_{oc} 10^5–10^6) than low molecular weight PACs (K_{oc} ~10^3–10^4) to

TABLE II. GC–MS scan identification of low molecular weight PACs after HPLC separation from the base/neutral organic fraction (I) of mature oil sands fine tailings pore water

PAC Identified	GC Retention Time (min)	MS% Quality Match[a]
1-Methyldibenzothiophene	33.1	Partial scan comparison[b]
2/3-Methyldibenzothiophene	32.4	Partial scan comparison
4-Methyldibenzothiophene	31.7	Partial scan comparison
Phenanthrene	29.3	80
2-Methylphenanthrene	33.1	80
4-Methylphenanthrene	33.2	80
3-Methylphenanthrene	33.8	80
1-Methylphenanthrene	34.0	80
3,6-Dimethylphenanthrene	37.4	83

[a]MS Library: NBS45K on Hewlett-Packard Chemstation.
[b]Partial scan comparison with standard PAC compound run under the same GC–MS conditions. Signal peak strength was not strong enough for comparison to the MS library.

organic carbon in sediments (Southworth, 1979; DHHS, 1995), and heteroatom PACs have lower K_{oc}s than PAHs (Means et al., 1982; Banwart et al., 1982). In the wet landscape system, the solubilities of low molecular weight PACs will allow them to cycle through the water-capping layer, while high molecular weight PACs should remain bound to the MFTs sediment.

Bioaccumulation of PACs by aquatic organisms depends on PAC concentrations in the water column and is influenced by the rates of physical and loss processes such as volatilization, solar degradation, and biodegradation relative to release from the pore water. The rates of loss processes are seasonal: in winter, temperatures and insolation in central Alberta are low, and lake surfaces are ice-covered. Most PACs have little tendency to escape into the atmosphere [e.g., K_H for benzo[*a*]pyrene = 0.034 Pa m^3/mol (ten Hulscher et al., 1992)]; this process takes place only at the water–air interface, and so it is influenced by wind speed, water turbulence, water temperature, and water column depth (Smith et al., 1978; Southworth, 1979; Achman et al., 1993). Photooxidation and direct photolysis of PACs in direct sunlight are intrinsically fast (half-lives of hours to days) (Smith et al., 1978; Marr, 1994; Zepp and Schlotzhauer, 1979), but only occur in the upper layers of the water column, where the ultraviolet component of sunlight penetrates most effectively.

Many bacteria, fungi, and algae degrade PACs (Atlas, 1977; Foght et al., 1985; Heitkamp and Cerniglia, 1989; Bumpus, 1989; Mueller et al., 1997) oxidatively with dissolved oxygen >0.7 mg/L and more slowly under anaerobic conditions (Borden et al., 1989; Sharak-Genthner et al., 1997; Coates et al., 1997). Although Foght et al. (1985) and Wyndham and Costerton (1981) reported that hydrocarbons degrade particularly slowly and incompletely in the Syncrude tailings ponds, the prevailing temperatures (5–6°C) are lower than those described by most other authors. Degradation rates of PACs at oil- and bitumen-contaminated sites are much higher than those found at pristine sites, due to an enriched population of hydrocarbon degraders (DHHS, 1995). Although high concentrations of surfactants inhibit PAC biodegradation (Deschenes et al., 1996; Tiehm, 1994), low concentrations have the opposite effect by increasing bioavailability (Soeder et al., 1996).

Endogenous microflora degraded the PACs in MFTs pore water in the dark at 3°C, over a 129 day experiment. Experiments were carried out in the presence of air in the head space of the bottles. There was a trend toward faster biodegradation when the surface area for microbial attachment was increased by adding glass beads, but it was not consistent for all congeners (Table III). We used 50 g of 3-mm diameter beads to give a surface area equivalent to the inside surface area of a

TABLE III. The initial and final concentrations and average biodegradation rates (*k*) of eight PACs by a mixed culture of natural microorganisms in the tailings pore water

