University of Prince Edward Island

Faculty of Veterinary Medicine
Summary of Dissertation

Submitted in Partial Fulfilment
of the Requirements for the

DEGREE OF MASTER OF SCIENCE

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Mechanisms of Naphthenic Acid Toxicity

Most of Alberta's crude oil is extracted via surface mining, producing large volumes of waste water or oil sands process-affected water (OSPW). This water cannot be released, resulting in the creation of tailings ponds, which contain numerous toxic compounds. Naphthenic acids (NAs) are considered one of the principle toxicants in OSPW. These carboxylic acids have been shown to be acutely toxic and have a number of sublethal effects on multiple organisms. Despite this knowledge, the mechanism of action of NAs has remained elusive. Based on the known mechanisms of resin acids, carboxylic acids from pulp and paper mill effluent, it was hypothesized that NAs would disrupt mitochondrial energetics by uncoupling oxidative phosphorylation (OXPHOS), inhibiting the electron transport system (ETS), and causing oxidative stress represented by increased reactive oxygen species (ROS). Two model adamantane NAs, 3,5-dimethyladamantane-1-carboxylic acid and 3-hydroxy-adamantane-1-carboxylic acids, were also examined to determine if they were suitable surrogate toxicants for NA mixtures. For this thesis, NAs were extracted and purified from a 17-year-old tailings. Mitochondria were isolated from rainbow trout (Oncorhynchus mykiss) livers and the mitochondrial oxygen consumption, membrane potential, and hydrogen peroxide production were measured simultaneously using the Oroboros respirometry system following exposure to different doses of NAs. Real-time flow cytometry was used to measure mitochondrial membrane potential and oxidation state. Results showed that OSPW-derived NAs were capable of completely inhibiting the ETS and dramatically increasing ROS. Oxidative uncoupling of mitochondria also occurred, but to a lesser degree than ETS inhibition and ROS stimulation. Adamantane acids showed certain similar effects to the OSPW-NA mixture, suggesting that some adamantane compounds may be able to act as toxicant surrogates. In conclusion, the mechanism of action of NAs appears to be complex and these compounds likely act via multiple mechanisms, wherein mitochondria may be an important target.