FOCUSED STUDY ACTIVITY WORK PLAN

General Information

*** Decision Pool A: Workplan approved but at a reduced funding level.** *** Approved at $424,000 (17/18 levels)** * Deliverables for this level of funding are to be clarified and an amended workplan submitted before March 23, 2018 to the Oil Sands Monitoring Secretariat. * It is a requirement of funding that key members of the project team participate in a Biological Monitoring Integration Workshop and the Deposition Integration Monitoring Workshop to be informed by the Oil Sands Monitoring Secretariat * Decisions on future funding beyond 18/19 are dependent upon the outcomes of these workshops *Funding expectations: as a minimum an annual progress report is required by February 28, 2019. All publications or products resulting from this work requires acknowledgement of funding from the Oil Sands Monitoring Program and are to be provided to the Oil Sands Monitoring Secretariat for tracking and any programmatic communications purposes. Work funded through the Oil Sands Program will be available for public dissemination.		
Work Plan Unique Identifier:	*WL-IC-11-1718*	
Focused Study Activity Title:	**Amphibian and Wetland Health: Investigation of Wetland Ecosystem Health**	
Focused Study Category:	Investigation of Cause or Potential Ecological Impact	
Geographic Location (*choose from drop-down menu. If Project Location is in more than one area choose from second drop-down***)**	Lower Athabasca River	More than 2 Locations (Described in Detailed Monitoring Plan)
Monitoring Site(s) Coordinates *(latitude and longitude)*	See appended list below	
Project Leader:	Bruce Pauli	
Organization and contact information:	**Environment and Climate Change Canada (ECCC)** Ecotoxicology and Wildlife Health Division Science and Technology Branch Environment and Climate Change Canada National Wildlife Research Centre 1125 Colonel By Drive, Ottawa, ON N1H 0H3 Tel: 613 998-6690; email: bruce.pauli@canada.ca	
Date Study initiated:	2011	
Monitoring Category:	Biotic Response Monitoring	
Strategic Objective of Focused Study: *(From OSM long-term plan; choose from drop-down menu)*	Objective B2: Investigate the causal mechanisms of a known important biotic relationship in relation to Oil Sands Developments Results from monitoring of wetlands and contaminant burdens in wetland bioindicator species (e.g. wood frogs, *Lithobates sylvaticus*) in	

	the oil sands region has revealed detectable levels of contaminants, including heavy metals and polycyclic aromatic compounds (PACs), both in the wood frogs and in the wetlands where they breed. Through integration with other components of the oil sands monitoring program, this focused study/investigation of cause has the following strategic objectives: (i) to assess levels of these high priority contaminants in wetlands, (ii) to assess contamination of the food web in these wetlands (iii) to track the sources of the contaminants measured, and (iv) to assess the biotic response/potential effects of the contaminants on receptor organisms in the wetlands. A major objective of this focused study/investigation of cause project is to conduct integrated evaluations of contamination of the food web of these wildlife bioindicator species to determine where the contaminant burdens in the animals are coming from and what the effects might be. The overall strategic objective of this focused study/investigation of cause is to establish the monitoring of contaminant burdens and effects in bioindicator wildlife species in wetlands as a validated long-term monitoring strategy for the evaluation of potential environmental impacts of oil sands industrial activities.
Hypotheses: *(Briefly outline the specific hypotheses that your focused study is aiming to address)*	The work will test the hypotheses that 1) contaminant burdens in the tissues of bioindicator wildlife species in wetlands and in the wetland aquatic environment near oil sands industrial operations are not different from burdens measured in animals and samples that are collected from remote "reference" areas, 2) oil sands industrial operations are not contributing to the increase in contaminant burdens in the tissues of these bioindicator species and their wetland habitats, and 3) contaminant burdens in the bioindicator wildlife species collected in the region are below a level where toxicological effects resulting from the animals exposure to oil sands-related contaminants are occurring.
Deliverables: *What tangible goal (s) and/or product(s) will the monitoring produce and when?*	Tangible goals and products from this focused study/investigation of cause and the associated monitoring that will be conducted during the project include the following. <u>Near-term deliverables:</u> 1. Measurements of the levels of contaminants likely to cause adverse human/environmental health effects in the oil sands region and downstream (i.e. in the Peace-Athabasca Delta and in Wood Buffalo National Park and into the Northwest Territories), 2. Measurements of food web contamination and contaminant fate, dynamics, cycling, and sources, 3. Assessments of the effects of oil sands-related contaminants on focal bioindicator or "sentinel" species, 4. Assessment and validation of effects biomarkers for wildlife exposed to oil sands chemicals of concern in target wetland bioindicator species (as validated biomarkers do not exist for oil sands contaminant mixtures, their development, validation and

	establishment is required). <u>Longer-term deliverables:</u> 1. Based on species present, establish long-term wetland health monitoring sites by collaborating with other oil sands monitoring programs (water and air deposition), 2. Choose the most appropriate sites and install the appropriate monitoring instruments and equipment for risk-based monitoring of wetland health, 3. Using established or newly-developed biomarkers and toxicity endpoints, establish toxicity thresholds and correlate these with contaminant concentrations measured at long-term monitoring wetland sites,, 4. Using the results from the preceding tasks, establish a robust and credible monitoring program for wetlands undergoing anthropogenic change in the Alberta Oil Sands region (AOSR). These tangible goals and products will be produced throughout the 3-year duration of the study, with the final product being delivered by the end of the three year period.

