Potential to Use Animals as Monitors of Ecosystem Health in the Oil Sands Region – July 2013 Update

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Oil Sands Research and Information Network

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The Oil Sands Research and Information Network (OSRIN) is a university-based, independent organization that compiles, interprets and analyses available knowledge about managing the environmental impacts to landscapes and water impacted by oil sands mining and gets that knowledge into the hands of those who can use it to drive breakthrough improvements in regulations and practices. OSRIN is a project of the University of Alberta's School of Energy and the Environment (SEE). OSRIN was launched with a start-up grant of $4.5 million from Alberta Environment and a $250,000 grant from the Canada School of Energy and Environment Ltd.

OSRIN provides:

- **Governments** with the independent, objective, and credible information and analysis required to put appropriate regulatory and policy frameworks in place
- **Media, opinion leaders and the general public** with the facts about oil sands development, its environmental and social impacts, and landscape/water reclamation activities – so that public dialogue and policy is informed by solid evidence
- **Industry** with ready access to an integrated view of research that will help them make and execute environmental management plans – a view that crosses disciplines and organizational boundaries

OSRIN recognizes that much research has been done in these areas by a variety of players over 40 years of oil sands development. OSRIN synthesizes this collective knowledge and presents it in a form that allows others to use it to solve pressing problems.

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REPORT SUMMARY

This review is focused on the effects of contaminants on wildlife and the potential for using wildlife as sentinels for human and environmental health. Some wildlife are permanent residents of the boreal forest encompassing the oil sands region, while many others are seasonal residents using this area as breeding grounds (i.e., migratory birds), both providing the potential for ongoing research into the biological effects of contaminants from oil sands activities.

Wildlife species may act as sentinels, or early warning systems, providing insight into the effects of contaminants on environmental and even human health. In the oil sands, both field and laboratory studies have used wildlife as bioindicators and/or sentinels of ecosystem health. The great majority of this research has focused on aquatic ecosystems and organisms.

Fish exposed to oil sands process affected water (OSPW), or water plus sediments from tailings ponds, and water from wetlands receiving oil sands effluents, have shown a range of detrimental physiological effects including increased detoxification activity by the liver, alterations in growth, hormonal disruption, abnormalities in hematological variables, pathologic changes in the gills, and increased mortality rates relative to fish from reference sites. Studies indicate that mature, reclaimed wetlands in the oil sands, those seven years or older, can support viable populations of locally important amphibians, whereas the younger wetlands retain toxic compounds which cause detrimental health effects such as decreased survival, delayed development, and increased rates of malformation.

Most research on birds has also focused on aquatic toxicology. Growth alterations (smaller skeletal size and body mass) have been reported in waterfowl raised on wetlands receiving oil sands effluent. Migrating waterfowl are at risk of landing on tailings ponds with floating bitumen, despite considerable efforts to design and deploy deterrent systems, and mass mortalities have resulted. Increased levels of mercury (Hg) in the eggs of water birds, and a positive correlation between Hg and naphthenic acid (NA) concentrations, suggests a common source of exposure for birds nesting on lakes that receive water from the Athabasca River downstream from the oil sands region. On the mine lease areas, reclaimed wetlands have in most years supported active populations of tree swallows during the breeding period and rearing of the offspring. However, stochastic events such as many days of cold, wet weather can cause severe stress resulting in high mortality rates. Studies of risk to mammals from tailings pond water suggest that terrestrial wildlife is unlikely to develop acute toxicity from NA exposure, although negative health effects may occur from repeated, or long-term exposures.

We have identified a conspicuous gap in knowledge related to effects of airborne contaminants on any species. Birds may prove especially valuable as sentinels because of the unique anatomy and physiology of their respiratory system (birds are more sensitive to airborne contaminants than mammals of similar size). As well, as pointed out in the report from the Royal Society of Canada, "quantifying these emissions is notoriously difficult and the data available in the National Pollutant Release Inventory on this subject do not provide enough detail to know what sources have been estimated nor how valid the numbers are"; and, "the subject of non-point

(fugitive) emissions of air contaminants from mines and tailings ponds is highly uncertain and currently available estimates are unlikely to be entirely valid".

One approach to better understand the effects of emissions on wildlife (and warm-blooded animals in general) could be through research on birds of prey (raptors). Raptors such as the American kestrel (*Falco sparverius*) could provide integrated insight into food web, as well as air borne exposure to environmental contaminants over time. Together with concurrent studies of their prey species, such as small mammals inhabiting reclaimed terrestrial areas, this type of work has the possibility of generating information relevant to the health of a range of animals in that ecosystem. Other studies of wildlife sentinels of ecosystem health could be based on herbivores. Domestic sheep and goats could serve as surrogates for caribou, moose and other ungulates naturally found in this region, for assessing health effects from deposition and accumulation of particulate air contaminants on vegetation. A final option would be to use small mammals such as mice and voles as sentinels of ecosystem health. Such species reflect the quality and quantity of local vegetation, readily populate any available area and serve as food for mammalian and avian predators.

For the oil sands as well as other petroleum producing regions, major emissions of interest are volatile organic compounds, hydrogen sulfide, sulphur dioxide, nitrogen dioxide, ozone and particulate matter, whereas aquatic contaminants related to the petrochemical industry are polycyclic aromatic hydrocarbons, naphthenic acids, sulphate ions, ammonia and trace metals. Once in the environment, complex interactions among contaminants and substrates along with inherent chemical characteristics will determine the fate of these compounds.

Extraction and production of bitumen from the oil sands produces compounds of environmental concern in the form of emissions perceived to pose risks to flora and fauna in local and downwind regions, and in the form of great volumes of liquid tailings. Research on wildlife species, used as either monitors, or indicator species, can provide early warning and predictive information regarding exposure and effects of contaminants from oil sands activities that would complement the huge ongoing investment into air and water monitoring systems.

Appendix 2 (added in Jul 2013) provides a summary of findings from a 2012 study of tree swallows with a focus on air-borne compounds, using these insectivores as sentinels, as described above.

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1 INTRODUCTION

The purpose of this report is to assess the potential of using wildlife species as monitors of ecosystem health in the oil sands region. The report summarizes current publications regarding the effects of contaminants released from the oil sands region on wildlife including mammals, birds, fish and amphibians.

This work is based heavily on peer-reviewed scientific papers, but also includes important information available through other major reports.

The production from the oil sands in northern Alberta makes this province the largest producer of crude oil in Canada. The major deposits are found in the Athabasca, Peace River and Cold Lake regions (Simpson et al. 2010). The estimated reserves found in these deposits are over 170 billion barrels of recoverable oil, comprising 30% of the world's oil reserves making them the third largest, after Saudi Arabia and Venezuela. In 1975, the Alberta Oil Sands Environmental Research Program (AOSERP) was created to coordinate research projects focused on potential environmental effects derived from oil sands development (Anderson et al. 1979). Early research focused on the effects of metals on fish (Sprague et al. 1978), and the effects of tailings on invertebrates including arthropods (Hilchie and Ryan 1980), leeches and worms (Duguay 1997). For more information about early environmental and toxicological research on the oil sands, refer to Jantzie (1977) and Jantzie et al. (1979).

1.1 Characteristics of Wildlife as Monitors of Ecosystem Health

Wildlife species play a role as indicators, monitors and sentinels of environmental health. While these three concepts are often used interchangeably, 'indicator' and 'monitoring' species describe those used to detect the presence and effects of contaminants in their environment. A sentinel species, on the other hand, is defined by the National Research Council as "an animal system used to identify potential health hazards to other animals or humans" (Glickman et al. 1991).

There are desirable characteristics that wildlife species must have to be useful and valuable as sentinels of human and environmental health (Figure 1). For example, they must fulfill relevant biological characteristics such as being sensitive to a particular contaminant so exposure will produce measurable biological effects. Sentinels must also fulfill methodological characteristics such as being practical to sample, tolerant to interference if they are not being lethally sampled, sampling must be cost effective, and it must be of interest to the public and public policy makers (Basu et al. 2007, Burger 2006). Animals such as birds of prey, caribou, bears and other large mammals capture the imagination and interest of the general public, more than invertebrates and lower life forms. Importantly, in the last decade there has been considerable effort on behalf of some researchers to develop non-lethal and minimally invasive techniques for collecting detailed biological information on subclinical (subtle) toxicity (Stebbins et al. 2009). These studies require larger bodied animals that can be handled and tested multiple times with minimal "investigator impact".

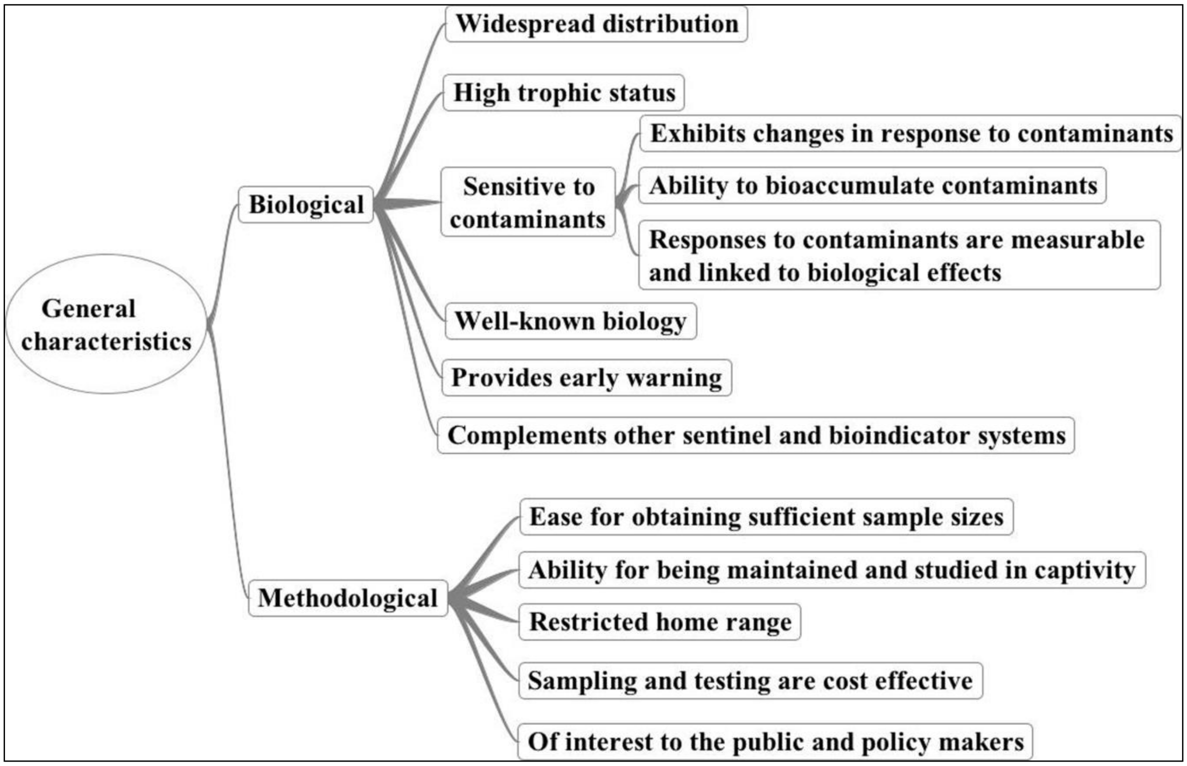

Figure 1. Characteristic proposed for animals to be useful sentinels of human and environmental health

Figure created from Basu et al. 2007, Burger 2006, Hollamby et al. 2006

A wide range of species is reported in the literature being used as sentinels, including avifauna, mammals, fish, amphibians and invertebrates. For further description and discussion on the value of animals as sentinels for human, animal and environmental health, see Basu et al. 2007 and references therein. Specific examples of sentinel wildlife, birds, amphibians and fish inhabiting the oil sands region are discussed in section 5.

1.2 Overview of Ecosystem Heath Impacts and Concerns

Evaluating ecosystem health requires a transdisciplinary approach in which a number of specialists collaborate with the overall aim of improving human health and well-being. The idea is to assess the resilience, organization and vitality of the ecosystem (Connell 2010). The main purpose of an ecosystem health approach is to "better understand the connections between nature, society and health, and how drivers of social and ecosystem change will ultimately also influence human health" (Wilcox et al. 2004). Examples of involved disciplines are ecology, biology, epidemiology, human medicine and public health, veterinary medicine, ecotoxicology, social and political sciences.

Collaborative interaction among experts is key for ecosystem health related research. Basu and coworkers describe one approach to studying ecotoxicology which entails measuring contaminant concentrations in certain biota, and then extrapolating what the potential effects may be of similar concentrations to other species (Basu et al. 2007). They base projections of effects on standard toxicological criteria such as the lowest observed adverse effects levels (LOAEL) and tissue residue value (TRV). To give an example from the oil sands, researchers analyzed mercury (Hg) concentrations in walleye from the lower Athabasca River. From these data, they noted that the concentration of this metal in walleye had increased over 29 years, and determined that these concentrations represent a human health risk since they exceed the subsistence fisher consumption guidelines from both the US EPA and Health Canada (Timoney and Lee 2009).

While measuring contaminant concentrations is of value, research on wildlife focused on assessing biological effects of contaminants provides a more integrated and meaningful understanding of what the presence of these contaminants actually do, biologically, to local species (Basu et al. 2007, Gentes et al. 2006, Hersikorn and Smits 2011, Pollet and Bendell-Young 2000). The study of physiological effects in sentinel biota would be useful for applications in veterinary and human medicine, ecotoxicology and public health.

The advantage of a multi-disciplinary approach is that it fosters a "mindset open to new avenues of inquiry into human health and well-being, allowing investigators to see opportunities that are otherwise obscured by disciplinary inquiry" (Connell 2010). Society is finally recognizing that the collaboration between biological and social sciences provides a key component for understanding and for implementing responses to the human-induced changes in ecosystems (Wilcox et al. 2004).