Compound	Glass Beads[a]	Initial Concentration (ng/L)	Final Concentration (ng/L)	Average Biodegradation Rate (*k* [ng/L/d])[b]
Naphthalene	+	366 ± 73	11 ± 2	2.5
	−	300 ± 60	40 ± 8	2.3
Fluorene	+	180 ± 36	1.5 ± 1	1.4
	−	169 ± 34	71 ± 14	0.76
Phenanthrene	+	406 ± 81	2.9 ± 1	3.1
	−	382 ± 76	56 ± 11	2.5
1-Methylphenanthrene	+	132 ± 26	1.5 ± 1	1.01
	−	108 ± 21	69 ± 14	0.30
Dibenzothiophene	+	55 ± 11	1.7 ± 1	0.40
	−	50 ± 10	8.3 ± 2	0.32
4-Methyldibenzothiophene	+	116 ± 23	2.9 ± 1	0.88
	−	107 ± 21	65 ± 13	0.32
Fluoranthene	+	17 ± 3	2.0 ± 1	0.11
	−	14 ± 3	6.8 ± 1	0.06
Pyrene	+	16 ± 3	1.4 ± 1	0.11
	−	14 ± 3	12 ± 3	ND[c]

[a]Glass beads increased the surface-to-volume of the glass container.
[b]Average biodegradation rate was calculated from the [final concentration − initial concentration]/129 days.
[c]No detectable average biodegradation rate or half-life.

500-mL bottle, thus doubling the surface area of the glass. No losses of PACs were observed in sodium azide-treated or gamma-irradiated samples, apart from initial losses due to radiolysis in the latter case; see Fig. 2, for the loss of phenanthrene. Because most of these curves did not follow first-order kinetics, the data in Table III are reported as average degradation rates $([c]_{final}-[c]_{initial})/129$ days. It is not meaningful to report degradation half-lives, and the method of calculation assigns the largest rates to the compounds initially present at the highest concentrations.

Acute Toxicity

Acute toxicity of the pore water extracts was studied using the Microtox assay, which is based on the inhibition of bioluminescence of the marine bioluminescent bacteria (*V. fischeri*, strain NRRC-B-11177). Light measurements are made before and after exposure of the organism to the toxicant, and an EC_{50} is calculated as the sample concentration resulting in a 50% reduction of light intensity (Symons and Sims, 1988; Microbics Manual, 1994).

The Microtox acute toxicity results for 10 low molecular weight PACs are presented in Table IV. Five were not acutely toxic to *V. fischeri* at the maximum concentration tested. Positive responses were characterized by linear dose-response curves as assessed using the Microbics software (Microbics Manual, 1994). The Microtox results for the MFTs pore water are presented in Table V. The acidic fraction of the pore water, containing the naphthenic acids, was ~100 times more toxic than the base/neutral fraction, consistent with previous findings (FTFC, 1995b and references therein; CEATAG, 1998; Yong and Ludwig, 1988). Acute toxicity due to naphthenic acids was reported to disappear

TABLE IV. Microtox acute toxicity results for some model PACs identified in the tailings pore water using the modified 45% test (Microbics Manual, 1994)

Compound	EC_{50} (at 15 min; μg/mL)
Anthracene	>2.3
2-Methylanthracene	>2.3
9-Methylanthracene	4.4
Phenanthrene	0.06
1-Methylphenanthrene	>4.5
Dibenzothiophene	0.4
Pyrene	>4.5
7,8-Benzoquinoline	1.5
Acridine	2.7
Carbazole	>4.5

TABLE V. Microtox acute toxicity results for the base/neutral (I) and acid (II) organic fractions of the tailings pore water

	Concentration of MFTs Pore Water Tested[a]	EC_{50} (at 15 min, % of Sample v/v)[b]
Fresh fine tailings[c]		20–40
Fraction I	200×	48
Fraction II	200×	No survivors
10× dilution	21×	19
25× dilution	8×	58

[a]Concentration of the fraction of pore water tested.
[b]EC_{50} value for fresh fine tailings pore water from the Syncrude settling pond [Nelson et al. (1993) and Mackay and Verbeek (1993) cited in FTFC (1995b)].
[c]EC_{50} value at 15 min as percentage of the concentrated extracted sample of tailings pore water.

