Ecotoxicity Assessment

of Treated Oil Sands Process-Affected Water (OSPW): 2019 Toxicity and Mesocosms Studies

A third party Science Report commissioned by the Office of the Chief Scientist

Alberta
Environment and Parks
Office of the Chief Scientist

Ecotoxicity Assessment of Treated Oil Sands Process-Affected Water (OSPW): 2019 Toxicity and Mesocosms Studies

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Alberta's Environmental Science Program

The Chief Scientist has a legislated responsibility for developing and implementing Alberta's environmental science program for monitoring, evaluation and reporting on the condition of the environment in Alberta. The program seeks to meet the environmental information needs of multiple users in order to inform policy and decision-making processes. Two independent advisory panels, the Science Advisory Panel and the Indigenous Wisdom Advisory Panel, periodically review the integrity of the program and provide strategic advice on the respectful braiding of Indigenous Knowledge with conventional scientific knowledge.

Alberta's environmental science program is grounded in the principles of:

- *Openness and Transparency.* Appropriate standards, procedures, and methodologies are employed and findings are reported in an open, honest and accountable manner.
- *Credibility.* Quality in the data and information are upheld through a comprehensive Quality Assurance and Quality Control program that invokes peer review processes when needed.
- *Scientific Integrity.* Standards, professional values, and practices of the scientific community are adopted to produce objective and reproducible investigations.
- *Accessible Monitoring Data and Science.* Scientifically-informed decision making is enabled through the public reporting of monitoring data and scientific findings in a timely, accessible, unaltered and unfettered manner.
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Learn more about the condition of Alberta's environment at: environmentalmonitoring.alberta.ca.

The Office of the Chief Scientist and Third Party Science Reports

The Chief Scientist, Alberta Environment and Parks, provides scientific oversight for the provincial environmental science program, which includes commissioning of scientific technical reports to inform Alberta Environment and Parks. Commissioning of third party technical reports is intended to fill gaps in current knowledge as part of building a credible and reliable body of knowledge which stands up to the scrutiny of the experts in a particular field of science and can be trusted to inform policy and management actions.

Upon request from the Minister, the Science Advisory Panel, the Department, or if the Chief Scientist deems it necessary, the Office of the Chief Scientist will engage independent expertise to undertake work to develop technical reports. The Office of the Chief Scientist acts as a neutral broker to bring together relevant experts from across scientific and Indigenous knowledge systems to evaluate, review and recommend improvements to the scientific foundations of ongoing science and monitoring programs or issue-focused applied research or monitoring activities. In upholding the principles of the environmental monitoring and science program, with the aim of building public trust in the credibility of scientific inputs to evidence-informed decision making processes, all third-party scientific reports will be publically available.

The Oil Sands Process Affected Water Science Team

Formation, role and structure

The Oil Sands Process Affected Water (OSPW) Science Team was formed in January 2018 by the Chief Scientist, Alberta Environment and Parks. The OSPW Science Team was established to provide independent, credible scientific information regarding the potential release of treated oil sands process water to the Lower Athabasca River by Syncrude Canada as part of its evaluation of a coke-slurry water treatment process. This information is intended to inform decision-making processes of government regulatory bodies (i.e., Alberta Energy Regulatory, Alberta Environment and Parks, Environment and Climate Change Canada). For the purpose of the Science Team work and the evaluation of the Syncrude proposal, OSPW is defined as water in tailings ponds that is recycled internally as a part of bitumen extraction process and for material transport including ore and tailings solids.

The OSPW Science Team is tasked with providing scientific information on **three focal areas** of work:

1. **Determining the toxicity of OSPW treated using Syncrude's coke-slurry treatment process.** This includes identifying relevant biological and ecological endpoints for toxicity testing as well as the specific details related to concentrations and exposure durations to quantify both acute and chronic toxicity. Endpoints used in toxicity testing includes those that reflect standardized testing plus those of value to Indigenous communities.
2. **Creating an enhanced environmental monitoring system for a focal reach of the Lower Athabasca River**. The design will build on Provincial and Federal designs and decision criteria, and incorporate culturally and locally relevant criteria based on Traditional Ecological Knowledge. The design will ensure a sufficient understanding and characterization of the baseline environmental conditions.
3. **Designing the requirements, parameters and conditions required for a quantitative modeling assessment of environmental impacts and a human ecological health risk assessment to evaluate and predict the effects of the release of treated OSPW to the Athabasca River**. The prediction system must address projections of the environmental fate and distribution of discharged compounds, potential cumulative effects on riverine water quality and ecosystem structure and function, and implications for human health.

For each of the three focal areas the OSPW Science Team will:

1. create study designs that will be integrated into work plans,
2. oversee the deployment of the work plans, and
3. provide and communicate findings to key stakeholders and government decision-makers.

The OSPW Science Team includes technical experts from academia, industry, Alberta Environment and Parks, the Alberta Energy Regulator (AER), Environment and Climate Change Canada (ECCC) and holders of Indigenous and local knowledge. The work of the OSPW Science team also supports efforts of the Integrated Water Management Working Group, a multi-stakeholder working group with representatives from industry, Indigenous Peoples, Environmental Non-Governmental Organizations, and Federal, Provincial and Municipal governments. The Integrated Water Management Working Group provides advice to Alberta Environment and Parks on water management issues for the oil sands sector, including the potential for the release of oil sands process affected water, as outlined in Alberta's Tailings Management Framework.