1.3 Potential to Act as Sentinels of Human Health

Animal models have long been used in scientific research across many disciplines. In the 19th century animals were used as the standard for mine safety (caged canaries were kept in coal mines to detect carbon monoxide and methane) (Brown et al. 1997). The vast majority of animal research has the ultimate goal of extrapolating the results for application on, and to the benefit of humans. Most toxicology studies aim to predict how the observed effects of toxic compounds on animals might affect humans.

For example, NO_2 was used as a test compound for developing a scaling method to predict effects in people based on data from dogs, guinea pigs, mice, rabbits and rats (Book 1982). Alarie (1981) demonstrated that an animal bioassay evaluating the irritating properties of a number of chemical substances yielded a good correlation for predicting safe levels of exposure for humans. Somers et al. (2004) used a sentinel mouse model to test the potential toxicity of urban air pollution in an industrial city. Mice were held outside in cages with, or without a high-efficiency particle air (HEPA) filtration unit. After finding elevated mutation rates in the mice without the HEPA filters, Somers could predict that air pollution, particularly exposure to particulate matter, posed a genetic risk for humans and wildlife.

The use of animals as sentinels for risk to human health is not without controversy. Stahl (1997) recommends caution regarding the use of mammalian and non-mammalian species as sentinels for human health due to the problems inherent in extrapolating data from animals to complex human health endpoints such as cancer. This researcher recommends conducting appropriate toxicological studies (using laboratory animals) under controlled conditions instead of depending on other species. However, many researchers emphasize the great value of sentinel species as early warning systems. They propose that study designs can be improved so they address epidemiological criteria that could prove causality between exposure to contaminants and health outcomes. Well-designed research can enhance our understanding of the link between exposure and effects of environmental contaminants in wildlife and permit prudent extrapolation to human health (O'Brien et al. 1993, Rabinowitz et al. 1999).

To strengthen and add value to studies using wildlife as sentinels of environmental health, novel methods for data collection such as geographical information systems (GIS) and mathematical modeling techniques can help overcome some of the challenges with interspecific extrapolations (Chion et al. 2011, Green et al. 2006, McLane et al. 2011).

2 CHEMICALS OF CONCERN

2.1 Air

Of the many chemicals of concern in air, special attention is placed on the following substances:

1. volatile organic compounds (VOCs) that are primarily represented by benzene, toluene, ethylbenzene, and the three isoforms of xylene (commonly collectively referred as to BTEX)
2. hydrogen sulfide (H_2S) known also as sour gas
3. sulphur dioxide (SO_2)
4. ozone (O_3)
5. nitrogen dioxide (NO_2), and
6. particulate matter (PM).

2.1.1 *Volatile Organic Compounds*

Volatile organic compounds (VOCs) are a broad collection of chemical substances composed of different numbers of carbon atoms. The volatility of this family of compounds is due to their high vapour pressure; therefore VOCs exist as gases in the atmosphere at ambient pressure (Gosselin et al. 2010).

2.1.1.1 Benzene

The commercial sources of benzene are derived from petroleum (petrochemical and petroleum refining industries) and from coal. In the United States, benzene is one of the most abundant chemicals produced (Wilbur et al. 2008). In the Athabasca oil sands, benzene was among the top 20 substances released to the air in the largest quantities in 2009 (Table 1).

Table 1. Top 20 Substances Released to Air for 2009 Reported to the NPRI by Oil Sands Operations.

Data were reported to National Pollutant Release Inventory (NPRI) by Oil Sands operators (table provided by Cristian Mihelle, Air Quality Research Division, Environment Canada).

Rank	*Substance*	*Releases to Air (Tonnes)*
1	Sulphur dioxide	115,295
2	Volatile Organic Compounds (VOCs)	43,914
3	Nitrogen oxides (expressed as NO_2)	40,157
4	Carbon monoxide	21,593
5	PM - Total Particulate Matter	5,668
6	PM_{10} - Particulate Matter <= 10 Microns	3,512
7	Xylene (all isomers)	2,946
8	$PM_{2.5}$ - Particulate Matter <= 2.5 Microns	1,856
9	Toluene	1,519
10	Hydrogen sulphide	878
11	n-Hexane	600
12	Ammonia (total)	599
13	Ethylbenzene	560
14	1,2,4-Trimethylbenzene	534
15	Sulphuric acid	434
16	Cyclohexane	291
17	Carbonyl sulphide	166
18	Propylene	160
19	Carbon disulphide	147
20	Benzene	124

Benzene (C_6H_6) is a chemical substance used extensively in industrial processes worldwide. In warm-blooded vertebrates, the most common toxic effects associated with benzene are hematotoxicity (i.e., disruptions of the blood-forming tissue), and cancer of white blood cell lines (leukemia) (Smith 2010, National Toxicology Program 1986). For this substance to be toxic, it must first be metabolized by exposed animals (Smith 2010). This metabolism is a two-step process which begins when benzene is taken up by the liver where, during phase I reactions, a family of detoxification enzymes referred to as cytochrome P450s, oxidize benzene into soluble metabolites including phenol, catecol, hydroquinone, and *t,t*-muconic acid (Smith 2010).

Subsequently, phase II reactions (secondary metabolism that normally occurs in the liver) of these metabolites occurs also in bone marrow where reactive oxygen species are then produced exacerbating the toxic effects on this tissue (Atkinson 2009).

The carcinogenic mechanisms of benzene remain unknown (Smith 2010), however, a combination of events including inhibition of cellular replication, oxidative stress, breakage of DNA strands, together with exposure factors (duration and frequency of exposure, and concentration of metabolites) are the mechanisms responsible for benzene's ability to cause cytotoxicity (damage to individual cells) that can eventually progress to carcinogenicity (Atkinson 2009, Smith 2010). It is suggested that most likely there is no safe level of benzene exposure (no evidence of a threshold) and therefore, any level of exposure must be considered to pose a level of risk of toxicity (Smith 2010).

2.1.1.2 Toluene

As with most organic solvents, toluene (also known as methylbenzene), a clear liquid refined from petroleum, is widely used for industrial processes as well as common household uses (paint thinners, adhesives, etc.) (Jacquot et al. 2006). In contrast to benzene, toluene does not require metabolism to exert its toxic effects. The route of exposure is through the lungs, from which it is rapidly distributed to the brain where it produces its most toxic effects (Bruckner et al. 2008, Jacquot et al. 2006). Toluene in the parent form accumulates in the myelin sheaths around the neurons and inhibits their function, making the central the nervous system the primary target organ. Symptoms of toxicity range from mild problems such as dizziness and headaches, to more severe effects such as respiratory depression and may proceed to death (Bruckner et al. 2008) depending on the dose of exposure (Huang et al. 1992). In addition to the central nervous system effects, toluene is an irritant to the nasal mucosa (Jacquot et al. 2006) which may impair olfactory function. It has been shown to be cardiotoxic, producing both tachycardia (increased heart rate) and bradycardia (decreased heart rate) (Einav et al. 1997), as well as teratogenic (described as toluene embryopathy) (Pearson et al. 1994). Incongruously, in humans and laboratory animals, the toxic effects of toluene can be reduced with concurrent exposure to benzene (Inoue et al. 1988); this "protective" interaction has also been reported in birds experimentally exposed to environmentally relevant concentrations of benzene and toluene (Olsgard et al. 2008).

2.1.1.3 Ethylbenzene

This compound is liquid at room temperature, evaporates readily (due to its high vapor pressure), and is poorly soluble in water. It is mainly used for manufacturing styrene but is also used as a solvent (Masten et al. 1994, Mellert et al. 2007). Inhalation appears to be the most important source of exposure (Cragg et al. 1989, Masten et al. 1994). Ethylbenzene distributes throughout the body, its metabolism occurring mainly in the liver where it is biotransformed through hepatic P450 enzymes. Its metabolites are primarily eliminated in the urine (United Nations Environment Programme 2005), but it can also be eliminated through the lungs. Engstrom et al. (1985) found the pattern of ethylbenzene's metabolism was altered with concurrent exposure to xylenes.

Ethylbenzene is reported to have a low acute toxicity but it produces irritation of the eyes at high concentrations and dermatitis has been reported after repeated exposures (Cragg et al. 1989). In addition, it is a carcinogen in animals but this type of effect has not been described for humans (United Nations Environment Programme 2005).

2.1.1.4 Xylenes

This colorless liquid has similar properties to those described above. It is heavily used as a solvent in the petroleum (Bahrami et al. 2011) and medical industries (Langman 1994), as an antiknock additive in engines, and as an intermediate for other compounds such as plastics, paints, adhesives and dyes (Jacobson and McLean 2003). Xylene exists in three isomers: ortho- (*o*), meta- (*m*) and para- (*p)* (Jacobson and McLean 2003). Commercially available xylene is a mixture of these isomers.

Similarly to the previously described VOCs, xylene enters the body primarily through the respiratory system (Bruckner et al. 2008), with approximate 5% being exhaled unchanged (Bahrami et al. 2011). Xylene can also enter the body through the dermal exposure (liquid in contact with the skin) (Langman 1994). Of the inhaled volume, approximately 64% will reach the systemic circulation (circulatory system) being taken up by the liver where it is metabolized and excreted in urine (Jacobson and McLean 2003).

The toxic effects of this substance are mostly linked to central nervous system depression, also called narcosis. However, studies on animal models have shown disruption of the respiratory system, causing difficulty breathing (dyspnea) and low blood oxygen (cyanosis) plus toxicity to blood cell production resulting in low red blood cell counts (anemia), depressed white blood cell counts (leukocytopenia), and blood clotting problems (thrombocytopenia) (Langman 1994).

2.1.2 *Hydrogen Sulfide*

Hydrogen sulfide (H_2S) is a coloured gas heavier than air, which evaporates easily from aqueous solutions. It presents a characteristic smell (sometimes called a rotten egg smell) perceptible at 0.2 ppm, reaching an unpleasantly strong smell at concentrations of 3 ppm in air (Reiffenstein et al. 1992). Hydrogen sulfide in the environment comes from both anthropogenic and natural sources. The petroleum industry (refineries, coke ovens), natural gas plants (H_2S is the most abundant sulphur compound present in natural gas), kraft paper mills, farms (from manure slurry tanks), tanneries and other industrial activities where incomplete combustion of sulphur-containing coal occurs, are examples of anthropogenic sources. Natural sources include volcanic activity (gases, sulphur springs, marine volcanic vents), decomposition of animal and plant material by bacterial organisms, crude oil and natural gas reserves (Beauchamp et al. 1984, Reiffenstein et al. 1992).

This gaseous compound enters the body primarily through inhalation and, due to its fat solubility (also called lipophilicity), it rapidly penetrates biological membranes including those of the respiratory system. Once absorbed, it is oxidized in the bloodstream and in the liver with the resultant sulphates being excreted in the urine (Beauchamp et al. 1984). Interestingly, H_2S is produced endogenously (in microscopic amounts) in brain and other tissues of mammals (humans, rat and cattle) and acts as a neurotransmitter/protectant despite being extremely toxic to humans (Ishigami et al. 2009, Kamoun 2004, Kimura 2002).

The toxic effects of H_2S appear to be similar in all eukaryotic cells (those containing a nucleus), thus affecting humans and animals in a comparable way, with lethal effects within minutes when organisms are exposed to concentrations of 100 ppm in air (Reiffenstein et al. 1992). The effects of this toxic gas are not cumulative and therefore toxicity is concentration-dependent rather than time of exposure-dependent (Beauchamp et al. 1984).

While this gas exerts its toxic effects in nearly all organ systems, the most serious target tissues are the central nervous and respiratory systems. Acute exposure will cause fatigue, vertigo, sudden anxiety, unconsciousness and respiratory failure, whereas chronic, lower level exposure can produce sore throat, chest pain, pulmonary edema and dyspnea along with psychological problems such as depression, insomnia and amnesia (Agency for Toxic Substances and Disease Registry 2006, Reiffenstein et al. 1992). Secondarily affected organs include the eyes (due to exposed mucous membranes), sense of smell, or olfactory system (causing olfactory paralysis at >100 ppm in air which prevents the exposed person/animal from detecting the danger) and the gastrointestinal tract producing nausea, vomiting and diarrhea (Reiffenstein et al. 1992).

Among animals, birds are more sensitive to the effects of H_2S than mammals (Reiffenstein et al. 1992), except for chickens which appear to be more resistant than humans (Brown et al. 1997). Hydrogen sulfide is toxic to fish at every stage of development, being a concern particularly during harvesting in fish farms (Reiffenstein et al. 1992). In vegetation, it produces leaf burns and necrosis at high concentrations while plant growth is stimulated at low concentrations (Beauchamp et al. 1984).

2.1.3 *Sulphur Dioxide*

Sulphur dioxide (SO_2) is a colorless gas, highly soluble in water and possesses a strong odor perceivable at concentrations of 3 ppm in air. Toxicologically, it is the most relevant of a group of the sulphur containing compounds called oxides of sulphur (SOx) (Gosselin et al. 2010). It is produced by anthropogenic activities that burn coal and oil (fossil fuels) primarily, and from natural sources such as volcanic eruptions and microbial activity (Brychkova et al. 2007). This substance primarily enters the body through inhalation (upper respiratory ways), although oral and dermal routes of exposure have been described (Costa 2008). From the respiratory tract, it is absorbed rapidly into the blood stream (the higher the concentration the faster its absorption) where it reaches the liver and is metabolized into sulfate. This metabolite is then excreted through the urine (Agency for Toxic Substances and Disease Registry 1998).

