

FOCUSED STUDY ACTIVITY WORK PLAN

General Information

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 (<i>choose from drop-down menu. If Project Location is in more than one area choose from second drop-down</i>)	Lower Athabasca River	More than 2 Locations (Described in Detailed Monitoring Plan)
Monitoring Site(s) Coordinates (<i>latitude and longitude</i>)	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: (<i>From OSM long-term plan; choose from drop-down menu</i>)	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, <i>Lithobates sylvaticus</i>) in the oil sands region has revealed detectable levels of contaminants, including heavy metals and PAHs, 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 strategic objectives to: (i) assess levels of these high priority contaminants in wetlands, (ii) assess contamination of the food web in these wetlands (iii) track the sources of the contaminants measured, and (iv) 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	

	<p>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.</p>
<p>Hypotheses: <i>(Briefly outline the specific hypotheses that your focused study is aiming to address)</i></p>	<p>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’ exposures are occurring.</p>
<p>Deliverables: <i>What tangible goal (s) and/or product(s) will the monitoring produce and when?</i></p>	<p>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.</p> <p><u>Near-term deliverables:</u></p> <ol style="list-style-type: none"> 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 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 and establishment is required). <p><u>Longer-term deliverables:</u></p> <ol style="list-style-type: none"> 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 and instrument the most appropriate sites with the appropriate equipment for risk-based monitoring of wetland health, 3. Using established biomarkers and toxicity endpoints, establish

	<p>toxicity thresholds and correlate these with contaminant concentrations measured at long-term monitoring wetland sites,,</p> <p>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).</p> <p>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.</p>
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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, PAHs, 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 Air 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 2017/18, 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 long-term monitoring of wetlands in the oil sands. 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), and diffuse gradient thin films (DGTs)) 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 exposure and effects such as the use of molecular biomarkers and oxidative stress, and frog

immunological markers, and exposure by examining distribution of metals and naphthenic acids in wood frogs, and uptake and depuration of PAHs 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, 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 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 to be used 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. 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 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.). PAHs could be detected in both wood frog tissues and SPMDs. The latter technique also revealed that wetlands could be distinguished with respect to their PAH 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 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 (Guterrez et al. 2014a, 2014b, 2015; Orihel et al. 2016) and 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 the 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 the 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 these 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 wetland ecosystem health 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 will be 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 contamination from 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 will be assessed with the assistance of Dr. Tom Harner, AQRD, who will provide passive air samplers for deployment at our monitoring wetlands, and who will analyse, 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

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).

Year 2017-18

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, PAHs, 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.

At intensive wetland monitoring sites, we will deploy passive sampling devices (e.g. SPMDs, DGTs, POCIS, PMDs) and collect and assess water, sediment, plants, and wood frog tadpoles for the presence of contaminants of concern. Basic water quality measurements will also be taken at each of these wetland sites. At these sites, passive sampling devices will be used for two purposes: (1) to assess time-weighted average accumulation of contaminants in wetland monitoring sites, and (2) to provide extracts from field-deployed passive samplers that will be used to assess the toxicological potential of the sampled wetland contaminants by measuring the dose-response of biochemical and molecular endpoints using *in vitro* assays. For example, the use of biomarkers, such as ethoxyresorufin-*O*-deethylase activity (EROD) and markers of oxidative stress will be used to determine if there may be a biotic response in wood frog tadpoles exposed to oil sands-related contaminants of concern *in situ*.

At extensive monitoring sites, a subset of the activities being conducted at the intensive monitoring sites will occur. For instance, in the 2017-18 pilot year, extensive monitoring sites may only be identified, assessed for their suitability for inclusion in a long-term monitoring program, and possibly sampled for general water chemistry and contaminant burdens (through grab samples of water, sediment cores, and deployment of SPMDs and other passive sampling devices such as DGTs).

In further health assessments of the wetland biota 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.

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.

Year 2018-19

- Abiotic and biotic samples proposed for collection 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 2017-18

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 2018-19

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 PAHs in wood frogs, as in 2017-18, e.g. 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.

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 PAHs 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.

References:

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Data Management

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

Deliverables	Timeframe
Data Collection Period: <i>Field work</i>	Start : 2017-04-03 End: 2018-03-30
Data Analysis Period: <i>Laboratory analysis and QA/QC of data</i>	Start : 2017-09-11 End: 2018-03-30
Data Release Date: <i>Metadata and data consistent, complete and meet basic standard format for publication in Open Data; on or linked to JOSM portal</i>	2018-11-01
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

Provide information on the anticipated reports / publications. (Insert additional rows if needed)

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, PAHs and naphthenic acids and effects on wildlife bioindicator species <i>in situ</i> .	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.	2017-2020.
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 Responsibilities¹

¹Does not include all academic partners and collaborators

Role	Responsibilities	Resource Name/Organization
Bruce Pauli Project Manager	Design of focus study, field work, analysis of data, writing and interpretation	ECCC
Lukas Mundy Project Scientist	Design of focus study, field work, analysis of data, writing and interpretation	ECCC
Dr. Danna Schock Project Scientist	Design of focus study, field work, analysis of data, writing and interpretation	Keyano College, Fort McMurray
Wildlife Health Specialist	Laboratory analyses and research support for wildlife health endpoints	ECCC
Technologist	Processing of samples and laboratory analyses	ECCC

Deliverables (Year 1) If your Focus Study is longer than 1 year then complete **Appendix C** for multi-year deliverables breakdown Provide a summary of tangible quarterly deliverables. Identify major project areas (deliverables) and results that can be identified as a tangible goal. This could include: field work, lab work/ analysis, evaluation, data, reports, publications, SOPs etc. Do not define process as your Deliverable e.g. 'fly to Ft. McMurray to conduct fieldwork' or 'seek Director approval for report'.