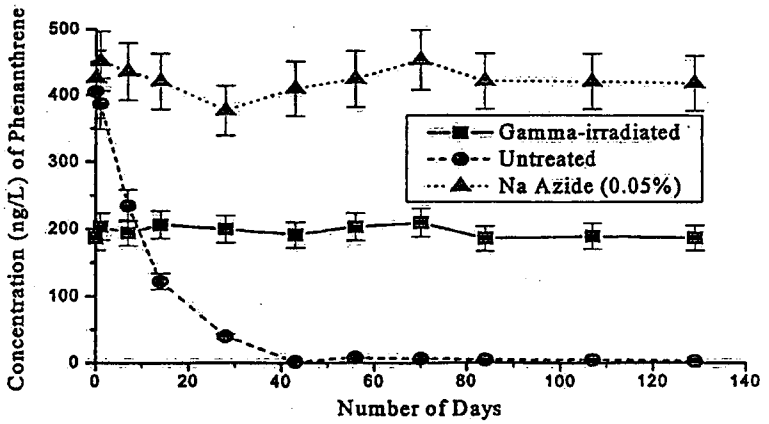


Fig. 2. Biodegradation of phenanthrene (with glass beads) by natural microbial flora in the MFTs pore water. Comparison of untreated, gamma-irradiated, and sodium azide (0.05%)-treated MFTs pore water.

within 1 year of the stoppage of fine tailings influx into a man-made pond system (Herman et al., 1993).

Ah Receptor and EROD Assays

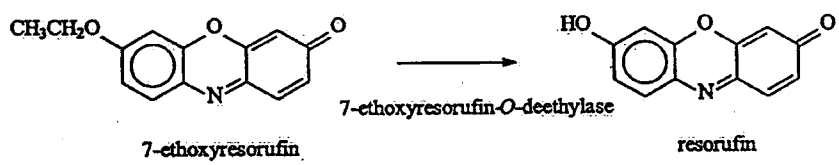
Many PACs induce the cytochrome P-450 monooxygenase enzymes by a complex mechanism that involves the initial binding of the xenobiotic to the intracellular Ah receptor protein (Bunce and Petrulis, 1998). Mutagenic and other toxic responses of PACs involve bioactivation through the P-450 system, converting the original xenobiotic to reactive intermediates such as epoxides (Josephy, 1997). We carried out an Ah receptor binding assay, in which individual PACs (and also concentrated pore water extracts) were allowed to compete in vitro with 10 nM [^{3}H]benzo[*a*]pyrene for a fixed aliquot of Ah receptor obtained from rat liver. Table VI shows the relative binding affinities of the PACs and gives 0.016 μg L^{-1} for the benzo[*a*]pyrene equivalence (BEQ) value.

Ligand binding to the Ah receptor is a necessary but not sufficient condition for inducing cytochrome P-450 (Petrulis and Bunce, 1999), and so we carried out EROD assays to show whether the PACs present in pore water were able to induce this enzyme. The EROD monitors the conversion of the synthetic substrate 7-ethoxyresorufin to the fluorescent derivative resorufin, catalyzed by cytochrome P-450 1A1 (Equation 1).

TABLE VI. Relative binding affinities of PACs by the Ah receptor assay

Compound	EC_{50} μM (RBA)[a]
Acridine	3.3 (0.003)
Benzo[*e*]pyrene	0.5 (0.021)
7,8-Benzoquinoline	1.0 (0.010)
Carbazole	1.5 (0.007)
Dibenzothiophene	4.1 (0.002)
Dibenzothiophene sulfone	2.8 (0.004)
2-Methylanthracene	0.4 (0.025)
9-Methylanthracene	0.4 (0.024)
1-Methylphenanthrene	1.5 (0.007)
3,6-Dimethylphenanthrene	1.2 (0.008)

[a]The reference radioligand was 10 nM [^{3}H]benzo[*a*]pyrene [EC_{50} = 10 nM, relative binding affinity (RBA) = 1.0]. For the 10^4-fold concentrated pore water extract, the calculated EC_{50} corresponded to a 1:65 dilution of the extract. Hence the BEQ of the 10^4-fold concentrated extract was 65 × 10 nM (0.65 μM), and the BEQ of the original pore water was 0.016 μg L^{-1}.



Equation 1

TABLE VII. EROD activities of PACs

Compound	EC_{50}	Highest EROD Activity (pmol/min/mg protein)[a]
Benzo[*e*]pyrene	Not reached	<2
7,8-Benzoquinoline	Not reached	<2
Carbazole	Not reached	<2
Dibenzothiophene	Not reached	<2
Dibenzothiophene sulfone	Not reached	<2
Anthracene	Not reached	<2
2-Methylanthracene	Not reached	<2
9-Methylanthracene	Not reached	<2
1-Methylphenanthrene	Not reached	<2
3,6-Dimethylphenanthrene	Not reached	<2
10^4-fold concentrated pore water extract	Not reached	<2 (10^2-fold concentrated)
Benzo[*a*]pyrene	2.0×10^{-7} M	46 (10^{-6} M)
TCDD (positive control)	1.9×10^{-11} M	50 (10^{-9} M)

[a]Values in parentheses in Column 3 are the concentrations of substance at which the highest EROD activity was observed. For the 10^4-fold concentrated pore water extract, the EC_{50} was not reached, even without dilution, and the highest EROD activity reached was less than 2 pmol/min/mg protein.