Detailed Study Plan

(Please provide detailed information on the specifics of your focused study including – (**keywords, hypothesis and the assumptions and constraints behind your hypothesis**)

Provide a maximum of 10 key words that describe this project. Use commas to separate them:

Wildlife health, contaminants, bioindicator species, amphibians, wetlands, mercury, PACs, oil sands, cumulative effects, ecosystem health

Describe how you will test your hypotheses:

Overview: The hypotheses related to this Focus Study/Investigation of Cause project are included above. A key aspect of how we will test our hypotheses is through enhanced cooperation with other researchers, i.e. with other groups in ECCC (scientists from the Water Science and Technology Directorate (WSTD) and the Atmospheric Science and Technology Directorate (ASTD) and the Canadian Wildlife Service), with Alberta Environment and Parks, the Alberta Biodiversity Monitoring Institute (ABMI), Health Canada, Alberta Health and Wellness, and academia. Also during 2018/19, a crucial aspect of this workplan is ongoing communication and planning with proposed study partners and a wide range of multi-disciplinary potential collaborators to further develop and refine the longer term work plan and strategy for scientifically-defensible long-term monitoring of wetlands in the oil sands region. Our plan is to accomplish this through regular discussion with principal investigators and ECCC partners, and via workshops. This is crucial for the development of recommendations for the most appropriate techniques and ecosystem components to include in a long-term monitoring program for wetlands in the oil sands.

In summary, to test the hypotheses of this project we will:

- Continue field work to study wetlands and monitor amphibian health,
- Continue collection of biotic and abiotic samples at wetlands to characterise wetlands and assess contaminant burdens and effects,
- Continue examination of use of passive sampling devices (e.g. polar organic contaminants integrated samplers (POCIS), semi-permeable membrane devices (SPMDs), permeable membrane devices (PMDs), diffuse gradient thin films (DGTs), and polyurethane foam (PUF) samplers) to monitor contaminants in the environment and to generate data to compare to levels in biota with the aim to both monitor exposure and reduce animal use,
- Continue examination of the most appropriate endpoints to determine effects,
- Continue laboratory studies to examine, in our wood frog sentinel species, the means of assessing effects from exposure to oil sands CoCs (such as the use of molecular biomarkers and oxidative stress, and frog immunological markers) and exposure itself by examining distribution of metals and naphthenic acids and uptake and depuration of PACs in wood frogs.

Evidence supporting the need for a Focus Study/Investigation of Cause
During the JOSM Amphibian and Wetland Health monitoring program we measured levels of metals including mercury and methylmercury, as well as polycyclic aromatic compounds (PACs) and naphthenic acids (NAs) in wood frogs and their wetland breeding sites. At some sites, concentrations of some of these contaminants were at levels that require further investigation (see below), particularly regarding mercury. Also requiring further investigation is the effects that may be occurring in biota that are resident in these wetlands and are exposed to these contaminants. We have also discovered spatial relationships between contaminant concentrations in wetlands and distance to oil sands industrial operations for some measures; further study is required to understand these patterns. The need for this study is absolutely required following a risk-based approach to monitoring: we have established the likelihood of the stressor (contaminants) being present from our baseline monitoring of wetlands in the AOSR, and we know that the impact of that stressor could potentially be very high with respect to the risk to wetland and ecosystem health, to biodiversity, to the preservation of ecosystem components and ecosystem resilience, and to the preservation of ecosystem function, which is necessary for stakeholders.

Among the contaminants of concern, metals are of concern because of their potential toxicity, their bioaccumulation and, in some cases, biomagnification. Therefore, we plan to continue to investigate spatio-temporal patterns of metals of concern in wetlands and amphibians, including mercury (Hg), arsenic (As), cadmium (Cd), lead (Pb) and selenium (Se). In previous JOSM monitoring activities we measured these metals in water and wood frog (*Lithobates sylvaticus*) samples collected from 13 wetlands multiple times between May to September, to collect data to establish baseline measures and for use in spatial variation analyses of the contaminant patterns across the AOSR. Data on general water chemistry were also collected. As an example, median levels (with ranges) of Hg, As and Pb in water were 2.08 (0.34-21.3) ng/L, 0.72 (<DL-22.2) µg/L and 0.05 (<DL-0.66) µg/L, respectively, and varied among sites. However, since Hg was detected in all samples, further monitoring is warranted to establish patterns and examine food web contamination/exposure and source attribution for this contaminant of concern. In amphibian tissues, median Hg, As and Se levels in tissues were 0.09 (0.02-0.41), 0.21 (<DL-12.79), and 0.65 (<DL-1.59) µg/g dw, respectively. Concentrations of Hg and Se varied among sites. Hg, Cd and Se did not vary among wood frog life stages, whereas As and Pb were significantly higher in tadpoles compared to recent metamorphs and adults. Linear mixed models indicated that, in general, variation in tissue metal concentrations was not related to distance from upgraders nor to time (within or across years), but was related to other metals present in water and tissues, and in a few cases, to the amphibian life stage sampled. These findings are driving the design of the Focus Study/Investigation of Cause being followed here; using a risk-based approach to monitoring, we have established the likelihood of the stressor and the potential high impact related to the possible effects to biota from their exposure to that stressor.

PAC levels were measured in wetland breeding habitat, in wood frog tissues, and using semi-permeable membrane devices (SPMDs), the latter as a passive sampling technique (Mundy et al. *in review*). PACs could be detected in both wood frog tissues and SPMDs. The latter technique also revealed that wetlands could be distinguished with respect to their PAC levels by the distance the wetland was from major industrial activities including upgrader facilities. This information supports other findings with respect to the spatial pattern of PAC contamination across the region. Very limited studies have been conducted on the toxicity of PACs to amphibians, fewer still on the toxicity to amphibians of the PAC mixtures that occur in the environment as a result of industrial processes, and almost none on the toxicity of oil sands-related PAC contaminants and wood frogs, outside of the studies we have conducted ourselves with our academic partners (Bilodeau et al., Gallant et al., Orihel et al.). Again using a risk-based approach, since we have established the likelihood of exposure to PACs, we need to assess effects of PACs on our focal bioindicator species the wood frog, we need to establish biomarkers for wood frog exposure to PACs and appropriate toxicity endpoints and thresholds, and we need to correlate these with contaminant concentrations measured in wetlands in the AOSR to determine the overall impact to ecosystem health from this exposure.