Deliverable(s) (please provide enough information to support status reporting)
Q1 – April to June 2017
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 2017
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 2017
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 2017
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

Detailed Financial Breakdown – Year 1 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	\$25,000	\$
Field Costs	\$7,100	\$
Fleet Use	\$0	
Data Management	\$0	\$
Internal Lab Analysis	\$0	\$
Consumable Materials & Supplies (<i>storage locker, Fort McMurray</i>)	\$4,680	\$
Consumable Materials & Supplies (<i>SPMDs, POCIS samplers, PMDs, DGTs</i>)	\$9,860	
Sub-Total	\$46,640	\$
O&M - Travel		
Field Work	\$13,800	\$
Conferences (<i>identify conference</i>)	\$	\$
Meeting (<i>identify meeting</i>) <i>For one PI to attend the Oil Sands Science Symposium and the Oil Sands Integrated Workplanning meetings</i>	\$	\$
Sub-Total	\$13,800	\$
O&M - External Contracts :		
External Lab Analyses (PAHs in tissues)	\$14,688	\$
External Lab Analyses (<i>organics in water</i>)	\$9,429	
External Lab Analyses (<i>metals in water</i>)	\$3,850	
External Lab Analyses (<i>metals in sediment</i>)	\$3,850	
External Lab Analyses (<i>DOC</i>)	\$1,196	
External Lab Analyses (<i>chlorophyll-a</i>)	\$1,209	
External Lab Analyses (<i>basic water chemistry</i>)	\$2,990	
External Lab Analyses (<i>PAHs in SPMDs</i>)	\$10,200	
External Contract (<i>exposure experiments and analyses</i>)	\$21,760	

Budget requirements – List areas that require budget expenditures: (ADD OR DELETE BUDGET CATEGORIES AS REQUIRED)	OS Funding	External Funding (outside JOSM)
External Contract (<i>investigating effects of nutrient limitations and ground truthing eDNA measures</i>)	\$6,000	
External Contract (<i>metals and naphthenic acids in wood frogs, Postdoctoral Visiting Fellow</i>)	\$30,000	
External Contract (<i>exposure experiments and analyses, naphthenic acids in wood frogs</i>)	\$6,000	
External Contract (<i>exposure experiments and analyses, PAHs in wood frogs</i>)	\$1,240	
External Contract (<i>data management</i>)	\$10,000	
External Contract (<i>sediment-based exposure experiments and analyses, PAHs in wood frogs</i>)	\$8,000	
Sub-Total	\$130,412	\$
Salaries:		
Principal Investigators	\$0	\$
Technical / Professional Assistants	\$233,148 ¹	\$
Sub-Total	\$233,148¹	\$
Total Salaries¹	\$233,148	\$
Total O&M	\$190,852	\$
2017-2018 GRAND TOTAL *	\$424,000*	\$

¹Includes 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:		
Organization:	Signature:	Date:
Project Approved by:		
Dr. Monique Dubé (AEP)		Dr. Kevin Cash (ECCC)
Signature		Signature
		
Date		Date

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		\$233,148	
c) Field Staff						
d) WLSL Laboratory Services Unit						
2) Operations and maintenance						
a) Facilities (storage locker)	\$4,680		\$4,680		\$4,680	
b) Equipment	\$7,100		\$7,100		\$7,100	
c) Lab analysis						
d) Data management						
e) Field work	\$13,800		\$13,800		\$13,800	
f) Helicopter Cost	\$25,000		\$25,000		\$25,000	
g) Shipping						
h) Fleet Use						
3) Consumable Materials and supplies						
a) Sampling devices (POCIS, SPMDs, PMDS, DGTs)	\$9,860		\$9,860		\$9,860	
4) Travel						
a) Conferences and meetings						

5) Dissemination & Engagement					
a) Translation (if required)					
b) Stakeholder Engagement					
c) Indigenous Peoples Engagement					
6) External Contracts					
a) Analyses: PAHs in tissues	\$14,688		\$14,688		\$14,688
b) Organics in water	\$9,429		\$9,429		\$9,429
c) Analyses: metals in water	\$3,850		\$3,850		\$3,850
d) Analyses: metals in sediment	\$3,850		\$3,850		\$3,850
e) Analyses: DOC	\$1,196		\$1,196		\$1,196
f) Analyses: Chlor-a	\$1,209		\$1,209		\$1,209
g) Analyses: water chemistry	\$2,990		\$2,990		\$2,990
h) Analyses: PACs in SPMDs	\$10,200		\$10,200		\$10,200
i) Exposure and effects studies, wood frogs	\$21,760		\$21,760		\$21,760
j) eDNA studies	\$6,000		\$6,000		\$6,000
k) NAs in wood frogs	\$30,000		\$30,000		\$30,000
l) PACs in wood frogs	\$1,240		\$1,240		\$1,240
m) Data management	\$10,000		\$10,000		\$10,000
n) Sediment exposure trials	\$8,000		\$8,000		\$8,000
Grand Total¹	\$424,000		\$424,000		\$424,000

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

APPENDIX C –Years 2 and 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 (2018- 2019)
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.
Q4 – January to March 2019
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.

Year 3 (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.