In general, SO_2 is known for its deleterious effects in humans, animals (e.g., birds – Brown et al. 1997), plants (Brychkova et al. 2007), and the environment through acid rain formation. The main toxic effects are limited to the respiratory tract where it causes a wide range of effects depending on the concentration and duration of exposure. For example, the effects range from respiratory tract irritation, bronchoconstriction and excess mucus secretion problems at low concentrations (1 to 5 ppm in air) of short duration, to severe respiratory complications and even death when concentrations reach >100 ppm in air (Agency for Toxic Substances and Disease

Registry 1998, Costa 2008). Numerous studies in humans and animals have been conducted on the toxicity of SO_2 using various concentrations at different exposure times and it is important to note that animal data concur with the findings in humans regarding effects of SO_2 toxicity (Agency for Toxic Substances and Disease Registry 1998).

2.1.4 *Ozone*

Ozone (O_3) occurs naturally in the upper atmosphere (stratosphere) where it acts as a protective barrier against UV radiation and maintains a natural equilibrium with its formation and degradation processes (Mustafa 1990). However, it can be formed from the reaction of VOCs and NOx with sunlight causing its accumulation in the lower atmosphere (troposphere) where it is known as ground-level ozone (Costa 2008). Anthropogenic emissions of VOCs and NOx are major contributors in ozone formation (Mustafa 1990).

Ground level ozone is an important photochemical air pollutant because of its potential for destructive oxidation in numerous biological systems (Kelly et al. 1995). Ozone exposure occurs only through inhalation (Kelly et al. 1995) and due to its poor water solubility and its reactivity, it produces the majority of its toxic effects within lung tissue (Mustafa 1990, Pryor and Church 1991, Pryor et al. 1995).

Ozone is toxic at low concentrations (sublethal concentrations occur at 1 to 2 ppm) and is lethal at concentration of 4 to 10 ppm in air (Mustafa 1990). It appears that permanent damage is not likely to occur after single dose exposures at low concentrations and, therefore, duration and concentration are important factors to consider (Costa 2008). The pattern of exposure is another important factor. Episodic exposures can produce more severe respiratory damage (specifically thickening of the lung interstitium) than continuous exposure to lower concentrations (Costa 2008).

Ozone's toxicity is due to its direct action (oxidation and destruction) on cells and through the formation of free radicals and other compounds (referred to as ozone cascade) (Mustafa 1990, Pryor et al. 1995). The respiratory system is the main target organ producing structural and biochemical damages (such as airway hypersensitivity, hypersecretion of airway mucus, increased epithelial permeability and neutrophil infiltration) (Pryor and Church 1991, Pryor et al. 1995). Other organs can be also affected, although to a lesser degree and include the central nervous system, endocrine system (decrease in thyroid hormone secretion) and hematopoietic system (increased fragility of red blood cells) (Mustafa 1990).

2.1.5 *Oxides of Nitrogen*

Oxides of nitrogen refer to a number of compounds that contain nitrogen and a varying number of oxygen atoms. Among these, nitrogen dioxide (NO_2), is the compound of greatest interest. Nitrogen dioxide is a colorless liquid which volatilizes readily at room temperature (>21°C). It is heavier than air, has a sharp, strong smell, is a highly reactive compound and is the most intensely monitored and toxicologically important constituent of the family (Azoulay-Dupuis et al. 1983, Costa 2008). It is produced from high temperature combustion of fuels and is released from automobile exhaust, emissions from industrial and power plants, and farm silos

(Costa 2008, Gosselin et al. 2010). It is used as a component of rocket fuel, in the production of explosives, and as an intermediate source for manufacturing lacquers and dyes (Agency for Toxic Substances and Disease Registry 2002).

Nitrogen dioxide is a major air pollutant in industrialized and urban areas and is an indicator of traffic-related emissions (Azoulay-Dupuis et al. 1983, Hogan et al. 2006). This compound contributes to the formation of ground-level ozone as described above (Gosselin et al. 2010). It produces irritation of the respiratory tract and its toxic effects are due to: (1) direct contact of the gas with lung cells, (2) production of free radicals and, (3) alteration of the immune response (Agency for Toxic Substances and Disease Registry 2002). At acute and high doses it produces coughing, nausea, abdominal pain, fatigue, bronchospasm, pulmonary and circulatory collapse and even death (Agency for Toxic Substances and Disease Registry 2002). At low concentrations and chronic exposures, NO_2 appears to increase susceptibility for bacterial and viral respiratory infections (Costa 2008, Gardner 1984), likely due to its effect on the immune system.

2.1.6 *Particulates*

Particulate matter (PM) includes a complex, highly variable mixture of precursor gases (formed in the atmosphere from emissions) plus primary particles derived from processes dependent on burning of fossil fuels (Davidson et al. 2005). Therefore, particulate matter has a carbonaceous center to which a number of organic and inorganic compounds attach, such as hydrocarbons, acid aerosols, metals and biological material (de Kok et al. 2006).

These particles differ in chemical composition, depending on the emission sources, and can be composed of sulfates, nitrates, ammonia, chloride, elemental and organic carbon, crustal metals (derived from soil, dusts and minerals) and biological material such as bacteria and pollen (Harrison and Yin 2000). In addition, PM may also contain trace elements and strong acids such as nitric and sulfuric acid (from the oxidation of NO_2 and SO_2, respectively) (Costa 2008). PM is classified according to aerodynamic diameter into the following size fractions: PM_{10} of 10 μm, $PM_{2.5}$ of <2.5 μm and ultraparticles, or $PM_{0.1}$, of <0.1 μm in diameter (Schwarze et al. 2006).

Particulate matter originates from natural sources such as forest fires, dusts storms and gaseous sulphur (volcanic activity and vegetation decay), as well as from anthropogenic sources related to the combustion (usually inefficient) of coal and other hydrocarbons (Davidson et al. 2005).

The toxic effects of PM are generally inversely related to their size, with the smallest aerodynamic particles being the ones with higher toxic potential due to their greater capacity to penetrate deep within lung tissue (Costa 2008). Smaller particles appear to be more capable of producing free radicals. PM may be directly toxic to cells, can produce pro-inflammatory cytokines, and can damage genetic material (DNA) and therefore, is capable of producing mutations. In general, short-term exposure to PM is associated with asthma and bronchitis, or acute respiratory infections, while long term exposure is associated with degenerative respiratory diseases, arteriosclerosis, and lung cancer (de Kok et al. 2006).

In addition to health effects in humans and animals (mostly based on studies with laboratory animals), PM has an effect on climate (warming effect) and can alter visibility (Davidson et al. 2005). Finally, the toxic risks from PM represent a real challenge since their size and composition vary tremendously over time and space with many factors that can modify them. Air quality assessments use only the total mass of particles suspended in air while disregarding their composition.

2.2 Water

2.2.1 *Polycyclic Aromatic Hydrocarbons*

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds composed of carbon and hydrogen atoms with two or more fused carbon rings (Walker 2001). They are commonly found in mixtures rather than as separate compounds, and are hydrophobic (hydrophobicity increasing with increased number of carbon atoms) (Timoney and Lee 2011). Natural sources of PAHs include geologic deposits of coal and crude oil, forest fires, vegetation decay, and formation by microbial degradation (Gabos et al. 2001, Usenko et al. 2010). Anthropogenic sources (specifically, incomplete fossil fuel combustion) account for most PAHs released to the atmosphere (Baek et al. 1991).

Polycyclic aromatic hydrocarbons can be found in most biota in water, sediments and soil, as a result of discharges from industrial plants, pipeline leaks, and precipitation, but also can be found suspended in the atmosphere attached to dust particles and/or vapors (usually lower carbon PAHs such as from petrogenic sources) (Agency for Toxic Substances and Disease Registry 1995). Because of their ubiquitous presence in biotic systems, PAH exposure can occur through the consumption of contaminated food and water, through direct absorption by the skin and through inhalation (Agency for Toxic Substances and Disease Registry 1995).

The toxic effects of PAHs occur after these compounds have been metabolized/activated through hepatic CYP 450 enzymes, with damage being related to genetic mutations that eventually lead to cancer (Agency for Toxic Substances and Disease Registry 1995, Walker 2001). Affected body systems include the liver, skin, as well as the immune system (Agency for Toxic Substances and Disease Registry 1995).

2.2.2 *Naphthenic Acids*

Naphthenic acids (NAs) are chemically stable, complex mixtures of carboxylic acids commonly classified by their number of carbon atoms and their structure (Headley and McMartin 2004). These varied groups of compounds have different toxicological, chemical and physical properties depending on their non-volatile and polar characteristics. Their high solubility in water allows NAs to act as surfactants (Clemente and Fedorak 2005).

Naphthenic acids are natural constituents of petroleum (crude oil and bitumen deposits), their concentrations varying depending on the oil source (e.g., approximately 2% in bitumen samples from the Athabasca oil sands) (Clemente and Fedorak 2005). Even with the current analytical technologies, NAs represent a challenge for analysis and characterization due to their complex and varied composition (Clemente et al. 2003, 2004, Clemente and Fedorak 2005, Dillon et al. 2011, Zhao et al. 2012).

Since NAs are components of bitumen deposits, and bitumen extraction processes require a significant amount of water, NAs (from the Athabasca oil sands) are found dissolved in oil sands process water and become concentrated in large tailings ponds (for example tailings ponds contain >100 times the concentration of NAs found in rivers near the Athabasca oil sands) (Clemente and Fedorak 2005, Headley and McMartin 2004). In these tailings ponds, NAs will be present in the water column and deposited in the sediments. Other sources of NAs in water are effluent discharges and wastewater from petroleum refineries, oil spills, mixing and erosion of groundwater (from river banks oil deposits) (Clemente and Fedorak 2005, Headley and McMartin 2004) and releases from industrial uses (Brient 1998).

These compounds are used in industrial processes as fuel and lubricating additives, in wood preservatives, as emulsifiers for agricultural insecticides and as oxidative catalysts (Headley and McMartin 2004). While NAs appear to be persistent in the environment, microbial degradation occurs reducing their concentration (and toxicity) and presenting a means for possible remediation approaches for oil sands tailings pond waters (Clemente and Fedorak 2005, Headley and McMartin 2004).

Naphthenic acids are known for their toxicity to aquatic organisms (fish, algae, microorganisms) and mammals, which seems to be related to their surfactant activity (Rogers et al. 2002). The surfactant-like qualities tend to interfere with cell membrane functions. These compounds are reported to be cytotoxic, capable of producing alterations in blood cells (red and white blood cells), toxic to the liver (liver appears to be the target organ for mammals) and occasionally lethal (after acute, high dose exposures) (Headley and McMartin 2004, Rogers et al. 2002). Effects of NAs have been observed on plant water conductance, larval development of fish, and liver function in mammals (Gentes et al. 2007b).

2.2.3 *Sulphate Ions*

Sulphate is commonly found in low levels in aquatic environments (freshwater) where plants and bacteria can use the sulphur for metabolic processes (Davies 2007). While some lakes have a naturally high concentration of sulphate ions, increased concentrations of this ion occur due to anthropogenic inputs including fossil fuel combustion, mine and industrial wastewaters, agricultural runoff and domestic sewage (Davies 2007, Nriagu 1978).

Sulphate ions can be toxic to aquatic organisms; however, complex interactions of many factors (such as other ions and total dissolved solids), determine the toxic effects and responses of organisms to sulphate (Soucek and Kennedy 2005). For example, toxicity is reduced by increased water hardness and chloride concentration (Kennedy et al. 2005).

2.2.4 *Ammonia*

Ammonia (NH_3) is a colorless gas with strong, sharp odor. It can be found in the atmosphere in its gaseous form but also it is commonly found soil and water bodies due to its high water solubility. Natural sources of NH_3 derive from animal and vegetation decay along with the natural breakdown of animal manure and decomposition of sewage by microorganisms (Randall and Tsui 2002). Anthropogenic sources are associated with industrial processes particularly in the agricultural sector (Agency for Toxic Substances and Disease Registry 2004). While most of the NH_3 present within ecosystems arises from natural sources, the anthropogenic NH_3 determines eutrophication (excessive addition of nutrients into water bodies) and acidification (low pH) processes of ecosystems (Behera and Sharma 2011).

The effects of NH_3 will depend on the route of exposure and the state of this compound – inhalation exposure produces severe irritation (burns) of the exposed tissues such as respiratory tract, eyes and skin; whereas tissue necrosis follows exposure to water-dissolved NH_3 (Agency for Toxic Substances and Disease Registry 2004). In elevated concentrations, NH_3 can cause central nervous dysfunction characterized by convulsions, coma and even death (Randall and Tsui 2002). Since toxicity of NH_3 is far greater within aquatic as compared to terrestrial ecosystems, fish are severely affected by this compound (Rattner and Heath 2003).

2.2.5 *Trace Metals*

Metals are naturally present within most ecosystems; they often are ubiquitous and very persistent in the environment (Liu et al. 2008). Unlike most of the contaminants described above, metals (as elements) are not created (nor destroyed) by human activity but their concentration is changed in the biosphere (Liu et al. 2008).

Sources of metals released to the environment include stationary coal combustion, wood combustion, internal combustion, metallurgical processes, mining, cement production, and solid waste incineration (Pacyna 1986). Oil sands developments, including bitumen upgraders, release airborne emissions of antimony, arsenic, beryllium, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, thallium and zinc, which are considered priority pollutant elements (PPE) (Kelly et al. 2010).

Trace metals can be present in water bodies, soil and air, and therefore, the exposure routes are mainly through ingestion and inhalation. Trace metals readily move within ecosystems through water and air and they can get deposited on sediments and soils for example, or be taken up by vegetation, invertebrates and vertebrates. Currently, concerns of exposure and toxic effects are related to low, chronic exposure rather than acute and high doses (Liu et al. 2008).