Further evidence supporting the need for this Focus Study/Investigation of Cause study is that an equivalent level of effort as described above for PACs has not been accomplished for naphthenic acids (NAs), and large data gaps exist in our knowledge of the levels of NA contamination in wetlands in the AOSR and potential effects on ecosystem health. Again following a risk-based approach, we have already collected baseline information on levels of NAs in wetlands and wood frogs in the AOSR, and have initiated studies examining effects of NAs in wood frogs using laboratory and mesocosm studies (Guiterrez et al. 2014a, 2014b, 2015; Orihel et al. 2016); the findings have necessitated this Focus Study/Investigation of Cause.

Finally, because of the large data gaps that exist in our knowledge concerning the levels and effects of all classes of oil sands-related chemicals of concern (PACs, metals and NAs) in wetlands, along with the high risk to the environment of these contaminants, we will follow a risk-based approach to study all three classes of contaminants in natural wetlands, in the laboratory and in mesocosms, over the entire three year duration of this Focus Study/Investigation of Cause project.

Focus Study/Investigation of Cause Overall Design

The Deliverables as mentioned above are guiding the design of this study. We plan to monitor the levels of contaminants that are likely to cause adverse human/environmental health effects in the wetlands of the oil sands region and downstream (in the Peace-Athabasca Delta and WBNP and into the NWT); we will measure contaminant burdens in our focal bioindicator species and in its food web, and contaminant fate, dynamics, cycling, and sources to the study wetlands. We will assess effects of oil sands-related contaminants measured in the wetlands on focal species inhabiting those wetlands. We will assess and validate effects biomarkers for wetland wildlife exposed to those contaminants, and develop biomarkers where they don't exist. The overall goal is to use all of the compiled information to design a robust and credible wetland health monitoring program for the AOSR.

To complete all this, this Focus Study/Investigation of Cause is comprised of two major components: field investigations of the relationships between the levels of high priority contaminants in wetlands and wetland ecosystems and the location of those wetlands in relation to oil sands industrial activity, and laboratory exposure experiments to examine relationships between the exposure of wood frogs to the high priority contaminants we are measuring and the biotic response of the animals. The goal of the latter component is to establish the most appropriate toxicological endpoints to use in a long-term wetland monitoring program for the oil sands. The laboratory investigations will cover a range of potential assessment endpoints by examining various physiological, enzymatic, immunological, endocrinological, morphological, and metabolomics and other genomics-based response variables.

To establish the sources and levels of contaminants, field monitoring of wood frogs and other components of wetland ecosystems during three field seasons will be used to evaluate the health of wood frogs and their wetland ecosystems in wetlands that are near to or farther removed from oil sands industrial operations. Starting in Year 1, contaminant sources were studied on an on-going basis through collaborations with air monitoring, snow monitoring, and water/sediment scientists, with food web contaminants assessments, and with the collaboration of various government and academic analytical chemists. If resources permit, the study design includes "core" monitoring wetlands monitored during the JOSM program, as well as wetlands that may be visited in cooperation with other oil sands monitoring activities. The goal is to have a "structured" design that includes matched or paired wetlands, instrumented with the appropriate passive samplers for air, water and sediment, with different levels of contaminant inputs, and including reference wetlands in areas outside of oil sands deposits, reference wetlands located on deposit but with little influence from oil sands industrial operations, wetlands near oil sands industrial operations, and wetlands situated across the landscape in such a manner that they follow a gradient of airborne deposition, so that the farthest-afield wetlands would receive contaminants only from aerial deposition.

Links to depositional rationalization

Amphibian wetland study sites are currently situated both inside and outside of the surface mineable region of the Athabasca oil sands deposit. Amphibian and wetland health monitoring sites were selected based on a number of criteria, including where they are located geographically with respect to the major sources of airborne emissions, and their "depositional" distance downwind from those sources based on information on prevailing winds. Extensive site appraisal occurred, and evaluation and selection was conducted and careful planning undertaken to ensure sites were located at varying distance to oil sands industrial development and infrastructure; some are situated at sufficiently remote locations that the only deposition source is airborne emissions from industrial infrastructure (i.e. "removing the fleet" etc.). Further, sites are situated across an atmospheric depositional gradient (high to low deposition). Depositional data, specific to the oil sands region for anthropogenic contaminants of concern (e.g. polycyclic aromatic hydrocarbons and metals, including mercury) influenced our site selection (e.g. Kelly et al. 2009, 2010). Furthermore, data generated from ECCC snow sampling activities, continues to influence our site selection and monitoring design (e.g. Kirk et al. 2014; Kirk, personal communication, 2017). In addition, actual deposition to our monitoring wetlands during the course of the amphibian breeding season in 2017 was assessed with the assistance of Dr. Tom Harner, AQRD, who provided passive air samplers for deployment at our monitoring wetlands, and who analysed, as a valuable in-kind contribution, the polycyclic aromatic compounds gathered by the samplers in these passive sampling devices, as in a previous collaboration (Cruz-Martinez et al. 2015).

STUDY DESIGN

The study is divided into a number of inter-related components that will provide samples and information for an integrated assessment of wetland health in core monitoring wetlands, as follows:

Monitoring health in an amphibian sentinel species

The use of amphibians as model organisms for studying environmental change, including interactions with toxicants, is a burgeoning field (Sparling, 2000). There have been very few studies on the exposure of free-ranging wildlife to contaminants such as PACs, naphthenic acids and heavy metals, and the potential mixtures of those contaminants, in areas impacted by oil and gas industrial activities and the ecological significance and the effects of this exposure are poorly understood, especially in species such as amphibians that are sensitive to the effects of contaminants, non-migratory, and are exposed to contaminants in both the aquatic and terrestrial environments (Hopkins, 2007). Given the paucity of information on the effects of these contaminants on reproductive success, physiological and immune function, and population level responses, investigative studies on amphibian health in the oil sands region are warranted.

Additional health assessments of amphibians at both intensive and extensive monitoring sites, the presence and pathogenicity of important amphibian diseases, such as *Ranavirus* and chytrid fungus (*Batrachochytrium dendrobatidis*), and any other pathogenicity observable with gross diagnostic techniques, will be monitored in boreal wood frog populations in the study area.

