Ecotoxicology and Environmental Safety 124 (2016) 285–295

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

# Small mammals as sentinels of oil sands related contaminants and health effects in northeastern Alberta, Canada

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ARTICLE INFO

*Article history:*
Received 11 August 2015
Received in revised form
28 October 2015
Accepted 1 November 2015

*Keywords:*
Oil sands
Wildlife
Biomarkers
Bioindicators
Reclamation

ABSTRACT

The extraction of bitumen in areas of northeastern Alberta (Canada) has been associated with the release of complex mixtures of metals, metalloids, and polycyclic aromatic compounds (PACs) to the environment. To mitigate effects on ecosystems, Canadian legislation mandates that disturbed areas be reclaimed to an ecologically sustainable state after active operations. However, as part of reclamation activities, exposure to, and effects on wildlife living in these areas is not generally assessed. To support successful reclamation, the development of efficient methods to assess exposure and health effects in potentially exposed wildlife is required. In the present study, we investigated the usefulness of two native mammalian species (deer mouse *Peromyscus maniculatus*, and meadow vole *Microtus pennsylvanicus*) as sentinels of oil sands related contaminants by examining biomarkers of exposure and indicators of biological costs. Tissue residues of 31 metals and metalloids in kidneys and muscle, activity of the hepatic detoxification enzyme EROD (as a biomarker of exposure to organic contaminants), body condition, and the relative mass of liver, kidney, spleen, and testes were compared in animals from one reclaimed area and a reference site. Deer mice from the reclaimed site had higher renal levels of Co, Se and Tl compared to animals from the reference site, which was associated with reduced body condition. Lower testis mass was another feature that distinguished mice from the reclaimed site in comparison to those from the reference site. One mouse and one vole from the reclaimed site also showed increased hepatic EROD activity. In marked contrast, no changes were evident for these variables in meadow voles. Our results show that deer mouse is a sensitive sentinel species and that the biomarkers and indicators used here are efficient means to detect local contamination and associated biological effects in native mammals inhabiting reclaimed areas on active oil sands mine sites. These field-derived findings can be used by risk assessors to fill possible data gaps for mammalian wildlife in science-based environmental risk assessments for oil and gas projects.



## 1. Introduction

Extraction and processing of bitumen from Canadian oil sands, whose production is projected to steadily increase by an average of 4% annually until 2030 (CAPP, 2014), pose growing environmental concerns. Besides loss of habitat and subsequent reduction in ecological function associated with mining activities (Johnson and Miyanishi, 2008), extraction of bitumen results in the release of complex mixtures of metals, metalloids, and polycyclic aromatic compounds (PACs) to surrounding water bodies, air, soils and vegetation (Kelly et al., 2010; Bari et al., 2014; Cho et al., 2014). Due to the persistence, bioaccumulative properties, and potential toxicity of many of these contaminants, which occur naturally in oil sands deposits, threats to wildlife and human health are possible.

Once energy resources have been extracted on the oil sand leases of northeastern Alberta, Canadian legislation mandates that the disturbed areas be reclaimed to an ecologically sustainable state (Johnson and Miyanishi, 2008). This implies the re-establishment of functional ecosystems, which can support traditional uses, diverse wildlife habitats, recreation, and possibly commercial forestry. However, government regulations have vague standards on "equivalent land capability" (Alberta Environment and Sustainable Resource Development, 2013). As required under the *Environmental Protection and Enhancement Act*, operators apply for a reclamation certificate once reclamation is complete.

Wildlife monitoring programs form part of environmental risk assessments (ERAs) to evaluate the efficacy of reclamation practices to ensure stable, productive, and self-sustaining ecosystems

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http://dx.doi.org/10.1016/j.ecoenv.2015.11.001
0147-6513/

(Alberta Environment, 2010; Hawkes et al., 2011). Several mammalian, avian, and amphibian species have been identified as sentinels, which are organisms suitable to assess effects of local contaminants and allow predictions about implications for domestic animal and human health (Smits and Fernie, 2013). However, to the best of our knowledge, ERAs conducted on reclaimed areas at the oil sands region do not typically evaluate the potential toxic effects on local exposed wildlife. Yet, reclaimed sites are adjacent to active surface mining and upgrading activities, acting as receptors of important loads of airborne pollutants (Kelly et al., 2010; Bari et al., 2014; Cho et al., 2014). Studies reporting exposure and health effects of oil sands related contaminants in wildlife on or near reclaimed wetlands, include birds (e.g. Cruz-Martinez et al., 2015), amphibians (e.g. Hersikorn and Smits, 2011), and fish (e.g. Van Den Heuvel et al., 1999). However, no investigations have examined native terrestrial mammals. Small mammals (rodents), which are early successional terrestrial wildlife colonizing reclaimed lands (Hawkes et al., 2011), are considered useful, sensitive, and accessible sentinels to evaluate ecological and health risks from pollutants (O'Brien et al., 1993).