The toxicity caused by metals will vary greatly. In general (Liu et al. 2008), metals affect biological systems due to their:

1. chemical structure
2. reactivity
3. capacity to bind cell receptor and inhibit cellular function (inhibition of enzymatic processes for example)
4. capacity of non-essential metals to "mimic" essential metals (such as lead that mimics calcium), and

5. oxidative damage.

2.2.6 *Particulates*

The effects of particulates, in this case dissolved particulates, have been covered in section 2.1.6.

3 EXPOSURE

Biological effects from exposure to contaminants will only occur if the chemical and/or its metabolites (after it has been biotransformed by the body) reach the target organs at sufficient concentrations to cause different levels of toxicity (ranging from sublethal to lethal) (Eaton and Gilbert 2008). The major routes of exposure to contaminants are: (1) ingestion (through the gastrointestinal tract), (2) inhalation (through the lungs and respiratory system), and (3) dermal or percutaneous (through the skin) (Eaton and Gilbert 2008).

Exposure routes will vary depending on the species and whether they inhabit terrestrial or aquatic environments (Figure 2). For example, fish can readily absorb contaminants directly from sediments and the water column though their gills since these are permeable respiratory surfaces, and amphibians can absorb contaminants through their skin, whereas these are not routes of exposure for whales, seabirds and other aquatic and semi-aquatic vertebrates (Walker 2001).

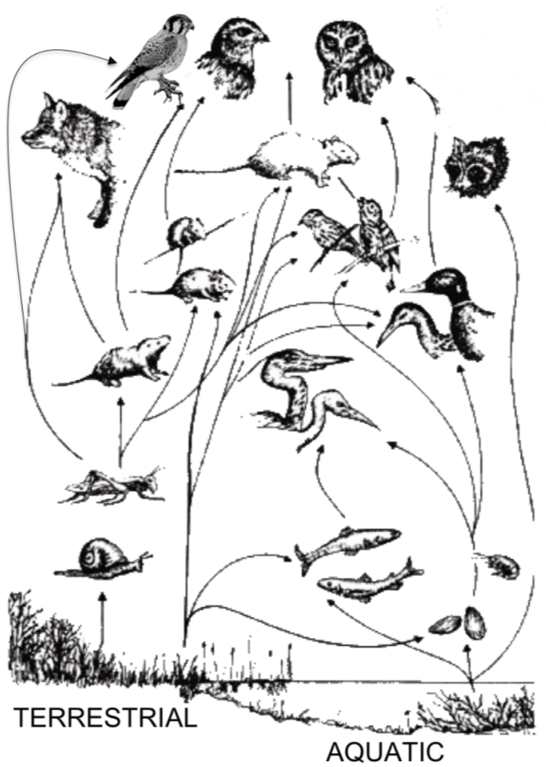

Figure 2. Simple web food structure showing lower trophic organisms, plants, algae and invertebrates which fix nutrients (autotrophs) and produce high-energy organic compounds.

As lower trophic organisms are consumed by higher trophic species, energy is transferred up the food web. Contaminants released to air, water or soil can be incorporated into terrestrial and aquatic food webs. Animals in high trophic positions such as birds of prey and large carnivores are valuable as sentinels of ecosystem health since they provide an integrated assessment of possible effects from bioaccumulation of contaminants.

3.1 Sources

3.1.1 *Air*

To extract crude oil (bitumen) from the oil sands and make it a useful product, a number of complex industrial processes occur. It is from these processes that contaminants are released to air as emissions, and the aquatic environment as effluents. Emissions arise from the following activities (Environment Canada 2010, Gosselin et al. 2010):

1. mining
2. *in situ* recovery of bitumen
3. extraction of bitumen from sand
4. refining and upgrading
5. waste management
6. dry tailings dust
7. vehicles
8. heavy equipment (haulers and cranes), and
9. non-point sources known as fugitive emissions from leaky storage units, valves, pipelines, etc.

3.1.2 *Water*

Activities such as mining, *in situ* recovery and upgrading are dependent upon water for bitumen extraction and processing (Gosselin et al. 2010). Oil sands processing at mines results in production of contaminated water, commonly referred to as oil sands process water (OSPW) or process-affected water. This OSPW is not discharged into the environment; rather it is contained in tailings ponds behind large dykes on the mine sites. The dykes are permeable; however seepage is collected and returned to the ponds or may contribute to existing wetland, or produce new wetlands. Volatile compounds such as benzene can evaporate from tailings ponds or wetlands containing process-affected water (Gosselin et al. 2010; Madill et al. 2001). Refinery discharges are another main source of environmental contaminants (Wrona et al. 2000).

The Regional Aquatic Monitoring Program (RAMP) reports that the contaminants in the Athabasca River system are due to natural leaching from oil sands deposits (Regional Aquatic Monitoring Program 2008). However, other studies have indicated that water from the Athabasca River and its tributaries is affected by contaminants released as emissions and as aquatic discharges from oil sands related activities (Kelly et al. 2009). Alberta Environment and Water's Minister has committed to implementing recommendations to upgrade the province's oil sands monitoring programs (Dillon et al. 2011).

3.2 Movement and Deposition between Terrestrial and Aquatic Ecosystems

After formation from anthropogenic sources, the fate of contaminants in ecosystems will depend on chemical, physical and biological factors (Walker 2001). For the purpose of this report, the following chemical characteristics will be reviewed:

1. Polarity, which refers to the solubility of a compound in water (greater polarity means increased water solubility)
2. vapor pressure, which relates to the volatility of compounds (the higher the vapor pressure the more volatile), and
3. chemical stability, which refers to how the contaminants react (transformation, degradation, etc.) with physical, chemical and microbiological processes.

For example, PAHs can exist in gaseous form and be transported in air for long distances getting deposited far from the source of origin and/or can be associated with particulate matter in water-soluble forms, which end up in the sediments in tailings ponds (Baek et al. 1991, Gosselin et al. 2010). Contaminants found in surface water such as tailings ponds have the potential for distribution into sediments (usually those with high molecular weight and poor water solubility), groundwater and surface waters (Gosselin et al. 2010, Walker 2001).

Once in the atmosphere, contaminants can be deposited to terrestrial and aquatic ecosystems through wet or dry deposition. Wet deposition refers to the process of contaminants being washed out of air (during rainfall) or rained out after the contaminants have accumulated in water droplets of clouds. Dry deposition occurs when contaminants land on surfaces (water, soil, sediment, vegetation). For example, benzene is deposited on water surfaces through both wet and dry deposition and later volatilizes into the atmosphere (Wilbur et al. 2008). A similar process occurs with PAHs although snowfall appears to be a better sink for these compounds than rainfall (Baek et al. 1991). When the snowpack melts, contaminants are dispersed through river systems, and as with SO_2 (and NO_2 but to a lesser degree), contribute to acidification of ecosystems (Valdez et al. 1987). Specifically for the oil sands region, a recent study reported that within 50 km of upgrading facilities (Suncor and Syncrude), substantial deposition of airborne particulates (in the snowpack) occurred, equivalent to 11,400 metric tons in a 4 month period (Kelly et al. 2009).

In addition to transport and deposition, contaminants may degrade in air, water and soils. Photochemical (photolysis) and chemical oxidation are important for degradation of VOCs, PAHs, and O_3 that occur in the atmosphere. Microbial degradation of VOCs (Wilbur et al. 2008) and polyaromatic compounds (Kelly et al. 2009) under aerobic conditions in water and soil result in degradation contaminants. Reactions of PAHs with SOx, NOx and O_3 also results in PAH degradation (Baek et al. 1991).

3.3 Season and Length of Exposure

The oil sands industry has been developing since the 1960's with a major increase of operations in the 1990s (Schindler 2010). By 2020, oil sands production is estimated to increase three-fold (Giesy et al. 2010). Therefore, such long-term activities, which are releasing a variety of chemicals to the air and water on a continuous basis, represent a major source of exposure for organisms inhabiting the region.

Exposure to contaminants will vary depending if wildlife are permanent inhabitants of the region – such as fish and ungulates (bison, deer, caribou, moose) – subject to constant exposure, or if they are migratory species. Birds that use the boreal forest in the oil sands mining areas as breeding grounds will have seasonal exposure of adults and hatch year birds. In the case of those born in the oil sands region, their growth and development are physiologically critical times and therefore the effects of contaminants might confer future detrimental consequences (Gentes et al. 2007a, b). The effects of oil sands activities on regional wildlife are covered in section 5.

4 OVERVIEW OF METHODOLOGY

4.1 Sampling Locations

The study sites for the majority of the projects covered in this literature review were approximately 40 km north of the city of Fort McMurray but extended as far as 75 km north and south of the city. Most of the studies were conducted on wetlands and ponds on Syncrude's and Suncor's mining lease areas. Freshwater bodies where fish and water were sampled and/or collected, included the Athabasca, Peace, Muskeg, Steepbank and Ells rivers, Mildred Lake and Gregoire Lake. Figure 3 provides a detailed description of sampling locations from the oil sands and Fort McMurray region.

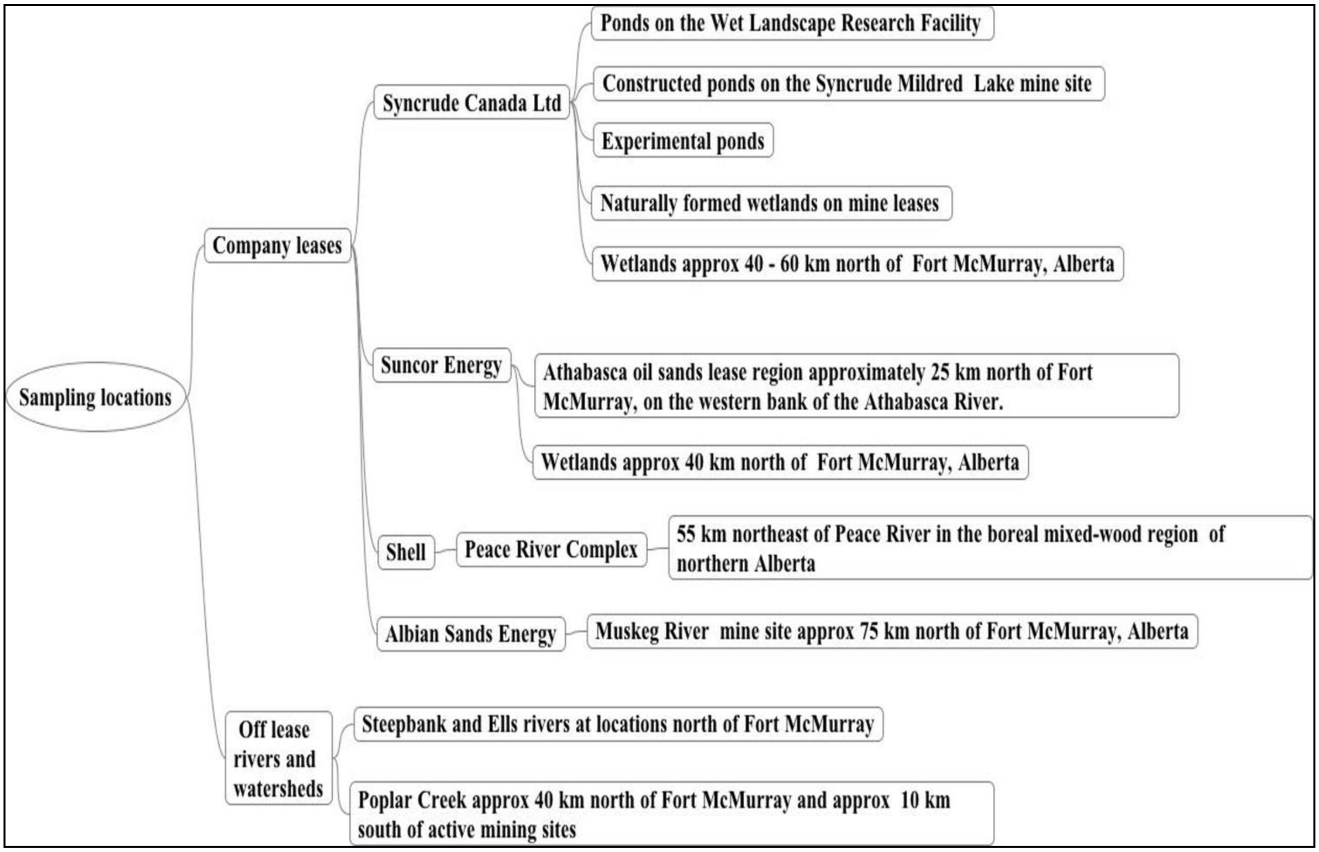

Figure 3. Sampling locations on company leases and off lease sites of the Athabasca oil sands region.

4.2 Sampling Methods

The sampling methodology can be categorized into (1) field studies that focused on natural exposure of contaminants on wildlife, and (2) laboratory studies that evaluated animals' responses after experimental exposures. The methodology varies depending upon the species. Detailed descriptions for the studies that have been carried out with different species under both field and laboratory settings are shown in Figure 4.