Water chemistry of study wetlands including ground water inputs

Water chemistry data is critical in the determination of wetland health and quality. Collecting water chemistry data addresses two key strategic objectives outlined for this focused study, and will enable us to assess high priority contaminants in wetlands, and track sources of contaminants measured. In addition to basic water chemistry measurements (pH, conductivity, nutrient inputs, total dissolved solids, dissolved carbon, chlorophyll A, etc.), concentrations of oil sands-related contaminants (metals, PACs, and NAs) will be assessed at wetland study sites. Water chemistry measurements will also be taken to characterize groundwater inputs. These additional sample collection activities will be performed in collaboration with Greg Bickerton (ECCC, Senior Hydrologist, Watershed Hydrology and Ecology Research Division; personal communication 2018). Previous wetland monitoring completed under JOSM focused on study sites situated across an atmospheric depositional gradient was conducted with limited consideration of attempting to delineate groundwater or surface water inputs. The groundwater pilot activities proposed in 2018/19 will focus on key wetland sites located within the AOSR in an effort to determine whether contaminants of concern previously detected in certain wetlands are the result of natural or anthropogenic processes.

Assessments of plant health in study wetlands (includes contaminant burdens in "sentinel" plant species and periphyton)

Macrophyte Vegetation and Algae

Wetlands are made up of diverse and variable vegetation and algae communities, often used in describing types of wetlands (National Wetlands Working Group, 1997). Both macrophytes and algae play several important roles in wetlands that contribute to the functionality of the ecosystem. As primary producers, they provide food at lower levels of the food chain and supply energy to the system (Fennessy et al., 2002; Stevenson et al., 2002). Algae is common in the diet of wood frog tadpoles (Schriever & Williams, 2013), the sentinel species of concern in this focus study. Vegetation and algae are used as habitat for numerous other taxa such as macroinvertebrates and amphibians. They also have a role in cycling nutrients, as well as improving water quality by taking up contaminants (Fennessy et al., 2002; Stevenson et al., 2002). In addition, several plant species found in the AOSR are considered to be culturally significant species, such as bog cranberry (*Vaccinium oxycoccus* (L.) MacM.) and ratroot (*Acorus americanus*), among others (Geribaldi & Straker, 2009). These qualities make vegetation and algae important features of wetlands.

Vegetation is a good indicator of wetland health and is used by several organizations for wetland monitoring. The United States Environmental Protection Agency (USEPA) published a series of documents detailing protocols for several elements of the environment that can be used for evaluating the condition of a wetland, including vegetation and algae (Fennessy et al., 2002; Stevenson et al., 2002). Other reputable organizations and conservation authorities use similar methods and have detailed guidelines for monitoring wetland vegetation (GLCWC, 2008; O'Reilly, Roy, Bowers, & Paudel, 2010). Plants are used for monitoring because their community structure responds quantifiably to anthropogenic stressors such as the introduction of nutrients, metals, or other contaminants (Fennessy et al., 2002). There are many community based metrics that can be studied including species richness, evenness, biodiversity, proportion of native species, etc. (Fennessy et al., 2002). These variables can be considered independently for comparison between regions (GLCWC, 2008), or can be applied collectively to form an Index of Biotic Integrity (IBI). A vegetation-based Index of Biotic Integrity (vIBI) has been developed for wetlands specific to the AOSR to assess the health of reclaimed wetlands. The vIBI would be a useful method of quantifying the health of any wetland in the region and has the potential to demonstrate a disturbance gradient response (Raab & Bayley, 2012). Watershed management agencies in the U.S. are incorporating a multi-metric IBI, such as the vIBI which has apparently proven useful as a tool in rapid assessment practices, and in circumstances that limit sampling to one site visit per year (Raab & Bayley, 2012; Rooney & Bayley, 2012).

Algae are found in the shallow water-saturated areas that occur in wetlands, and can also be plentiful in deeper water under the right conditions (Stevenson et al., 2002). A substantial portion of wetland metabolism is performed through algal processes. Wetland algae are well studied and commonly used indicators of biological integrity because they are sensitive to environmental changes; their community structure shifts in predictable patterns (Stevenson et al., 2002). They can be divided into two broad communities; unicellular phytoplankton that reside in the water column, and periphyton that form mats or films on wood, plants, sediments, and other surfaces in wetlands (Stevenson et al., 2002). Periphyton assemblages sampled from the sediments (epipelon) can give an understanding of conditions over several years of accumulation. Periphyton sampled using introduced surfaces (epilithon), on which the algae are left to grow for a portion of the season, provide a finer temporal resolution of environment conditions over several months as opposed to several years. Similar to macrophyte vegetation, species composition is used to calculate functional metrics such as relative abundances, and presence/absence of particular species or groups. The USEPA recommends comparing similarity of biomass and relative abundance of species between sites (Stevenson et al., 2002). Previous research has already shown the promise of using algae as a biotic indicator in the oil sands region by doing paleoecological assessments of sediment cores (Kurek et al., 2013), and finding links between taxonomic composition and oil sands contaminants (Leung et al., 2003).

Plants and soils have previously been collected by Environment and Climate Change Canada (ECCC) for investigation of oil sands contaminants (ECCC, 2018). Continued collection and testing of plant and algal tissues for contaminants is warranted to further understand how they influence the movement of contaminants through the wetlands. Furthermore, accumulation of contaminants in culturally significant plant species could have implications on human health.

Use of passive sampling techniques to monitor contaminants in study wetlands

At intensive wetland monitoring sites, we will continue to deploy passive sampling devices (e.g. SPMDs, DGTs, POCIS, PMDs, PUFs) in addition to the collection of other abiotic and biotic samples (e.g. water, sediment, plants, and wood frog tadpoles) in order monitor for the presence of contaminants of concern.