The assay involves the incubation of primary rat hepatocytes with the PAC or pore water extract for 24 h to allow time to induce cytochrome P-450 1A1, followed by addition of the substrate 7-ethoxyresorufin to the incubation mixture. After a further brief incubation, the rate of formation of resorufin is determined fluorimetrically. TCDD was used as a positive control. Of the PACs studied, only benzo[*a*]pyrene (B[*a*]P) gave a significant EROD response, and the responses of the concentrated pore water extracts were below the detection limit (Table VII). This result is consistent with the very low concentrations of the individual PACs in pore water (no compound above 1 μg/L, which corresponds to concentrations in the low nanomolar range); hence, we predict that the pore water from the oil sands mining operations should have undetectable EROD activity. Previous work (Petrulis and Bunce, 1999) has shown that PACs that are metabolizable by the hepatocytes display negligible EROD activity, because as soon as some P-450 1A1 is induced, it oxidizes the PAC, preventing further induction.

Mutagenicity Tests

The conversion of PACs into reactive metabolites by cytochrome P-450 catalyzed oxidation is implicated in mutagenesis and carcinogenesis (Amdur et al., 1991; Josephy, 1997). One might expect a parallel between the EROD activities and mutagenic potencies of individual PACs and of the whole pore water. Mutagenic potencies were determined using the Ames *Salmonella typhimurium* tester strains TA98 and TA100 and converted into benzo[*a*]pyrene equivalency factors (BEFs) (Madill et al., 1999). The total potency, or benzo[*a*]pyrene equivalence [BEQ, defined by Eq. (2)], was found to be 0.05 μg of B[*a*]P equivalents per liter of pore water [the value based on identified PACs in Table I was similar (0.14 μg of B[*a*]P equivalents per liter)]:

$$\text{BEQ} = \Sigma(\text{concentration of component } i) \times (\text{BEF of component } i) \quad (2)$$

Although other work from our laboratories (Brownlee et al., 1996) has shown positive mutagenic responses from the "solids" fraction of MFTs (with metabolic activation: +S9), we attribute this to the higher concentrations of the mutagenic four- to six-ring PACs in sediment. These PACs sorb strongly to sediment but have very low water solubilities.

CONCLUSIONS

Several PACs in MFTs pore water were characterized by GC–MS; overall concentrations were low. These PACs were found to be degradable by microorganisms naturally present in the pore water. Because of the difficulty of characterizing these PACs in pore water, several bioassays were used to exclude the possibility that important but unidentified toxic constituents might be present. The Microtox assay indicated that acute toxicity is associated principally with the naphthenic acid fraction of pore water extracts rather than with the base/neutral fraction; other work has shown that the acute toxicity dissipates fairly rapidly. Although Ah receptor binding assays indicated that some PACs present in pore water have moderate relative binding affinities compared with the reference toxicant B[*a*]P, the experimental and calculated activities of the pore water extracts were negligible in terms of benzo[*a*]pyrene equivalents. Even lower BEQ values are found in the EROD assay, because the PACs are metabolized by the hepatocytes competitively with the induction of cytochrome P-450 enzymes. The application of benzo[*a*]pyrene equivalency factors to the identified PACs suggested a slight mutagenic potency for MFTs pore water, but experimentally (Ames assay) this was below the limit of detection (Madill et al., 1999).

The importance of the present findings is that they do not suggest any impediment to developing the wet landscape option for the reclamation of oil sands fine tailings. The resulting artificial lakes are unlikely to show long-term toxicity toward aquatic life due to slow release of PACs from MFTs to the capping water. Comparison with work on the MFTs solids fraction suggests that the four- to six-ring PACs, which are of the greatest toxicological concern, are strongly sorbed to particulate matter and hence are unavailable in the water column. Although they could become bioavailable through ingestion by sediment-dwelling organisms, PACs are generally sufficiently reactive metabolically that significant distribution through the food web is unlikely.

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