In vitro assessment of pH-dependent uptake and toxicity of ionizable organic chemicals in fish

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Background and objectives

• Uptake and effects of ionizable organic chemicals (IOCs) in fish can differ as a function of ambient pH.
• As one example, the acute toxicity of oil sands process affected water (OSPWs) is ~ together with several non-polar chemicals – often associated with acidic chemicals, among others naphthenic acids.
• The goal of this study was to evaluate if permeation of some charged species from OSPWs can be used to screen for pH-dependent uptake of IOCs more rapidly than is currently achievable using in vivo.

Results: cytotoxicity of weak acids and fractions of OSPWs

• The speciation of ionizable organic chemicals (IOCs) depends on its acid dissociation constant (Ka) and the ambient acidity (pH value).
• Uncharged (neutral) species can easily permeate through lipid bilayers, while the charged species permeates to a significantly lesser extent.
• Paracellular permeability (i.e. through the paracellular space in between cells) is reduced by tight junctions, which are protein complexes that seal this gap.
• Under some conditions, weak acids can become trapped within cells ("ion trap" effect).

Results of chemical LC-HRMS analysis

• Less than 50% of the chemicals found in reconstituted OSPW following extraction from exposure media at non-cytotoxic concentrations (143 chemicals, m/z 118 to 355) in in vitro-cultured gill cell monolayers could cross membranes.
• Out of these, only two showed a monotonic decrease in permeability with increasing pH.
• Surprisingly, with increasing pH there was an increase in the permeability of 11 chemicals.
• Chemicals that caused pH-dependent cytotoxicity might have been retained within cells due to "ion trapping". Thus, we currently examine internal concentrations of chemicals in extracts of cells.

Conclusions

• The in vitro assay can be used to screen for pH-dependent uptake and toxicity of IOCs in fish cells.
• Thus, there is potential for this assay to be used as a tool for in vitro-in vivo extrapolation, and prioritization of chemicals in non-target chemical screenings.
• Permeation of some charged species from OSPWs at higher pH values may be explained by active transport, which currently is being investigated by use of a pharmacological approach.

Next steps: exposure of rainbow trout to OSPWs at various ambient pH values

• We will expose rainbow trout fry to diluted OSPWs for 7 days directly after swim-up while the ambient pH will be systematically varied (6.0, 7.4, 8.5) and concentrations of IOCs found in OSPWs determined in fry by means of LC-HRMS.
• Based on this data, together with previously determined partitioning coefficients, we will develop a model for in vitro-in vivo extrapolation of IOC uptake across fish gills.