SPMDs have been used with increasing frequency since their introduction in 1990, and are routinely deployed in water monitoring programs for a variety of applications (Esteve-Turrillas et al., 2007). Triolein-containing SPMDs, like those used in previous monitoring years, were originally designed to mimic the bioconcentration of organic contaminants in the fatty tissues of aquatic organisms as these membranes selectively accumulate dissolved hydrophobic compounds that are assumed to represent the bioavailable fraction of compounds (Lu et al., 2002). As a result, SPMDs have been added to biomonitoring studies to improve estimates of exposure to waterborne organic contaminant mixtures and in some cases, they have been used as surrogates for biomonitoring organisms in order to predict tissue concentrations of specific contaminants (Lu et al., 2002; Petty et al., 1998). In addition, POCIS samplers will be used to measure naphthenic acid accumulation in target wetlands within the vicinity of oil sands industrial development, including tailings ponds, and at reference wetlands, and Polyurethane Foam (PUF) samplers will be used at sites to measure the deposition of airborne PACs.

Overall, we will use passive sampling devices for three purposes: (1) to determine the presence, source, and time weighted average concentrations of hydrophobic organic contaminants in boreal wetlands and in the atmosphere (e.g. Polyurethane Foam (PUF) sampling system), (2) to compare concentrations of contaminants of concern in passive sampling devices and biomonitoring organisms (e.g. wood frog tadpoles and potentially benthic invertebrates) and (3) to provide extracts from field-deployed passive samplers that will be used to characterize the toxicological potential of a particular target wetland (see biomarkers and toxicogenomics section below for more detail).

Use of biomarkers, toxicogenomics and physiological measures to monitor health of wetlands fauna (e.g. EROD, oxidative stress, corticosterone, immune function and immune modulation etc.)

We are using biomarkers of exposure (i.e. changes in enzyme activity and other biochemical and molecular endpoints) to assess the degree to which organisms inhabiting wetlands situated inside and outside the AOSR are exposed to contaminants of concern. In addition to traditional analytical chemistry approaches that measure chemicals of concern in abiotic and biotic samples, the use of biomarkers provides an additional level of toxicological information with respect to whether concentrations of contaminants in a particular wetland are capable of inducing subtle biochemical and molecular changes in wetland fauna. The use of biomarkers of exposure is a much needed measure when dealing with certain compounds, especially PACs. For example, our work using passive sampling devices showed a great degree of variability in PAC concentrations across wetland study sites, with higher concentrations detected in sites located close to oil sands mining activities and bitumen upgraders. The same spatial pattern however, was not clearly replicated in wood frog tissues. The ability of biomonitoring organisms to metabolize and excrete xenobiotic compounds, like PACs, makes it difficult to simply rely on analytical chemistry techniques to inform the degree to which wildlife may be exposed to contaminants of concern.

EROD activity is an indirect measure of cytochrome P4501A enzyme induction, an enzyme involved in the phase I metabolism of xenobiotic compounds. EROD activity is a biomarker that has been used in a number of wildlife monitoring projects conducted previously in the AOSR. For example, increased EROD activity has been measured in tree swallows (Cruz-Martinez et al., 2015) and wood frogs (Hersikorn and Smits, 2011) inhabiting reclaimed wetland sites relative to reference sites. We will measure EROD activity in the liver microsomes of wood frogs collected from wetland monitoring sites and determine whether the assay is subtle enough to detect differences between tadpoles sampled from different wetlands inside and outside the AOSR.

Chemical extracts from passive sampling devices will be used to assess toxicity in wetlands sites. Crump et al., (2017) recently assessed the toxicity of various petcoke extracts in avian hepatocytes. We will use the same well-established, high throughput avian *in vitro* assay (i.e. primary embryonic hepatocyte assay), two well-characterized biochemical assays, and a custom-designed avian PCR array to elucidate biochemical and transcriptomic effects of passive sampling extracts. Biochemical assays include measures of EROD activity and total porphyrins. The PCR array measures 43 target genes, which provide coverage of numerous toxicity pathways that may be impacted by an organism's exposure to OS-related contaminant mixtures.

In addition to markers of exposure linked with xenobiotic metabolism, we aim to potentially measure markers of oxidative stress, compromised immune function, and hormonal changes (e.g. corticosterone), and possibly other physiological or endrocrinological biomarkers in wood frog tadpoles exposed to oil sands-related contaminants *in situ* and/or in controlled laboratory exposure experiments.

Use of conservation genetics tools to monitor the health of amphibian populations at wetland study sites

Population genetics provides important endpoints when evaluating the impacts of anthropogenic disturbances on resident wildlife. Understanding the health of amphibian populations at any given wetland requires an understanding of the population source-sink dynamics (Dias, 1996) associated with that wetland. For example, landscape-level population genetics approaches have demonstrated that amphibian breeding wetlands with higher Hg levels can actually serve as population "sinks" on the landscape, irrespective of the demographics and apparent health of the individuals encountered at that wetland (e.g., Wilson et al., 2012; Wilson and Hopkins, 2013). This is due to the rescuing effect of migrants from non-contaminated wetlands that act as source populations on the landscape. We will use well-established population/landscape genetics approaches (e.g. RADSeq molecular techniques; Davey and Blaxter, 2011) and ecological theory frameworks in conjunction with the contaminants data from our study wetlands to investigate the extent to which oil sands-related contaminants may be contributing to population 'sinks' in wood frogs in the AOSR.

Field Sampling Campaign Highlights:

- Continued field work to study wetlands and monitor amphibian health.
- Continued collection of biotic and abiotic samples at wetlands to characterise wetlands and assess contaminant burdens and effects.
- Continued examination of use of passive sampling devices (e.g. POCIS, SPMDs, PMDs, and DGTs) in comparison to levels in biota to monitor exposure and potentially reduce animal use.
- Continued examination of the most appropriate endpoints to determine effects using field-collected samples (supported by laboratory exposures using similar compounds and subsequent effects assessments for establishment of biomarkers of effects).