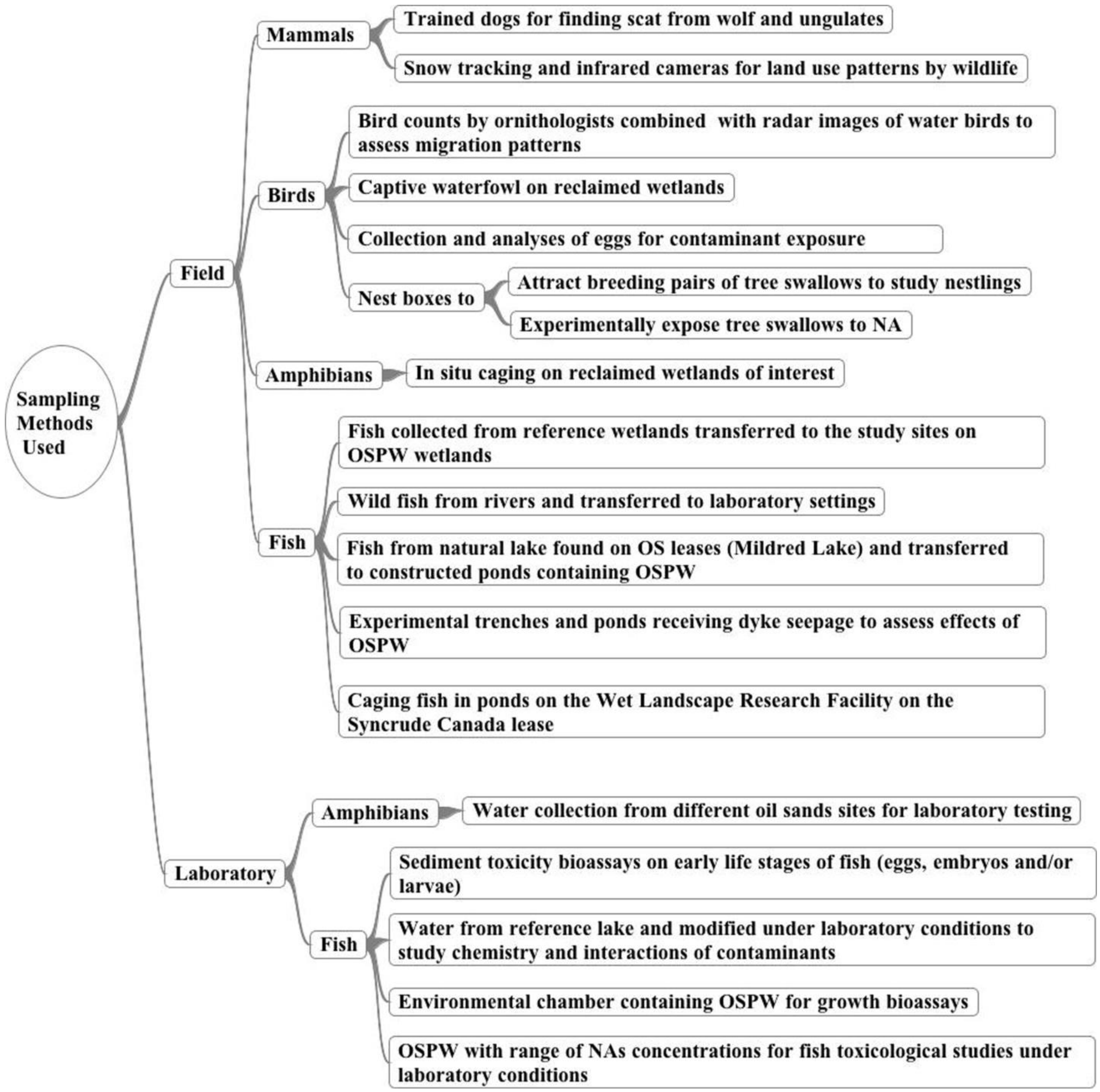

Figure 4. Field and laboratory methods used on different wildlife species at the oil sands region.

The number of samples required in any study will depend on the specific research questions, on the number of animals in the study population, on the ease of working with those animals, the restrictions imposed by Animal Care Protocols, etc. Fortunately, the number of samples/animals required to address specific research questions can be determined through a power analysis aimed at achieving the strongest statistical analysis possible. Power analysis refers to techniques used at the beginning of experiments (experimental design phase) to determine a proper sample size that will yield a statistically reliable and precise value to answer research questions (Browner et al. 2007).

4.3 Analyses

Both the field and laboratory studies are designed to assess exposure and biological effects from the oil sands on wildlife through measuring specific endpoints. An endpoint is a measurable variable or biomarker that reflects the health (or disease) status of organisms and/or population exposed to contaminants. While there are various kinds of endpoints used in research, those specific to this review are classified into biological, chemical and toxicological, and are shown on Figure 5.

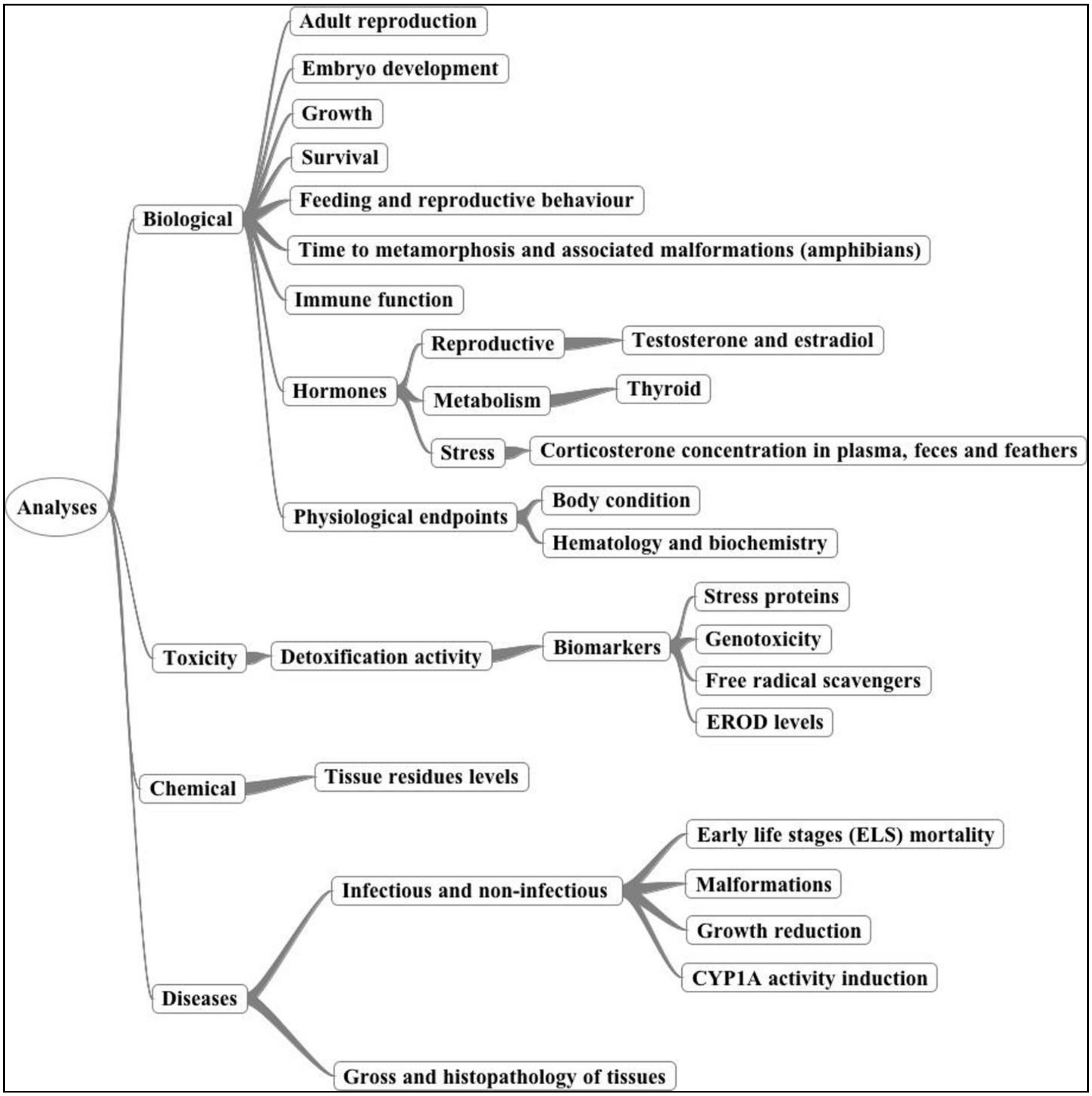

Figure 5. Different endpoints used on wildlife species at the oil sands region to assess the effects of contaminants.

4.4 Interpretation

The interpretation of results from toxicological studies must be based on the most sensitive endpoints, changes that occur at the lower concentrations of the contaminants of concern, or responses that occur after short term exposure to higher, but still environmentally relevant levels of the compound or mixture of xenobiotics. Changes in biochemical, morphometric, hormonal, or immunological reactions yield the most important insights regarding the biological consequences of such exposure.

It is important to recognize that interpretation of biological studies must be done in tandem with information generated by other specialists such as chemists, who, for example can collect objective data on air, water or soil quality. Research complementary to wildlife investigations could include passive and active air monitoring stations, water quality assessments and analysis of compounds of concern in soils, vegetation, and other biota. As stated by Kelly et al. (2009), "monitoring of air, the snowpack, spring snowmelt, and summer rain and vegetation is essential to identify and control sources of petroleum-based hydrocarbons and their potential environmental and human health impacts".

4.5 Length of Studies

The length of studies will depend on the wildlife species and the specific endpoints needed to answer the research questions. For field studies based on wildlife, an absolute minimum of two years is necessary to gather statistically usable data. This is to accommodate the uncontrollable fluctuations and vagaries in natural systems, such as extended periods of cold, harsh, unseasonable weather, massive forest fires that affect hunting and prey availability (and which we know contribute to airborne contaminants such as dioxins), or harsh conditions in the over-wintering areas of migratory species, affecting the size of the returning population, and in turn the number of animals available for study. Two or more years of data are also necessary to ensure that the information produced by collaborators from the different fields of expertise, also overlap temporally and spatially with the wildlife investigations.

4.6 Costs

Costs associated with both field and laboratory experiments will vary greatly depending on the complexity of the study, the time required to collect the information and samples based on the species life history, the equipment required, the logistical support offered by the industrial partners (such as housing during the field season), etc. The budget must cover data collection, consumables, sample storage, transport and analysis, manpower costs, logistical support in the field, vehicle and travel related costs, plus communication costs during the field season and for dissemination of results through publications and presentations.

A sample budget based on a three year study is provided in Appendix 1. The budget assumes it is a university-based study; work carried out by others may cost more or less.

5 EXAMPLES FROM THE LITERATURE

5.1 Domestic Animals

There appear to be no peer-reviewed publications relating to oil sands activities and domestic animals. However, because of the captive wood bison population managed by Syncrude on its active mine lease areas (see next section for details), it is relevant to discuss a series of studies on the effects of oil and gas emissions on cattle.

The Western Canada beef productivity study examined VOCs (benzene and toluene), SO_2 and H_2S on over 33,000 cows in 205 beef cow-calf herds from spring 2001 to the end of the calving season in 2002 (Waldner 2008b). Researchers (Bechtel et al. 2009a, Waldner 2008a, Waldner and Clark 2009) reported the following effects:

1. exposure to increasing concentration of benzene and toluene was associated with increased likelihood of respiratory lesions in calves older than 3 weeks of age that were exposed after birth
2. exposure to increasing levels of toluene was significantly associated with the decrease of an important subset of white blood cells (CD4 T lymphocytes) responsible for immunity and resistance to disease, and
3. increasing exposure to SO_2 during gestation was associated with increased odds of lesions in either the skeletal muscle or myocardium (heart muscle) and with increased risk of calf mortality if they were exposed during time of calving.

Despite the above findings, the authors found no associations between the exposure to these compounds and

1. the risk of lesions in the respiratory system in calves born alive in spring 2002
2. the risk of either abortion or stillbirth
3. increased odds of non-pregnancy or culling for pregnant cows, or
4. negative effects on the immune system in neonatal calves that were exposed *in utero*.

In addition, no associations were found between immune function in yearling cattle and airborne polycyclic aromatic hydrocarbons (PAH) and particulate matter (Bechtel et al. 2009b, Waldner 2009).

5.2 Wildlife

5.2.1 *Wood bison*

Syncrude Canada Ltd. has established a wood bison (*Bison bison athabascae*) herd on land surrounded by active oil sands mining activities and is adjacent to an active tailings pond. Pauls (1999) observed that:

1. the reclaimed land supporting the wood bison had comparable forage productivity and carrying capacity to that of bison pastures in other areas of Alberta

2. the bison had weight gains and calving rates comparable to those from ranched bison, and
3. the herd had similar husbandry problems as those experienced with other ranched bison in Alberta.

5.2.2 *Other Mammals*

No studies were found documenting health impacts of oil sands processing activities on mammals that are important in the region, including moose (*Alces alces*), caribou (*Rangifer tarandus caribou*), black bears (*Ursus americanus*), lynx (*Lynx canadensis*), gray wolf (*Canis lupus*), coyote (*Canis latrans*), white-tailed deer (*Odocoileus virginianus*), and mule deer (*Odocoileus hemionus*).

Wistar rats have been used as surrogates of terrestrial wildlife and exposed to various concentrations of NAs that were extracted and purified from tailings pond water. NAs were given by oral gavage to the rats at acute and subchronic doses to mimic worst-case scenarios. Researchers reported that while hepatic tissue damage occurred after both acute and subchronic doses, it is unlikely that terrestrial wildlife would develop acute toxicity from NAs present in tailings pond water; however, the development of negative health effects might occur upon repeated exposures (Rogers et al. 2002).

5.3 Birds

5.3.1 *Waterfowl*

Waterfowl represent a diverse group of avian species that depend on open, fresh water for many of their biological needs (feeding, reproduction, landing, roosting, resting). An important migratory flyway for waterfowl (and other shorebirds) follows the Athabasca delta and carries through the oil sands region (Bellrose 1976). Census-like daylight observations at Syncrude Lease #17 during fall migration detected over 25,000 waterfowl (geese, swans, ducks and sandhill cranes); and, observations during spring migration counted over 16,000 waterfowl flying over one tailings pond (Ronconi 2006, Timoney and Ronconi 2010).

