Assessing the mechanisms of toxicity of oil sands process affected water by use of a genome wide live cell array reporter system

Garrett Morandi, 2Guan Miao, 2Hattan Alharbi, 3Xiaowei Zhang, 3Alberto dos Santos Pereira, 3Jonathan W. Martin, 1,4,5John P. Giesy, 1Steve Wiseman 1Toxicology Centre, University of Saskatchewan, SK, Canada, 2State Key Laboratory of Pollution Control & Resource Reuse, School of Environment, Nanjing University, Jiangsu province, Peoples Republic of China, 3Department of Laboratory Medicine and Pathology, Division of Analytical and Environmental Chemistry, University of Alberta, Edmonton, AB, Canada, 4Department of Veterinary and Biomedical Sciences, University of Saskatchewan, Saskatoon, SK, Canada, 5State Key Laboratory in Marine Pollution, Department of Biology and Chemistry, City University of Hong Kong, Hong Kong SAR, Peoples Republic of China, 6Department of Zoology and Centre for Integrative Toxicology, Michigan State University, East Lansing, MI, USA.

Competing Interests: None.

INTRODUCTION

Oil Sands Process Affected Waters (OSPW) • OSPW is a by-product from the extraction and separation of bitumen in the Alberta Oil Sands(1), • OSPW is restricted to a zero-discharge policy and requires remediation before release(2), • OSPW has acute and chronic toxicity to a range of species(3, 4), • Causative agents and mechanism of toxicity are unknown but studies have shown that the dissolved organic fraction of OSPW is acutely toxic(5, 6), • Naphthenic acids, class of cyclic and acyclic compounds with a -COOH functional group, have been proposed as the main toxic constituents of the organic fraction of OSPW. • Oxidative stress might contribute to the toxicity of OSPW (5, 4).

Escherichia coli K-12 MG-1655 gene reporter system • Open-format approaches for investigating the adverse effects of chemicals have gained popularity in recent year(5), • The Live Cell Array (LCA) system facilitates measurement of promoter activity by use of transcriptionally fused fast-folding fluorescent proteins(7), • E. coli MG-1655 system is composed of 1820 promoters in its genome.

OBJECTIVES

1. Perform an effects-directed analysis (EDA) of the organic fraction of OSPW to identify compounds responsible for acute toxicity.
2. Assess the molecular mechanisms of toxicity of fractions of organic compounds from OSPW by use of the E. coli MG1655 gene reporter system.
3. Analyze gene expression profiles by use of DAVID(8) to identify enriched pathways.
4. Combine acute toxicity data and gene enrichment profiles to compare molecular mechanisms of toxicity between toxic and non-toxic fractions.
5. Compare results with chemical profile of fractions to identify chemicals in OSPW that cause toxicity.

MATERIALS & METHODS

1. OSPW Fractionation and acute toxicity assays

• ROUND 1 FRACTIONATION

- Figure 1. Fractionation of the OSPW organics. Round 1 fractionation was sequential-solvent extraction at pH 2 and 7. Round 2 fractionation was liquid-liquid washing at pH 2 and 12. Acutely toxic fractions were identified using A) The Microtox® system, B) 96-h growth and lethality test Chironomus dilutus and C) 96-h embryotoxicity test Pimephales promelas. The OSPW was collected from the Base mine lake and is the first end pit lake in the industry.

- Figure 2. 3.00E+06 6.00E+06 9.00E+06 1.20E+07

Figure 4. Fraction induced response in the number of differentially expressed genes from three fractions of OSPW organics.

- Table 1. Fraction induced response in the number of differentially expressed genes from three fractions of OSPW organics.

- Figure 5. Total abundance of species by heteroratom class based on the sum of the peak areas in the chromatograms of fractions of OSPW, by use of Orbitrap Mass spectrometry: A) NEF and NEF2 in ES1; B) NEF1 and NEF2 in ES1.

CONCLUSIONS

• An acutely toxic fraction (NEF2) of OSPW was identified using a suite of bioassays and contains the enriched chemical classes: ES1-02, -O-S, -O-S and ES1+: -O, -O2-, -O, -ON, -On.
• Gene expression analysis identified two processes that responded to the toxic fractions (NE, NEF) of OSPW: • Functional enrichment of the electron transport chain (uqH2, trc, ahpF)
• Pathway analysis revealed significant responsiveness in the KEGG pathway (Kyoto Encyclopedia of Genes and genomes) ubiquinone and terpenoid biosynthesis: specifically affecting genes related to Oxidative Phosphorylation.
• UbIC, UbIC→>2-fold down-regulation.
• Ionizable compounds are known to effect the electron transport chain...a role for naphthenic acids contributing to toxicity.

FUTURE WORK

• Continue EDA and fractionate NEF2 fraction.
• Screen fractions of NEF2 by use of LCA system.
• Investigate role of electron transport chain toxicity of BML-OSPW.

REFERENCES


ACKNOWLEDGEMENTS

1. Collaborative Research and Development Grant from the Natural Science and Engineering Research Council of Canada and Syncrude Canada Ltd. (NSERC-CRS) to J.P. Giesy and JW Martin.
2. Discovery Grants from the Natural Science and Engineering Research Council of Canada (NSERC) to J.P. Giesy.
4. Grants from the Helmholtz Association (Helmholtz) to J.P. Giesy.
5. J.P. Giesy was supported by the Canada Research Chair program, and an at large Research Chair at the Department of Biology and Chemistry and State Key Laboratory in Marine Pollution, City University of Hong Kong.
6. Syncrude Canada Ltd. for supplying the OSPW.