<u>Year 2018-19</u>

In summary, field assessments of contaminant burdens in wetland habitat and wetland biota and assessments of amphibian health will occur at "intensive" and "extensive" monitoring wetlands. For the intensive wetland site monitoring program, monitoring of oil sands-related contaminants of concern (e.g. metals, PACs, naphthenic acids) in wetlands and bioindicator species (wood frog - *Lithobates sylvaticus*) will continue at "core" monitoring sites that have been sampled since the inception of the project in 2011 (see below, and the Table below listing proposed sampling sites along with their location and sampling history). For the extensive wetland site monitoring program, additional wetlands will be integrated with other proposed wildlife toxicology focused studies (e.g. gull and tern egg contaminants monitoring), and with other oil sands monitoring programs where possible, in order to generate data that will increase our knowledge of the extent to which oil sands-related contaminants of concern are entering and moving through aquatic boreal food webs. Identification of potential wetland sites that might be sampled for the "extensive" wetland monitoring program will be pursued through discussions with other oil sands monitoring scientists.

Samples to be collected at "extensive" wetland sites will be determined based on the level of program integration we are able to achieve with other oil sands research and monitoring projects. For example, we hope to deploy passive sampling devices at remote wetland sites being visited by JOSM project partners

- Abiotic and biotic samples collected in 2017-18 for contaminants analysis will continue to be targeted in 2018-19. Additional food web samples, and sensitive indicators of wetland water quality e.g. benthic macroinvertebrates, will be collected. In addition, we plan continued alignment with other high priority wildlife toxicology focused study wetland locations, and with the overall wetlands monitoring program.

- We will continue our investigation of the use of passive sampling devices to monitor contaminants in wetlands and the possibility of also deploying biomonitoring organisms (e.g. caged mussels) to enhance passive sampling monitoring initiatives.

- Using information being provided by our on-going laboratory exposure and effects experiments, we will conduct additional testing and validation of novel biomarkers in the laboratory, in mesocosms and in natural wetlands to determine whether contaminants of concern have a measured biotic response in wood frogs.

- We will continue to monitor the presence of amphibian diseases, namely *Ranavirus* and chytridiomycosis, in boreal wood frog populations.

Year 2019-20

- Abiotic and biotic samples proposed for collection in previous years for contaminants analysis will continue to be targeted in 2019-20.

- Continued alignment with other high priority wildlife toxicology focused study aquatic locations.

- Continued investigation of the use of passive sampling devices to monitor contaminants in wetlands and the use of biomonitoring organisms (e.g. caged mussels) to enhance passive sampling monitoring initiatives.

- Additional testing and validation of novel biomarkers in the laboratory, in mesocosms and in natural wetlands to determine whether contaminants of concern have a measured biotic response in wood frogs.

- The presence of disease, namely ranavirus and chytridiomycosis, in boreal wood frog populations.

Laboratory Exposure Experiments:

Year 2018-19
Supporting laboratory and mesocosm exposure experiments have already been initiated for this monitoring program, as mentioned above (Bilodeau et al. 2017; Gallant et al. 2015; Guiterrez 2014a, 2014b, 2015; Orihel et al. 2016). In fact, laboratory studies associated with this study have produced the first results examining uptake, depuration and biological effects of oil sands-related contaminant mixtures in the wood frog *Lithobates sylvaticus* (Bilodeau et al. 2017; Gallant et al. 2015). Continued laboratory studies with academic partners will examine, in our wood frog sentinel species, the means of assessing exposure and the development and establishment of biomarkers of exposure and health in wood frogs. This involves the coordination across ECCC scientists and partners in government and university projects that are examining uptake and depuration, molecular markers of exposure, enzyme activity (ECCC), oxidative stress, bitumen toxicity to amphibians, NA and PAC toxicity to wood frogs, immune toxicity, nutrient limitation and environmental DNA, metal and NA body burdens, etc. The information to be generated will be used to establish robust wetland health monitoring systems for the oil sands.

Year 2019-20
Continued laboratory studies with academic partners to examine, in our wood frog sentinel species, the means of assessing exposure and effects endpoints such as molecular biomarkers and oxidative stress, frog immunological markers, distribution of metals and naphthenic acids in wood frogs, and uptake and depuration of PACs in wood frogs, as in 2018-19.

STUDY LOCATIONS

Site Name	Site Location	Species Sampled	Samples Collected	Year First Collected

Lucy Pond	Mineable oil sands	wood frog	wood frog, passive sampling devices, water, sediment, plants, contaminants, disease incidence	2011
Maqua Lake	Mineable oil sands	wood frog	wood frog, passive sampling devices, water, sediment, plants, contaminants, disease incidence	2013
Jetliner Pond	Mineable oil sands	wood frog	wood frog, passive sampling devices, water, sediment, plants, contaminants, disease incidence	2011
Jenny Pond	Mineable oil sands	wood frog	wood frog, passive sampling devices, water, sediment, plants, contaminants, disease incidence	2011
Tower Road	Mineable oil sands	wood frog	wood frog, passive sampling devices, water, sediment, plants, contaminants, disease incidence	2011
WF4	Mineable oil sands	wood frog	wood frog, passive sampling devices, water, sediment, plants, contaminants, disease incidence	2012
Gateway Pond	Mineable oil sands	wood frog	wood frog, passive sampling devices, water, sediment, plants, contaminants, disease incidence	2012

BM11	Mineable oil sands	wood frog	wood frog, passive sampling devices, water, sediment, plants, contaminants, disease incidence	2012
HAT-S5	Mineable oil sands	wood frog	wood frog, passive sampling devices, water, sediment, plants, contaminants, disease incidence	2013
JP302	Mineable oil sands	wood frog	wood frog, passive sampling devices, water, sediment, plants, contaminants, disease incidence	2012
JP311	Mineable oil sands	wood frog	wood frog, passive sampling devices, water, sediment, plants, contaminants, disease incidence	2012
NE7	Mineable oil sands	wood frog	wood frog, passive sampling devices, water, sediment, plants, contaminants, disease incidence	2012
Pat's Pond	Mineable oil sands	wood frog	wood frog, passive sampling devices, water, sediment, plants, contaminants, disease incidence	2013
WBNP Surprise Depression	Remote “reference” site	wood frog	wood frog, water, contaminants, disease incidence	2012
WBNP Toadlet Pond	Remote “reference” site	wood frog	wood frog, water, contaminants, disease incidence	2012