Previously, we conducted an experiment using laboratory mice as surrogates for small mammals in the wild to evaluate a mixture of metals (Pb, Cd, and Hg) and alkyl-PAHs at environmentally relevant concentrations in the oil sands region (Rodríguez-Estival et al., in press). Biomarkers of exposure (metal residues in kidneys and hepatic detoxification enzyme induction) were studied together with morphometric evaluations and biomarkers of oxidative stress which served as physiologically relevant, subclinical responses to contaminant exposure. Toxicological effects in the mice were evident through reduced organ and body size, as well as changes in homeostasis of dietary antioxidants (vitamins A and E in liver) and the glutathione antioxidant system in the testes. In the present study, we investigated the value of native small mammals on one reclaimed area in the oil sands as sentinels of contamination through using similar measures that proved sensitive in our previous experimental trial. Residues of essential and non-essential trace elements in the kidneys (the main organ for excreting metals) and muscle tissue reflected exposure to metallic inputs. The muscle was analyzed because of interest in the potential transfer of pollutants to muscle tissue more commonly consumed by humans, considering that small mammals are sentinels of other large, terrestrial herbivores such as game species. The induction of the hepatic detoxification enzyme, ethoxyresorufin-*O*-deethylase (EROD), was a biomarker of exposure to organic compounds such as PACs. Finally, changes in body condition and relative organ mass reflected the animal's overall health and quality. We hypothesized that small mammals on the reclaimed site compared with those on a local reference site would be exposed to higher contaminants from adjacent mining activities, which would be illustrated by related metals and metalloids in tissues, induction of EROD activity (reflecting exposure to organic contaminants), and by alterations in body condition and organosomatic indices. The ultimate goal of this research is to contribute data to fill the data gap for mammalian wildlife in science-based ERAs, and to support the future development of wildlife indicators for the Criteria and Indicators Framework for Oil Sands Mine Reclamation Certification (Alberta Environment and Sustainable Resource Development, 2013).

## 2. Materials and methods

### 2.1. Study area

This study was carried out in June 2014 on the Athabasca Oil Sands Region of northeastern Alberta, Canada, the world's largest active oil sands operations and surface mines (Cho et al., 2014). Within this region, one post-mining, reclaimed site adjacent to current mining and processing activities (M4), and one local but primarily up-wind reference site (REF) were selected. The reclaimed site on a company lease belonging to one operator, was within ~2 km of active surface mining and upgrading, and was reclaimed and planted 4 years previously. The predominant landscape, habitat conditions, and vegetation stage in this site is dominated by native plant species including herbs, forbs, shrubs, lichens, and some dispersed young trees (primarily aspen, *Populus tremuloides*) that have naturally invaded after initial plantations (Alberta Environment, 2010). The reference site (REF) was on public land ~40 km NE and generally upwind from the reclaimed site and any active mining activity. The REF was ~15 km NE from the "North QA site" designated as their "quality assurance" reference site by the atmospheric chemists Bari et al. (2014), and therefore is expected, as much as possible, to be beyond the range reportedly affected by airborne deposition of most oil and gas related contaminants. The selected REF was in an area burnt out by a massive wildfire in April–July 2011 (4 years ago), resulting in a successional vegetation stage essentially the same as at M4. Our reference site was within the geological area containing natural oil sands deposits, and lies within the same natural macroregion as the reclaimed site, matching the key criteria in the Reference Condition Approach for Monitoring Reclamation Areas in the Athabasca Oil Sands Region, a document which guides selection of reference sites for ecotoxicological research (Ciborowski et al., 2013).

### 2.2. Small mammal trapping and sample collection

We focused on two species of small mammals, deer mouse (*Peromyscus maniculatus*) and meadow vole (*Microtus pennsylvanicus*), which satisfy biological and technical criteria as excellent sentinels for human and environmental health risks in the terrestrial ecosystems affected by oil sands in northeastern Alberta (Hawkes et al., 2011). The trapping was carried out according to the method described by Hawkes et al. (2011) for live-trapping small mammals with some modifications. The handling and trapping protocol followed the standards for the ethical and humane treatment of animals (Animal Care Committee of the University of Calgary protocol AC14-0084), and were carried out with the Wildlife Research Permit (#54803) and Collection License (#54804), Environment and Sustainable Resource Development Division of the Government of Alberta (Canada).

Small mammals were trapped using Longworth style live traps baited with a mixture of peanut butter, oatmeal, and water. Bedding material was provided to avoid trap mortality from low night temperatures. At both REF and M4, traps were arranged in $6 \times 10$ grids (with the traps placed at 5–7 m intervals, unless terrain or circumstances dictated another arrangement), were checked twice daily (morning and late-afternoon), and were re-set/re-baited as necessary throughout the sampling period. Post-trapping, the captured rodents were placed into individual containers and immediately transported to laboratory facilities improvised in the field near the trapping sites.

Animals were placed into a bell jar and anaesthetized with a cotton swab soaked with the inhalation anesthetic, isofluorane (Baxter Corporation, Mississauga, ON, Canada). Once anaesthetized, they were weighed to the nearest 0.01 g using a digital balance, and the total length (body + tail, measured separately and together) to the nearest 0.1 mm using digital callipers. Animals were then euthanized by exsanguination via cardiac puncture, followed by cervical dislocation. Blood was immediately transferred into cryovials. The liver, kidneys, spleen, and testes were dissected and weighed to the nearest 0.001 g. Stomach contents,