Despite the presence of deterrent systems (Ronconi and St. Clair 2006), waterfowl landings on the numerous and vast tailings ponds is a growing concern (Timoney and Ronconi 2010). This is clearly exemplified by the mass mortality (>1,600 birds) that occurred at one of Syncrude's tailings ponds (Timoney and Lee 2009). Mortality associated with landings on tailings ponds occur as a result of the inherent toxicity of oil compounds and the effects on plumage such as loss flight impediment, loss of thermoregulation and waterproofing (Leighton 1993). Researchers estimated that a range of 458 to >5,000 birds die each year from oil sands related activities including non-waterfowl species such as birds of prey. However, the authors suggest that this mortality is only a fraction of the true count, since rigorous data are yet needed to get more accurate estimates for waterfowl mortality events (Timoney and Ronconi 2010).

In field studies, mallard ducklings (*Anas platyrhynchos*) were raised in wetlands formed by effluent from oil sands mining activities, or on reference wetlands to assess growth patterns (skeletal size and mass) and physiological condition (plasma metabolites). Ducklings reared in oil sands effluent wetlands were significantly smaller with lower body mass as compared to ducklings from the reference wetland (Gurney et al. 2005).

5.3.2 *Waterbirds*

Hebert et al. (2011) collected freshly laid eggs from common terns (*Sterna hirundo*), caspian terns (*Hydroprogne caspia*), ring-billed gulls (*Larus delawerensis*), California gulls (*Larus californicus*) and herring gulls (*Larus argentatus*), to measure levels of mercury (Hg), arsenic (As), 16 different PAHs and for stable nitrogen isotope analyses. Eggs were collected from lakes downstream of the oil sands region that receive water from the Athabasca River and from lakes not influenced by this river (Hebert et al. 2011). Hebert et al. 2011 report:

1. all samples had measurable levels of Hg, whereas PAHs and As levels were low
2. in most instances, Hg levels were increased in eggs from birds at higher trophic levels (as indicated by nitrogen isotope analyses)
3. eggs collected from the lake influenced by the Athabasca River had higher levels of Hg than expected (based on trophic position)
4. there is a possible common source of contamination (uncertain if its directly from oil sands industrial activities) since Hg levels were correlated with naphthalene levels, and
5. Hg levels in eggs have increased 40% in a 22-year period (1977 to 2009).

5.3.3 *Song Birds*

5.3.3.1 Tree Swallows

Throughout North America, this aerial insectivorous avian species is abundant and commonly found occupying open landscapes such as wetlands and lakeshores (Gentes et al. 2006). These habitats provide them with various types of insects (midges, horseflies, mayflies, beetles, etc.) and plant products (i.e., mayberries) that usually occur in close proximity (~100 m) to their nesting territory (McCarty and Secord 1999, Mengelkoch et al. 2004). Tree swallows are cavity nesters and produce between 4 to 7 eggs per clutch (Smits et al. 2000).

Rationale for research using this species includes (Gentes et al. 2006, Jones 2003, Smits et al. 2000):

1. ease of availability (widely distributed)
2. small size at adulthood which facilitates handling
3. due to their cavity nesting behavior tree swallows will readily occupy artificial nest boxes facilitating research logistics (capture and tractability)
4. the development of the insects they depend upon for feeding can occur on relatively polluted water bodies, making these birds useful sentinels of environmental health

5. they are tolerant of human interference continuing to care for their young in spite of investigator interference, and
6. much is known about this species since it has been used in numerous studies.

Specifically in the oil sands, tree swallow nestlings have been studied as a sentinel species by assessing several health variables to test the sustainability of experimental wetlands at different stages of maturation. These wetlands were created for bioremediation of OSPW and contain PAHs, salts and NAs along with varying amounts of unrecovered bitumen. The following paragraphs describe the projects' research findings.

Harms et al. (2010) assessed reproductive performance, nestling growth, body condition, immune function, and feather corticosterone as a surrogate measure of stress. Researchers reported that while these wetlands may have moderate to high concentrations of PAHs and NAs, there were no significant differences in reproductive performance (hatching and fledgling success, number and mass of eggs laid) for three reclaimed wetlands. Nestlings raised on the older, reclaimed wetland were significantly larger than those on the reference wetland. In addition, nestlings from this mature reclaimed wetland produced a stronger immune response (measured by a delayed-type hypersensitivity test). Finally, feather corticosterone concentrations were similar in the birds from the reclaimed and reference wetlands, although males from the younger (and therefore more contaminated) wetland showed higher levels of this stress hormone. These results demonstrate primarily positive biological outcomes on the reclamation sites (Harms et al. 2010).

Smits et al. (2000) obtained similar results when assessing reproductive, immune and physiological endpoints of tree swallows inhabiting six wetlands. Here, increased detoxification efforts (measured by the induction of ethoxyresorufin-0-deethylase [EROD]) were observed indicating toxicant exposure in these insectivorous birds. However, no significant differences were reported for reproductive success, nestling growth rate or immune response among the tree swallow populations living in these wetlands, demonstrating their ability to compensate for the contaminant exposure (Smits et al. 2000).

A series of studies by Gentes et al. assessed thyroid hormone levels, mortality rates, growth and survival, and EROD induction in relation to harsh weather and natural exposure of tree swallows to OSPW wetlands (Gentes et al. 2006, 2007a, b). They also carried out an experimental exposure of wild nestling tree swallows to NAs, a component of OSPW of major concern for toxicity to some classes of animals. Findings included elevated levels of thyroid hormones (T_3 in plasma and T_4 within thyroid gland), probably due to exposure to chemicals (PAHs) and environmental factors (food availability). Thyroid hormones increase in response to higher energetic demands required for day-to-day function. During a 10-day period of lower temperatures and heavy rainfall compared with average conditions in this region, mortality rates were over ten times higher on OSPW wetlands compared to the reference wetland, whereas similar mortality rates were reported among wetlands under normal weather conditions. The authors conclude "nestlings from OSPW-impacted wetlands are less able to withstand additional stressors, which could decrease their chances of survival after fledgling". Regarding nestling growth, no significant differences were reported for skeletal measurements of chicks among wetlands; however, chicks from OSPW wetlands weighed less than those living on the reference site. Finally, EROD induction (measuring detoxification efforts) was higher on the more contaminated wetlands (proportional increase in relation to wetland contamination) (Gentes et al. 2006, Gentes et al. 2007a). Regarding the experimental exposure to NAs in nestlings less than 1 wk old at the beginning of the exposures, there were no adverse effects of the NA exposure (1.5 mg/day/7 days) on health variables (nestling growth, hematocrit, blood biochemistry, organ weights, EROD induction). The time of exposure represented half the birds' lifespan (Gentes et al. 2007b).

The above studies provide evidence that the reclaimed wetlands on different oil sands sites have the capacity to sustain populations of tree swallows during the breeding and nesting periods. This may be due to the short-term exposure of the nestlings to the OSPW chemicals, but it does occur over their entire lives, from fertilization of the eggs to fledgling (leaving the nest) of the young birds. However, it is also clear that additional stochastic events may result in intolerable stress on tree swallows inhabiting OSPW, leading to severely reduced survival.

5.3.4 *Raptors*

We did not identify any peer-reviewed publications regarding experimental or field studies on raptors regarding the effects of oil sands. To our knowledge, the only work on birds of prey has been a project on American kestrel (*Falco sparverius*) chicks reared at two sites impacted by oil sands mining operations (Syncrude and Suncor) and at a reference site (not impacted by oil sands operations). This study examined DNA damage in red blood cells, using flow cytometry. DNA damage was significantly greater in the kestrel chicks raised on the Syncrude and Suncor sites as compared to the chicks from the reference site (Smits unpublished data).

5.4 Amphibians

From the ten species of amphibians found in Alberta, two are commonly found in the oil sands region: (1) the western toad (*Bufo boreas*) that inhabits the boreal forest, mountain regions and Aspen Parkland; and (2) the tree frog (*Lithobates* (*Rana) sylvatica*) that is known as a non-prairie species well adapted to cold (Russel and Bauer 2000). The diet of these anurans depends on their life stage. As tadpoles (larval stage), plant material present in aquatic environments is the main food source, whereas adult frogs depend on insects (Hersikorn et al. 2010, Russel and Bauer 2000). The eggs of these amphibians are deposited in the water, later giving rise to tadpoles that undergo metamorphosis to become adults (Russel and Bauer 2000).

Amphibians are considered indicators of environmental health (Cooke 1981) and have been studied as bioindicators of toxicity from industrial chemicals (petroleum, agriculture) and endocrine disrupting chemicals. Wood frogs and western toads have been chosen as study subjects for the oil sands since they are the most abundant amphibians in the region (Roberts et al. 1979), which simplifies collection of eggs and tadpoles, and because they would potentially occupy OSPW-affected wetlands for breeding (Hersikorn et al. 2010, Pollet and Bendell-Young 2000).

As part of the reclamation effort by the oil sands industry, wetlands formed from seepage of oil sands process water (OSPW) through containment dykes are considered as a viable option for bioremediation and further reclamation (Madill et al. 2001). However, while these OSPW-based wetlands would support populations of invertebrates, they would not support fish populations and would be unlikely to support other types of aquatic organisms.

Two species were used to test the above statement (Pollet and Bendell-Young 2000). Experiments were conducted on western toads (*Anaxyrus boreas*, formerly *Bufo boreas*) and wood frogs (*Lithobates sylvaticus*, formerly *Rana sylvatica*) exposed to water from OSPW wetlands to evaluate tadpole survival, growth, time to metamorphosis, and frequency of physical deformities (Pollet and Bendell-Young 2000). For *B. boreas*, the authors reported reduced growth and prolonged time to metamorphosis (developmental time) and for *L. sylvaticus*, decreased survival along with reduced growth occurred from exposure to OSPW as compared to water from reference wetlands. The authors concluded that OSPW wetlands would not support viable amphibian populations (Pollet and Bendell-Young 2000) but these exposures did not consider the anticipated benefits of the maturation of tailings ponds. Similarly, Gupta reported that the growth and survival of wood frog tadpoles are adversely affected when the animals are exposed to OSPW (Gupta 2009). Because of the anticipation that constructed reclamation wetlands become less toxic over time, researchers tested if the age of these wetlands had an effect on growth, development (time to metamorphosis) and detoxification activity in *L. sylvaticus* tadpoles. For tadpoles raised in young wetlands ($<$ 7 yrs), researchers report similar findings to those mentioned above, including thyroid hormone disruption, delayed metamorphosis, and higher levels of detoxification enzymes (measured by ethoxyresorufin-0-deethylase [EROD] activity). Conversely, tadpoles raised in more mature wetlands ($\geq$ 7yrs) developed similarly to tadpoles raised in reference wetlands, supporting the expectation that there is reduction of toxicity in reclaimed wetland over time (Hersikorn and Smits 2011).

The growth and development of tadpoles is dependent upon the availability of food resources of adequate quantity and quality (Newman 1998), as well as absence of acutely toxic 'process affected materials'. The studies described above indicate that native amphibians may be used as relevant and sensitive indicators of the sustainability and health of reconstructed ecosystems in the oil sands.

5.5 Fish

In Alberta there are 63 species of fish, 23 of which occur in the northern Athabasca River watershed. Large-bodied species such as walleye and northern pike inhabit the Clearwater and Athabasca rivers using the smaller tributaries for spawning. Small-bodied fish such as minnows are found abundantly throughout the watershed (www.ramp-alberta.org). In the Athabasca oil sands region, fish are considered an important resource for both subsistence and recreational purposes, and, are also considered an important biological indicator of ecosystem health and integrity (www.ramp-alberta.org).

5.5.1 *Yellow Perch*

Yellow perch (*Perca flavescens*) is an important sport fishing species with a well-known biology (Peters et al. 2007). Both laboratory and field studies have been conducted on this species to assess the effects of oil sands-related water. In field studies, native yellow perch from Mildred Lake (a natural lake situated on the oil sands region that does not contain OSPW) were collected and stocked into experimental ponds created from mature oil sands fine tailings and equal volumes of water (known as water capping approach for reclamation purposes) to assess physiological, population, chemical, biochemical and histopathological endpoints (Van den Heuvel et al. 1999b).

After 5 to 11 months, exposed fish had improved condition factors and increased fecundity when compared to perch from the non-exposed parent population. However, the exposed perch showed (1) higher mortalities exceeding natural rates (post stocking mortality) (Van den Heuvel et al. 1999b), (2) correlation between mixed function oxidase activity (detoxification efforts indicative of contaminant exposure) and PAH concentrations in bile (Van den Heuvel et al. 1999a), and (3) pathologic changes of the gill including tumors, fin erosion and large aneurysms (Van den Heuvel et al. 2000). The authors suggest that the greatest concerns for fish exposed to mixtures from oil sands wastewater are the adverse effects on survival rather than reproductive effects (Van den Heuvel et al. 2000).

In a study using a similar methodology for collecting yellow perch from Mildred Lake and stocking them into experimental ponds, researchers reported significant changes in liver and gill histopathological changes including hepatocellular degeneration and epithelial cell necrosis respectively, after 3 week exposure to aged OSPW (Nero et al. 2006). The authors suggest that histopathology changes along with gill morphometrics are sensitive indicators of exposure of fish to oil sands materials (Nero et al. 2006). Finally, Peters et al. (2007) experimentally exposed early stages of yellow perch to settling surface water (containing varying concentrations of NAs) and reported an increased incidence of deformities and reduced length at hatch (concentration dependent effect) (Peters et al. 2007).

5.5.2 *Fathead Minnow*

Fathead minnows (*Pimephales promelas*) are an indigenous and abundant species found in wetlands of northeastern Alberta (Bendell-Young et al. 2000). Research on this species has involved both laboratory and field studies using life stages from eggs and larvae to adult individuals.