WBNP Wetland 190	Remote "reference" site	wood frog	wood frog, water, contaminants, disease incidence	2012
WBNP Jessica Pond	Remote "reference" site	wood frog	wood frog, water, contaminants, disease incidence	2011
WBNP Galoot Lake	Remote "reference" site	wood frog	wood frog, water, contaminants, disease incidence	2011
Nagel Channel	Remote "reference" site	wood frog	wood frog, water, contaminants, disease incidence	2012
Antoinette's Pond	Remote "reference" site	wood frog	wood frog, water, contaminants, disease incidence	2012
Raelene's Pond	Remote "reference" site	wood frog	wood frog, water, contaminants, disease incidence	2012

Assumptions and Constraints behind the hypotheses and the testing methods:

(1) Compiled data support a conclusion that contaminant burdens in the tissues of bioindicator wildlife species are highly variable and no oil sands "signal" can be detected in the data using geospatial and spatial variation analyses,
(2) Contaminant inputs to the region come from various sources leading to the potential to confound the data,
(3) The compiled data do not provide a clear link to oil sands industrial operations as being the source of the increased contaminant burdens seen,
(4) Contamination and contaminant effects on both the food webs and the bioindicator wildlife species being studied in this program should be detectable and effects are measurable using a suite of diagnostic, bioassessment and biomarker techniques.

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Data Management

If this work generates data please summarize your project-level data management plan.

Deliverables	Timeframe
Data Collection Period: *Field work*	Start : 2018-04-02 End: 2019-03-29
Data Analysis Period: *Laboratory analysis and QA/QC of data*	Start : 2018-09-03 End: 2019-03-29
Data Release Date: *Metadata and data consistent, complete and meet basic standard format for publication in Open Data; on or linked to JOSM portal*	2019-11-04
Project-level Data Management Plan	This project is linked to the Wildlife Contaminants and Toxicology Biotic Response Synthesis Project and data collected during this Focus Study/ Investigation of Cause will be incorporated into the Oil Sands Wildlife Contaminants and Toxicology database being established by that project. From there the data can be assessed by the Synthesis Project activities, and can also be made available to the ECCC Open Data Catalogue, the ECCC Oil Sands Portal and the GoC Open Data Catalogue. Publishing in the Open Access literature will also occur when feasible.

Reporting and Publications

Expected Subject/Titles of Publications or Reports	Short Description of Publication or Report	Expected Year of Publication
Publications and reports on field monitoring of amphibian and wetland health: contamination of wetlands by metals, PACs and naphthenic acids and effects on wildlife bioindicator species *in situ.*	Results from field monitoring of amphibian and wetland health, conducted across the oil sands region, to investigate potential health impairment of these ecosystems and the bioindicator wildlife species that depend on them as a result of exposure to oil sands-related industrial contaminants.	2018-2020.
Publications and reports on experiments involving exposures of wood frogs to high priority oil sands contaminants of concern and the establishment of validated biotic response variables, toxicity assessment endpoints and SOPs.	Description of laboratory exposure experiments conducted with high priority oil sands contaminants to establish and validate biotic response variables, toxicity assessment endpoints and standard operating procedures.	2018-2021

Technical / Professional Roles and Responsibilities[1]

[1]Does not include all academic partners and collaborators

Role	Responsibilities	Resource Name/Organization
Project Manager	Design of focus study, field work, analysis of data, writing and interpretation	ECCC
Project Scientist	Design of focus study, field work, analysis of data, writing and interpretation	ECCC
Project Scientist	Design of focus study, field work, analysis of data, writing and interpretation	Keyano College, Fort McMurray
Groundwater Scientist	Support for determination of groundwater sources and inputs to wetlands	ECCC
Wildlife Health Specialist	Laboratory analyses and research support for wildlife health endpoints	ECCC
Oil Sands Technologist	Field work and processing of samples and laboratory analyses	ECCC
Oil Sands Technologist	Field work and processing of samples and laboratory analyses	ECCC

Deliverables (2018/19) If your Focus Study is longer than 1 year then complete **Appendix C** for multi-year deliverables breakdown.

Deliverable(s) (please provide enough information to support status reporting)
Q1 – April to June 2018
Field work logistics: Equipment maintenance, contract preparation, materials acquisition, planning, field sampling Field work in northern Alberta and the Peace-Athabasca Delta: deployment of sampling devices and collection of samples.
Laboratory work and logistics: Exposure experiment planning, contract preparation, meetings with academic partners and graduate students conducting exposure experiments, laboratory exposures, site visit to laboratories conducting experiments.
Q2 – July to September 2018
Field work in northern Alberta and the Peace-Athabasca Delta: deployment of sampling devices and collection of samples; field water samples analysed.
Laboratory work: Site visits for progress meetings with academic partners and graduate students conducting exposure experiments, laboratory and mesocosm exposures completed.
Q3 – October to December 2018
Samples prepared for analyses: water, tissues, SPMD and sediments from both field and laboratory exposures prepared for analyses, for contaminant concentrations, toxicity endpoints and biomarkers of exposure. **Data Product: Progress reporting**
Q4 – January to March 2019
Laboratory analyses: tissues, SPMD and sediment from both field and laboratory exposures analysed for contaminant concentrations, analysis of tissue samples for toxicity endpoints and biomarkers of exposure, submission of data to wildlife health oil sands database.
Data Product: Progress reporting

APPENDIX C –Year 3 Deliverables (Complete the following detailed breakdown. Provide a summary of tangible quarterly deliverables and your anticipated expenditures. Identify major project areas (deliverables) and results that can be identified as a tangible goal.)

