Effect of Oil Sands Process Water on Activity of Drug Efflux Transporters in Caco-2 Cells and Japanese Medaka

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BACKGROUND
- Oil sands process affected water (OSPW) is generated during extraction of bitumen from oil sands during surface mining.
- Fresh OSPW is acutely toxic to aquatic organisms. As OSPW ages in tailings ponds potency for acute toxicity decreases.
- Aged OSPW has chronic toxicity to aquatic organisms.
- Crude oil can inhibit cellular detoxification by inhibition of the activity of ABC transporters proteins that efflux parents or metabolites of organic compounds from cells.
- Members of the ABC family of proteins include P-glycoproteins (P-gp), multiple drug resistance proteins (MDR), and multidrug resistance-associated protein (MRP).
- It is not known if OSPW affects activity of ABC proteins.

OBJECTIVES
Determine if organic chemicals in fresh and aged OSPW affect activity of ABC proteins.

METHODS

OSPW was collected from two sources:
- Base Mine Lake (BML) – The first experimental end pit lake constructed in the oil sands industry. Established in 2013.
- TPW - Experimental reclamation pond constructed in 1993.

CACO-2 ASSAY
- Intestinal epithelial cells that express several ABC proteins, including MDR1/P-GP.

RESULTS – CACO 2 CELLS
Figure 3. Fold change accumulation of calcein-AM in Caco-2 cells exposed to different concentrations of the dissolved organic fraction relative to control of (A) Verapamil (positive control), B) BML-OSPW, and (C) fractions of BML-OSPW (** p < 0.5, *** p < 0.1, *** p < 0.01).
- Dose-dependent increase in accumulation of calcein-AM in cells exposed to different concentrations of, verapamil, an inhibitor of MDR1/P-gp.
- Dose-dependent increase in accumulation of calcein-AM in cells exposed to different concentrations of the dissolved organic fraction of BML-OSPW.
- Accumulation of calcein-AM was greater in cells exposed to the acid, basic, or neutral fractions of the dissolved organic fraction of BML-OSPW. Accumulation of calcein-AM was greatest in cells exposed to the basic or neutral fractions.

RESULTS – JAPANESE MEDAKA FRY
Figure 4. Fold change accumulation of calcein-AM in fry of Japanese medaka exposed to (A) MK571 (positive control), (B) different concentrations of the dissolved organic fraction of BML-OSPW or TPW-OSPW, and (C) fractions of BML-OSPW relative to control (** p < 0.5, *** p < 0.1, *** p < 0.01).
- Accumulation of calcein-AM was greater in fry exposed to 5 µM of MK571, an inhibitor of MRp activity.
- Accumulation of calcein-AM was greater in fry exposed to the dissolved organic fraction of BML-OSPW but not TPW-OSPW.
- Accumulation of calcein-AM was greater in fry exposed to 5 × of the dissolved organic fraction of BML-OSPW.
- Accumulation of calcein-AM was greater in fry exposed to the neutral or basic fractions, but not the acid fraction, of BML-OSPW.

DISCUSSION
- Dissolved organic chemicals in fresh OSPW collected from Base Mine Lake, inhibited activity of MRp proteins in vitro and in fry of Japanese medaka.
- Inhibition of MXR activity in fry of Japanese medaka occurred only at concentrations of the dissolved organic fraction of BML-OSPW that were greater than found in the BML end pit lake.
- Aging of OSPW attenuates this effect as accumulation of calcein-AM was not greater in fry of Japanese medaka exposed to the dissolved organic fraction of OSPW from the TPW experimental reclamation pond.
- Chemicals that cause the effect of the dissolved organic fraction of BML-OSPW are found in neutral and basic fractions, but the identities of these compounds are not known.

FUTURE WORK
- Chemical structures of the major components of basic and neutral fractions are being elucidated to understand structure-activity relationships.
- Polycyclic aromatic hydrocarbons (PAHs), which are found in tailings ponds, are excreted by ABC proteins. Studies are being conducted to determine if dissolved organic compounds in BML-OSPW impair excretion of metabolites of PAHs from cells, and whether this effect might have toxicological implications.

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