Researchers captured fish from pristine (reference) wetlands and transferred them to the study sites consisting of wetlands receiving oil sands effluent; the fish were were held *in situ* in wire mesh cages, to assess the stress response and acute lethality. Most of the fish from oil sands effluent wetlands were not able to survive for over 2 weeks and those that survived presented significant alterations in blood chemistry variables relative to fish from reference wetlands. The authors reported that changes in blood chemistry of fish represent a sensitive indicator of anthropogenic stressors and that this species is not capable of surviving in wetlands receiving oil sands effluent (Bendell-Young et al. 2000).

Siwik et al. (2000) reported alterations in growth of fathead minnows exposed under laboratory and field conditions to OSPW (wastewater). Laboratory larval growth bioassays demonstrated (1) no significant alterations in larval weight (dry weight), (2) increased growth for only the first seven days of the bioassay and, (3) decreased survival relative to reference fish populations. In field studies, involving maintaining eggs and larvae in mesocosms (outdoor, semi-controlled areas), fish were smaller or of similar length as compared to reference fish (Siwik et al. 2000).

Under field settings, water channels were constructed and ponds adjacent to a large dyke containing consolidated tailings (CT) were used to naturally expose (28 day period) fathead minnows to CT and to seeped wastewater from this dyke. Farrell et al. (2004) reported significant alterations on hematology (decrease in lymphocyte count) and gill morphology (cellular hyperplasia and hypertrophy) after 96 hrs of exposure. Prolonged exposure time resulted in increased mortalities, and no fish survived the 28 day exposure period (Farrell et al. 2004).

In experimental settings, sediment toxicity bioassays were conducted on early life stages (ELS) of fathead minnows. For this study, eggs, embryos and larvae were exposed to natural oil sands sediments and to tailings pond sediments. Increased hatching alterations and mortality rates (both concentration dependent), malformations and reduced growth were reported for fish maintained on natural oil sands sediment water and from tailings ponds containing sediment as compared to fish maintained on control water and from reference sediments (Colavecchia et al. 2004). In addition, eye pathological changes (poor retinal differentiation, optic fissures and microphthalmia) along with expression of CYP1A were reported on fathead minnow larvae (Colavecchia et al. 2007). The researchers suggest that since the CYP1A levels are related to larval mortality, the enzyme represents an indicator of adverse effects rather than just of exposure to contaminants (Colavecchia et al. 2007).

Kavanagh et al. (2011) also used laboratory exposures to assess reproduction in fathead minnows after exposure to aged OSPW. They reported that reproduction is affected as determined by reduced levels of sex steroid hormones (testosterone and 11-ketotestosterone in males, and estradiol in females), and reduced spawning rates (Kavanagh et al. 2011).

5.5.3 *Goldfish*

Goldfish (*Carassius auratus*) represent another fish commonly found in northern Alberta; they are considered a naive species and much is known about their development and reproductive endocrinology (Nero et al. 2006). Researchers conducted field studies by transferring goldfish from a naturally occurring lake within the Athabasca oil sands (Mildred Lake) into experimental ponds. The author reported that after 3 weeks of exposure to aged OSPW, significant changes in liver and gill histopathology occurred, including hepatocellular degeneration and epithelial cell necrosis respectively (Nero et al. 2006). Lister et al. (2008) caged goldfish in ponds containing OSPW (and in reference ponds) and reported that exposed fish had reduced capacity to produce steroid hormones noted by reduced levels of circulating (in plasma) sexual hormones (testosterone in males and 17B-estradiol in females) and by reduced basal sexual hormone levels (Lister et al. 2008).

5.5.4 *White sucker*

Early life stages of white suckers (*Catostomus commersoni*) were used in experimental exposures to tailings sediments and sediments from natural oil sands (downstream of the oil sands region) by the ELS sediment toxicity bioassay (35 days exposure period) (Colavecchia et al. 2006). White sucker eggs and larvae exposed to sediments from natural oil sands and to tailings sediments presented reduced growth, premature hatching, increased larval deformities and increased ELS mortality as compared with fish exposed to reference sediments (upstream of the oil sands region). In addition, they reported significant elevations in EROD activity of juvenile white suckers exposed to natural oil sands and tailings sediments (Colavecchia et al. 2006).

Young et al. (2008) conducted field studies on this species to determine the concentration of naphthenic acids (NA) in flesh, using single ion gas chromatography-mass spectrometry (GC-MS). For a total of four wild white suckers, they reported flesh NA concentrations ranging from <0.1 to 0.8 mg/kg^{-1} (Young et al. 2008).

5.5.5 *Japanese Medaka*

Early life stages of Japanese medaka (*Orizias latipes*) were experimentally exposed to tailings pond water (containing varying concentrations of NAs) and to a low NA-containing solution (Peters et al. 2007). The authors reported an increased incidence of deformities and reduced length at hatch (concentration dependent effect) on fish exposed to tailings pond water as compared to the low NA level solution (Peters et al. 2007).

5.5.6 *Brook Stickleback*

Brook stickleback (*Culaea inconstans*) were used by Bendell-Young et al. (2000) for field studies using *in situ* wire mesh cages in wetlands receiving oil sands effluent. Most of the fish from oil sands effluent wetlands were not able to survive more than 2 weeks, and those that survived presented significant alterations in blood chemistry variables relative to fish from reference wetlands. The authors concluded that this species would not to survive in wetlands receiving oil sands effluent (Bendell-Young et al. 2000).

5.5.7 *Slimy Sculpin and Pearl Dace*

Slimy sculpin (*Cottus cognatus*) and pearl dace (*Semotilus margarita*) were collected from reference sites, from sites exposed to natural weathering of oil sands and from sites exposed to oil sands mining and processing, to evaluate reproductive endpoints. While no consistent alterations were found in gonadal development, steroid hormone production was decreased in these forage species from both the natural weathering and mining sites when compared to fish from reference sites. In addition, EROD activity was elevated at both oil sands exposed sites relative to reference sites; fish from mining sites had 8 times higher EROD activity than that of natural weathering sites (Tetreault et al. 2003b). Further, the same authors experimentally exposed Ontario slimy sculpins (*Cottus cognatus*) to sediments from the above sites and reported that while detoxification activity (EROD induction) was similar to that of the oil sands free-living fish, this experimental exposure "is not a suitable surrogate for field studies" (Tetreault et al. 2003a).

5.5.8 *Trout*

Koning and Hrudey (1992) evaluated fish tainting (mal odor or taste) together with chemical analysis on muscle (fillets) and bile in rainbow trout (*Oncorhynchus mykiss*) exposed to four types of tailings ponds for 24 hrs. All the wastewaters tested produced a detectable odor change compared to fish from reference water (Mildred Lake). Bile and muscle contained alkylated benzene and phenols, pungent chemicals that were also detected in the tailings ponds. No negative effects on fish were reported, but this may have been influenced by dilution of the experimental water to prevent acute toxicity (Koning and Hrudey 1992).

Rainbow trout were exposed to NA for 96 hours in a laboratory to determine the concentration of NA in flesh using single ion GC-MS, a newly developed technique that allows for quantification of minute concentrations of these contaminants (Young et al. 2008). Researchers reported a magnification factor of 2 in the rainbow trout (exposed to 3mg of NA). This methodology was furthered applied to wild species (results described below in section 5.5.9).

In a recent study, primary cultures of trout hepatocytes were exposed to water extracts from intensive bitumen extraction areas within the oil sands, and to reference water extracts to determine the sub-lethal, toxic effects of such extracts. The water samples from tailings ponds and interceptor wells (both containing PAHs) produced (Gagne et al. 2011):

1. an increase in oxidative damage
2. an increase in genotoxicity
3. elevated levels of detoxification enzymes, and
4. diminished viability of trout hepatic cells.

5.5.9 *Other Wild Northern Fish*

Young et al. (2008) collected lake whitefish (*Coregonus clupeaformis*), northern pike (*Esox lucius*) and walleye (*Sander vitreus*) from the Athabasca River, north of Fort McMurray. Concentration of NAs in flesh ranged from <0.1 to 2.8 mg/kg^{-1}. No effects were reported in fish with these tissue concentrations (Young et al. 2008). This paper described a new method capable of detecting very low concentrations of NA in fish muscle.

Jardine and Hrudey (1988) used walleye tissue to determine the threshold odor levels of twelve compounds, including benzothiphene and dimethylnapthalene from the extraction and upgrading of bitumen which occur in oil sands process affected waters. This study only addressed odor thresholds and did not report toxicity in the exposed fish.

6 CONCLUSIONS

The extraction and production of bitumen in the oil sands produces compounds of environmental concern in the form of air emissions perceived to pose risks to flora and fauna in local and downwind regions, and in the form of large volumes of liquid tailings. For the oil sands region, airborne contaminants of interest are VOCs, H_2S, SO_2, NO_2, O_3 and PM, and aquatic contaminants related to the petrochemical industry are PAHs, NAs, sulphate ions, NH_3 and trace metals. However, some of these contaminants have both a gaseous and an aquatic phase. For example, some PAHs can readily volatilize into the atmosphere (especially those with fewer naphthalene rings), as well as being present in tailings ponds.

Once in the environment, complex interactions among contaminants along with inherent chemical characteristics will determine the fate of these compounds. Contaminants may be:

1. volatilized from aquatic media (such as benzene from tailings ponds) and be returned through dry or wet deposition processes
2. broken down in the atmosphere, usually by photochemical processes or through interaction with other contaminants (VOCs for example), or
3. persist in air, water, soil and biota, representing a persistent, internal cycling of contaminants (PAHs and trace metals for example)

Main sources of emissions include activities related to bitumen mining, extraction, refining and upgrading, as well as emissions from working vehicles and equipment as well as fugitive, or non-point source escaping gases. Also, due to the requirement of water for the bitumen extraction process, large volumes of OSPW containing traces levels of numerous contaminants are produced.

Laboratory, domestic and wild animals have been central to research aimed at understanding potential impacts of the oil sands industry on human health and well-being. As stated by the Agency for Toxic Substances and Disease Registry, "without laboratory animals, scientists would lose a basic method to obtain information needed to make wise decisions to protect public health" (Agency for Toxic Substances and Disease Registry 1998). Wildlife species have the potential to act as sentinels, or early warning systems, for environmental and human health. They have also been used as monitor, or indicator species, regarding exposure and effects of contaminants from oil sands activities. For wildlife to be useful sentinels of human and environmental health, they must have certain characteristics, such as:

1. ease of handling and sampling
2. be sensitive enough to the contaminants of concern that such exposure will produce a measurable response
3. be common, non-threatened, and fairly widely distributed in the region of interest
4. have well-known biology and life history traits
5. occupy higher trophic levels in the food chain, and

6. be tolerant of human interference, if the studies are not intended to conclude with the death of the animals being studied

In the oil sands, both field studies and laboratory studies have used wildlife as bioindicators and/or sentinels of ecosystem health. The great majority of this research has focused on aquatic ecosystems and organisms, and predominantly fish such as yellow perch and fathead minnows. The effects of oil sands contaminants on fish will depend on many factors such as dose of exposure, the specific contaminant(s) (especially PAHs and NAs), duration of exposure, and the age of the animal at first exposure.

Overall, fish exposed to oil sands affected waters such as OSPW, water (and sediments) from tailings ponds, and water from wetlands receiving oil sands effluent, present a range of detrimental physiological effects including increased detoxification activity (increased EROD levels), alterations in growth, hormone production and hematological variables, pathologic changes in the gills, compromised survival and increased mortality rates compared to fish from reference sites. Regarding locally important amphibians, under conditions of normal precipitation, it appears that mature oil sands wetlands, those 7 years old or older, are capable of supporting viable populations of amphibians whereas the younger wetlands retain toxic compounds which cause detrimental health effects such as decreased survival, delayed development, and increased rates of malformation.

Research on birds has also focused on aquatic toxicology. Waterfowl are at risk of landing on tailings ponds despite current deterrent systems and mass mortalities have been documented. In addition, growth alterations (smaller body size and mass) have been reported in waterfowl raised on wetlands receiving oil sands effluent. For waterbirds, increased levels of Hg and correlation of Hg concentration with NAs suggest a common source of exposure for birds nesting in lakes that receive water from the Athabasca River downstream from the oil sands. Reclaimed wetlands on different company leases in the oil sands have, in most years, supported active populations of tree swallows during the breeding period and rearing of the offspring. However, stochastic events such as sustained inclement weather may cause insupportable stress resulting in increased mortality rates in song birds (tree swallows) inhabiting these wetlands.

Aquatic toxicology research on mammals suggests that terrestrial wildlife is unlikely to develop acute toxicity from NAs exposure (from tailings pond water), although negative health effects might occur from repeated, or long-term exposures. To date there are no publications on effects of airborne contaminants on terrestrial wildlife, pointing out a gap in knowledge in this field. Studies of contaminants in large mammals, (moose and caribou for example, which are important country food), were not identified. Nor have the authors found any investigations of small mammals which are common to all terrestrial ecosystems, and which would make ideal, lower trophic levels sentinels, especially for particulate airborne contaminants that are deposited on the soil and vegetation upon which small mammals are dependent. Not only would such research provide insight into the productivity, diversity and resilience of the reclaimed terrestrial areas, but would add value to studies on animals such as mammalian carnivores and birds of prey which are dependent on these same small mammals for their sustenance.

This review focused on the effects of contaminants on wildlife and the potential for wildlife to be used as sentinels for human and environmental health. Other factors are reported to negatively affect wildlife such as habitat loss, habitat fragmentation and mortality events directly associated with mining operations, road construction, heavily contaminated tailings ponds, and overall development of the oil sands region and are covered in detail elsewhere (e.g., Dunne and Quinn 2009, Timoney and Lee 2009, Wasser et al. 2011). These stresses may act in a synergistic manner with contaminant stresses.