Year 2 (2019- 2020)
Deliverable(s) (please provide enough information to support status reporting)
Q1 – April to June 2019
Field work logistics: Equipment maintenance, contract preparation, materials acquisition, planning, field sampling
Field work in northern Alberta and the Peace-Athabasca Delta: deployment of sampling devices and collection of samples.
Laboratory work and logistics: Exposure experiment planning, contract preparation, meetings with academic partners and graduate students conducting exposure experiments, laboratory exposures, site visit to laboratories conducting experiments.
Q2 – July to September 2019
Field work in northern Alberta and the Peace-Athabasca Delta: deployment of sampling devices and collection of samples; field water samples analysed.
Laboratory work: Site visits for progress meetings with academic partners and graduate students conducting exposure experiments, laboratory and mesocosm exposures completed.
Q3 – October to December 2019
Samples prepared for analyses: water, tissues, SPMD and sediments from both field and laboratory exposures prepared for analyses, for contaminant concentrations, toxicity endpoints and biomarkers of exposure.
Q4 – January to March 2020
Lab analyses: tissues, SPMD and sediment from both field and laboratory exposures analysed for contaminant concentrations, analysis of tissue samples for toxicity endpoints and biomarkers of exposure, submission of data to wildlife health oil sands database.

Detailed Financial Breakdown – Year 2 of 3 (2017-2020)

Also complete **Appendix B** for the multi-year financial breakdown

Budget requirements – List areas that require budget expenditures: (ADD OR DELETE BUDGET CATEGORIES AS REQUIRED)	OS Funding	External Funding (*outside JOSM*)
O&M - Operations and Maintenance:		
Helicopter Costs	**$7,000**	$
Field Costs	**$75,000**	$
Fleet Use	**$0**	$
Data Management	**$0**	$
Internal Lab Analysis	**$0**	$
Consumable Materials & Supplies (*storage locker, Fort McMurray*)	**$6,000**	$
Consumable Materials & Supplies (*SPMDs, POCIS samplers, PMDs, DGTs, ARUs*)	**$5,000**	$
Sub-Total	**$93,000**	$
O&M - Travel		
Field Work	**$10,000**	$
Conferences (*identify conference*)	$	$
Meeting (*identify meeting*)	$	$
Sub-Total	**$10,000**	$
O&M - External Contracts :		
External Lab Analyses (PAHs in tissues)	**$6,250**	$
External Lab Analyses (*organics in water*)	**$16,500**	$
External Lab Analyses (*metals in water and sediment*)	**$7,000**	$
External Lab Analyses (*DOC*)	**$1,000**	$
External Lab Analyses (*chlorophyll-a*)	**$1,000**	$
External Lab Analyses (*basic water chemistry*)	**$3,000**	$
External Lab Analyses (passive samplers)	**$8,102**	$
External Contract (*conservation genetics for wetland health assessment*)	**$5,000**	$
External Contract (*isotopic analyses of groundwater and surface water samples*)	**$10,000**	$
External Contract (*exposure experiments and*	**$15,000**	$

Budget requirements – List areas that require budget expenditures: (ADD OR DELETE BUDGET CATEGORIES AS REQUIRED)	OS Funding	External Funding (*outside JOSM*)
analyses, PACs in wood frogs)		
External Contract (*exposure experiments and analyses, biomarkers in wood frogs*)	**$15,000**	**$**
Sub-Total	**$87,852**	**$**
Salaries:		
Principal Investigators	**$0**	**$**
Technical / Professional Assistants	**$233,148**[1]	**$**
Sub-Total	**$233,148**[1]	**$**
Total Salaries[1]	**$233,148**	**$**
Total O&M	**$190,852**	**$**
2017-2018 GRAND TOTAL*	**$424,000***	**$**

[1]Includes associated ECCC EBP, Accommodations, PWGSC Accommodations, and SCC costs
*Grand Total includes EBP, Accommodations, PWGSC Accommodations, and SCC costs

Appendix A - Approvals

Project Submitted by:		
Name: Bruce Pauli		
Organization: Environment and Climate Change Canada	Signature:	Date:

Project Approved by:		
Signature		Signature
Date		Date

Activity Planning Review and Evaluation

To be completed by OSM Administration

Date Completed	**Review type**	**Validated by** (insert name and title)
	Program Management review completed	

APPENDIX B – Detailed Multi-year Financial Breakdown (Complete the following detailed financial breakdown; add or delete categories as required)

Budget requirements	Year 1 (2017- 2018)		Year 2 (2018- 2019)		Year 3 (2019- 2020)	
	Cash	In-kind	Cash	In-kind	Cash	In-kind
1) Salaries and benefits						
a) Investigators						
b) Technical/professional assistants			$233,148		$233,148	
c) Field Staff						
d) WLSD Laboratory Services Unit						
2) Operations and maintenance						
a) Helicopter costs			$7,000		$7,000	
b) Field costs (field crew and field costs, truck rentals and shipping charges)			$75,000		$75,000	
c) Facilities (storage locker)			$6,000		6,000	
d) Equipment (equipment and consumable materials for field work, e.g. SPMDs)			$5,000		$5,000	
e) Lab analysis						
f) Data management						
g) Field work travel			$10,000		$$10,000	
3) Consumable Materials and supplies						
a)						
4) Travel						
a) Conferences and meetings						

5) Dissemination & Engagement						
6) External Contracts						
a) Analyses: PAHs in tissues			$6,250		$6,250	
b) Organics in water			$16,500		$16,500	
c) Analyses: metals in water and sediment			$7,000		$7,000	
d) Analyses: DOC			$1,000		$1,000	
e) Analyses: Chlorophyll-a			$1,000		$1,000	
f) Analyses: water chemistry			$3,000		$3,000	
g) Analyses: SPMDs and other passive samplers			$8,102		$8,102	
h) Conservation genetics			$5,000		$5,000	
i) Isotopic analyses of groundwater and surface water in wetlands			$10,000		$10,000	
j) Exposure (NAs and PACs) in wood frogs			$15,000		$15,000	
k) Biomarkers in wood frogs			$15,000		$15,000	
Grand Total[1]	($424,000)		$424,000		$424,000	

[1]Grand Total including EBP, Accommodations, PWGSC Accommodations, and SCC costs determined from Budget Calculations spreadsheet