7 RECOMMENDATIONS

Many wildlife species are permanent residents of the boreal forest encompassing the oil sands region, and many more are seasonal residents using this area as breeding grounds (i.e., migratory bird species), providing the potential for ongoing research focused on the effects of contaminants from oil sands activities.

We have identified a conspicuous gap in information related to effects of airborne contaminants on any species. Birds may be especially valuable to study because of the unique anatomy and physiology of their respiratory system (birds are more susceptible to airborne contaminants than mammals of similar size). As well, as pointed out in the report from the Royal Society of Canada, "quantifying these emissions is notoriously difficult and the data available in the National Pollutant Release Inventory on this subject do not provide enough detail to know what sources have been estimated nor how valid the numbers are"; and, "the subject of non-point (fugitive) emissions of air contaminants from mines and tailings ponds is highly uncertain and currently available estimates are unlikely to be entirely valid" (Gosselin et al. 2010).

One approach to study the effects of emissions on wildlife would be to conduct research on birds of prey (raptors) that use the oil sands region as breeding grounds. Raptors are of particular interest because, firstly, they are known to inhabit the area and are being encouraged to return to mined lands, as evidenced by perches and nest boxes that have been put up on reclaimed areas. Secondly, as top predators, raptors (1) provide integrated insight into compounds transferred through the food web, and (2) are directly exposed through inhalation of air borne compounds. Table 2 shows biological and ecological traits of raptors and other birds with potential to serve as sentinels and/or bioindicators of ecosystem health. Together with concurrent studies of small mammals inhabiting reclaimed terrestrial areas, this sort of work has the possibility of generating information relevant to the health of other wildlife in that ecosystem.

Table 2. Comparison of conservation status and ecological traits (nesting, habitat and feeding behavior) among raptor species and other sentinel wild birds.

Species	Conservation status	Nest type	Habitat	Feeding behavior
American Kestrel	Not threatened	Natural cavities Nest boxes	Open country with scattered trees	Small rodents Amphibians Dragon flies

Species	Conservation status	Nest type	Habitat	Feeding behavior
Ferruginous hawk	Threatened Population at risk	Nest in low trees, in the ground and on cliffs	Open country	Jack rabbits Ground squirrels
Swainson's hawk	Not threatened	Nest in isolated trees Open plains	Open plains	Small rodents Grasshoppers
Osprey	Not threatened	High platforms (Natural or artificial)	Lakes Rivers Seacoast	Fish
Broad-winged hawk	Not threatened	Nest in trees (Nests are difficult to identify) Woodland Prefer undisturbed forest near water	Woodland Prefer undisturbed forest near water	Small mammals Amphibians Reptiles Invertebrates
Northern harrier	Not threatened	Nest on ground	Freshwater marshes Open prairie	Rodents Invertebrates Carrion
Great-horned owl	Not threatened	Nest in trees	Forest, open country and semi-deserts	Varied diet Small mammals
Tree swallow	Not threatened	Natural cavities Nest boxes	Lakeshores Flooded meadows Marshes Streams	Insects

As a specific example, American kestrels (*Falco sparverius*) are an ideal model to assess natural exposure to contaminants associated with oil sands mining and extraction. These falcons have many of the characteristics required for a sentinel species. They have been widely used in a range of research areas (toxicological, biological, nutritional and behavioral studies), they are abundant, small sized and non-endangered. In addition, kestrels readily use artificial nest cavities or nest boxes; this facilitates attracting them to areas that investigators wish to study. Once a breeding pair has selected a nest box for laying eggs and raising offspring, it is relatively simple to work with the nestlings to collect data and biological samples. The shape and size of the nest boxes would also allow for the deployment of passive air monitors to collect quantitative data on exposure to specific contaminant emissions.

Other studies of wildlife as sentinels of ecosystem health could be based on herbivores. For example, deposition and accumulation of particulate air contaminants may be tracked using herbivores. Domestic animals (small ruminants - goats and sheep) may serve as surrogates for caribou, moose and other ungulates naturally found in this region. A number of factors could be investigated such as trace metals in blood, milk, muscle tissue and hair, and immunotoxicity and hormonal responses. If these surrogates represent ruminants destined for human consumption, gross and microscopic pathological examination of tissues can be performed.

Another potential wildlife sentinel is Syncrude's bison herd, which could be monitored similarly to the approach described for small ruminants.

A final recommendation would be to use small mammals such as mice and voles as sentinels of ecosystem health. Such species reflect the quality and quantity of local vegetation (including reclamation vegetation), and readily populate any available area. Studies with these herbivores, and shrews which are insectivores, would provide a meaningful indication of local habitat sustainability. These species also are a source of exposure for top predators that prey on them, thereby forming a food chain link with wildlife higher in the trophic level.

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9 GLOSSARY

9.1 Terms

Acute

The rapid onset of an event (disease, exposure, etc.).

Anemia

Diminished number of red blood cells.

Arteriosclerosis

General term describing the loss of elasticity of large vessels (arteries in particular).

Bioindicator

Biological species that can be used to determine the integrity of an ecosystem. The terms bioindicators, biological monitor and sentinel are often used interchangeably.

Biotransformation

Chemical modification on a chemical compound or contaminant (usually occurring in the liver).

Bradycardia

Disorder of the heart consisting of slow heartbeats.

Cardiotoxic

Chemical or substance that produces toxicity to the heart.

Chronic

The slow onset of an event (disease, exposure, etc.).

Cyanosis

Presence of blue or purple color of the mucous membranes due to inadequate levels of oxygen.

Dyspnea

Abnormal breathing patterns due to inadequate levels of oxygen within the body.

Edema

Swelling caused by fluid within tissues.

Embryopathy

Anomalies or developmental disorders occurring in the embryo.

Endocrine

Refers to hormones secreted by several glands (endocrine system).

Eutrophication

Increased concentration of nutrients (particularly phosphates and nitrates) occurring in water bodies.

Hematology

Science that studies the red blood cells and associated blood tissues.

Hematocrit

Measurement of the percentage of the volume of whole blood that is composed of red blood cells.

Hematopoietic

Tissue that forms blood cell lines.

Histology

Science that studies the microscopic anatomy of tissues.

Leucocytopenia

Reduced number of white blood (defense) cells.

Myelin sheath

Vane rich in lipids that surrounds axons in both the central and peripheral nervous systems.

Necrosis

Death of body tissue.

Pulmonary interstitium

The tissue between the air space epithelium in the lung, the vascular endothelium and the pleural mesothelium.

Tachycardia

Disorder of the heart consisting of rapid heartbeats.

Thrombocytopenia

Reduced number of platelets (blood clotting cells).

9.2 Acronyms

BTEX	benzene, toluene, ethylbenzene and xylene
CT	consolidated tailings
CYP 450	cytochrome P450
EROD	ethoxyresorufin-0-deethylase
H_2S	hydrogen sulphide

HEPA	high-efficiency particle air
Hg	mercury
LOAEL	lowest observed adverse effects level
NAs	naphthenic acids
NH_3	ammonia
NO_2	nitrogen dioxide
NOx	oxides of nitrogen
NPRI	National Pollutant Release Inventory
O_3	ozone
OSPW	oil sands processed water
OSRC	oils sands related compounds
PAHs	polycyclic aromatic compounds
PM	particulate matter
PPE	priority pollutant elements
RAMP	Regional Aquatics Monitoring Program
SO_2	sulphur dioxide
SOx	oxides of sulphur
TRV	tissue residue value
VOCs	volatile organic compounds

APPENDIX 1: Example of a Budget for a University-based Study on Wildlife Monitoring of Ecosystem Health

Funds Requested.	$ Year 1	$ Year 2	$ Year 3
i) Salaries and Benefits			
a) Students (summer assistant)	8,000	8,000	8,300
b) PhD graduate student (Post-Professional)	37,000	38,000	40,000
c) Technical/professional lab assistants ~ 1/3 time	18,000	18,000	18,000
ii) Toxicology analyses - wildlife tissues &-air monitoring	33,000	33,000	28,000
Pathology & biological sampling	6,000	6,000	4,000
iii) Field research depending on the species & requirements	10,000	4,000	4,000
-housing * *would increase by 150% without in kind support from industry partners*	25,000	25,000	25,000
iv) Materials, supplies, consumables	2,500	2,500	1,500
- sampling, preparation, storage, shipping	3,500	3,500	2,500
v) Project Travel - truck rental, petrol, field season	7,000	7,000	7,000

Funds Requested.	$ Year 1	$ Year 2	$ Year 3
a) Presentation at conferences (e.g., CONRAD January workshop, Aquatic Toxicity Workshop, SocEnvironToxAndChem)	1,500	2,500	2,500
SubTotal	151,500	147,500	140,800
vi) Overhead 30% of total	45,450	44,250	42,240
TOTAL	196,950	191,750	183,040
* In Kind (housing-summer crew (2) – Industry partners) (hotel suite or camp accommodations 3 mo)	18,000	18,000	18,000

APPENDIX 2: Exposure and Health Effects of Oil Sands-Related Emissions on Nestling Tree Swallows

INTRODUCTION

In this Appendix we describe a field study on nestling tree swallows in which we assess potential health effects from exposure to oil sands-related emissions. This field study is a practical approach to validating the recommendations in the body of the report and a test of its conclusions.

Tree swallows (*Tachycineta bicolor*) are an abundant species of insectivorous birds commonly found in open landscapes and wetlands. They are often used for ecotoxicology research because they are common across North America, they nest in cavities or accept nest boxes for breeding, and they tolerate human interference (Jones 2003). In the oil sands, tree swallows have been studied as sentinels of ecosystem health by assessing growth, survival and other health variables to test the sustainability of experimental wetlands at different stages of maturation.

METHODS

Tree Swallow Studies on Oil Sands Leases and Local Reference Areas

In 2012 we erected nest boxes at two industrial sites within 5 km of active mine pits, tailings ponds and processing plants on oil sands leases north of Fort McMurray

- lease site 1: 57° 17’ 48.8” latitude and -111° 23’ 24.3” longitude
- lease site 2: 56° 59’ 18.1” latitude and -111° 33’ 57.7” longitude

and at one reference site more than 100 km south of the industrial leased sites

- reference site: 56° 27’ 27.9” latitude and -111° 18’ 2.3” longitude

Determining Exposure

We assessed general exposure to contaminants by measuring detoxification enzymes in liver tissue and in growing feathers through the ethoxyresorufin-O-deethylase assay (EROD assay). We equipped the nest boxes with passive air monitors to measure airborne concentrations of volatile organic compounds (VOCs), polycyclic aromatic compounds (PACs), NO_2, and SO_2.

Toxicological Effects

We assessed the effects on:

1. the immune system through the T-lymphocyte-based phytohemagglutinin (PHA) skin test, and immune organ mass (somatic index), and
2. the endocrine system by measuring:
    a. the stress hormone, corticosterone, in feathers and plasma, and

b. the enzymes responsible for activation of thyroid hormones (i.e., deiodinases), which regulate basal metabolic rate, plus growth and development, as well as conducting histological studies of the thyroid gland.

RESULTS

Details and data from this study are presented in a manuscript for a peer reviewed journal, plus the PhD Thesis currently being prepared by L. Cruz-Martinez.

Exposure

Air contaminant levels were higher at the oil sands sites relative to the reference sites that were approximately 100 km south of the exposed sites:

- NO_2 and SO_2 (5-fold)
- VOCs (6-fold)
- PACs (up to 5-fold).

Compared to reference nestlings, tree swallows from the oil sands had significantly higher detoxification enzyme activity ($P = 0.001$) and decreased liver mass ($P = 0.02$). No detoxification enzyme activity was found in growing feathers via a new methodology that was being tested in our lab using the EROD assay.

Biological Effects

Immunotoxicity tests revealed suppression of the T-lymphocyte response in nestlings from the oil sands ($P = 0.001$) and decreased size of the bursa of Fabricius (the organ responsible for the B-lymphocyte/antibody response in birds) compared with those from the reference areas ($P < 0.001$). For the endocrine response, we found significantly lower baseline corticosterone levels in feathers ($P = 0.01$) from the oil sands nestlings. As well, thyroid activation enzymes (necessary to produce the biologically active form of the thyroid hormones), and thyroid glandular structure indicated higher activity of the thyroid hormone axis in nestlings from the industrial sites.

DISCUSSION

This pilot study confirms the value of measuring hepatic detoxification enzymes as a reliable indicator of exposure to local (in this case, oil sands related) contaminants. While the EROD assay was not sensitive enough to detect detoxification activity in growing feathers of these nestling, we established a methodology that requires further development and validation, but one we consider to be useful in the future as we refine non-lethal methods for investigating biological responses to environmental contaminants.

We noted a pattern of effects in the immune system (suppression) and the endocrine system (up-regulated activity) in nestlings from the industrial sites compared to those from the reference site. This pattern indicates that the nestlings being reared on the industrial sites have higher biological and energetic demands than those from reference areas. This increased metabolic demand associated with oil sands exposures was demonstrated by Gentes et al. (2006) who reported higher mortality in tree swallow nestlings on oil sands leases compared to those from control (reference) areas during stressful weather events, as well as increased intensity of parasitism in the oil sands birds (Gentes et al. 2007) which also indicates lower resources available for resisting infestation by parasites.

Studying wildlife as bioindicators of ecosystem status can provide predictive information on exposure and effects of contaminants from oil sands activities. This information complements ongoing air and water quality monitoring, and has the potential to serve as criteria for measuring ecosystem recovery related to the different reclamation and restoration strategies in current use by oil sands companies.

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