OSPW Science Team Report 1: Design of toxicity experiments to assess the efficacy of Syncrude's coke-slurry water treatment process

The following technical report, describes the suite of experiments required to assess the efficacy of Syncrude's coke-slurry process to treat oil sands process affected water. This work is foundational for the two additional focal areas related to designing an environmental effect-based monitoring program, and predictive modeling to assess potential effects on the environment and on human health, should a short-term trial release of treated OSPW be approved.

The full suite of toxicity tests for deployment in 2019 were identified by the OSPW Science Team and are accompanied with the following context as requested by Mikisew Cree First Nation, Fort McKay First Nation and Fort McKay Metis Community Association.

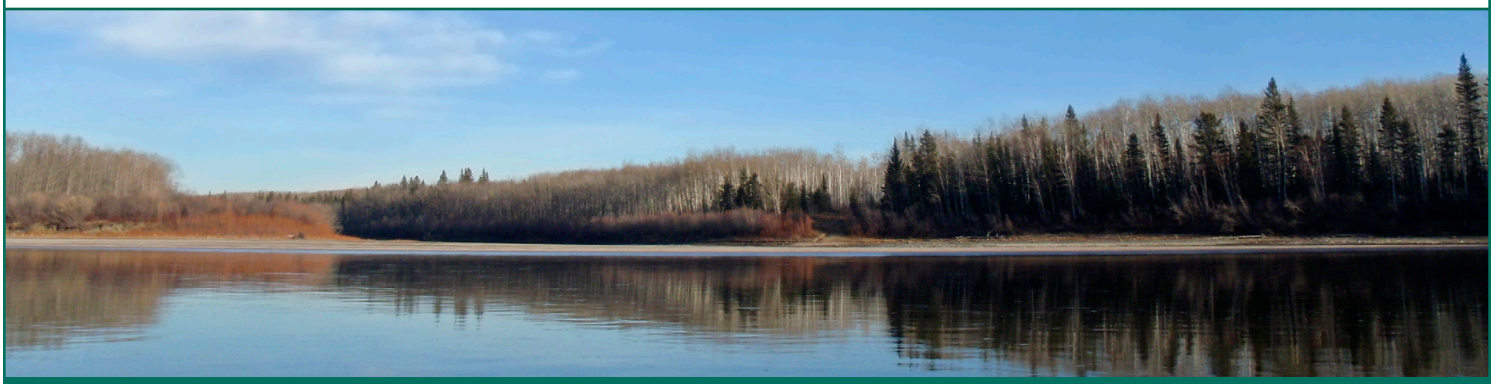
NOTICE

This document and material is provided by the Oil Sands Process Water Science Team for general information purposes only. The Oil Sands Process Water Science Team (OSPWST) is comprised of representatives of the Government of Alberta, Government of Canada, industry, academia, Mikisew Cree First Nation, Fort McKay First Nation and Fort McKay Metis Community Association. The information contained in this document may include views, opinions and recommendations of representatives of the OSPWST for the sole purpose of facilitating the work of the OSPWST. The information is not intended to provide the views, opinions, recommendations, endorsement or approval by either Mikisew Cree First Nation, Fort McKay First Nation or Fort McKay Metis Community Association of the release of oil sands process water. Further, take note that Mikisew Cree First Nation is opposed to the untreated release of process affected water that furthers the risk to the Peace Athabasca Delta and the community of Fort Chipewyan. Partially treated OSPW like the stream in the Syncrude Pilot application is not considered treated by MCFN.

Ecotoxicity Assessment of Treated Oil Sands Process-Affected Water (OSPW):

2019 Toxicity and Mesocosms Studies

May 2019



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ECOTOXICITY ASSESSMENT OF TREATED OIL SANDS PROCESS-AFFECTED WATER (OSPW):

2019 TOXICITY AND MESOCOSMS STUDIES

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APRIL 2019

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LIST OF ACRONYMS

AB	Alberta
BC	British Columbia
BTEX	Benzene, Toluene, Ethylbenzene, Xylene
CALA	Canadian Association for Laboratory Accreditation
DIC	Dissolved Inorganic Carbon
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
ECCC	Environment and Climate Change Canada
IC25	Inhibition Concentration at which 25% of a quantitative biological function is inhibited
LC50	Lethal Concentration at which 50% mortality occurs
NH_4-N	Ammonium, as N
NO_3-N	Nitrate nitrogen
OSPW	Oil Sands Process-Affected Water
PAH	Polyaromatic Hydrocarbon
SO_4	Sulphate
SRP	Soluble Reactive Phosphorus
TDP	Total Dissolved Phosphorus
TDS	Total Dissolved Solids
TIE	Toxicity Identification Evaluation
TOC	Total Organic Carbon
TP	Total Phosphorus
TSS	Total Suspended Solids
USEPA	United States Environmental Protection Agency

DISTRIBUTION LIST

The following individuals/firms have received this document:

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AMENDMENT RECORD

This report has been issued and amended as follows:

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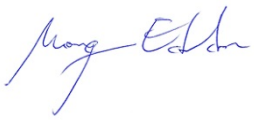
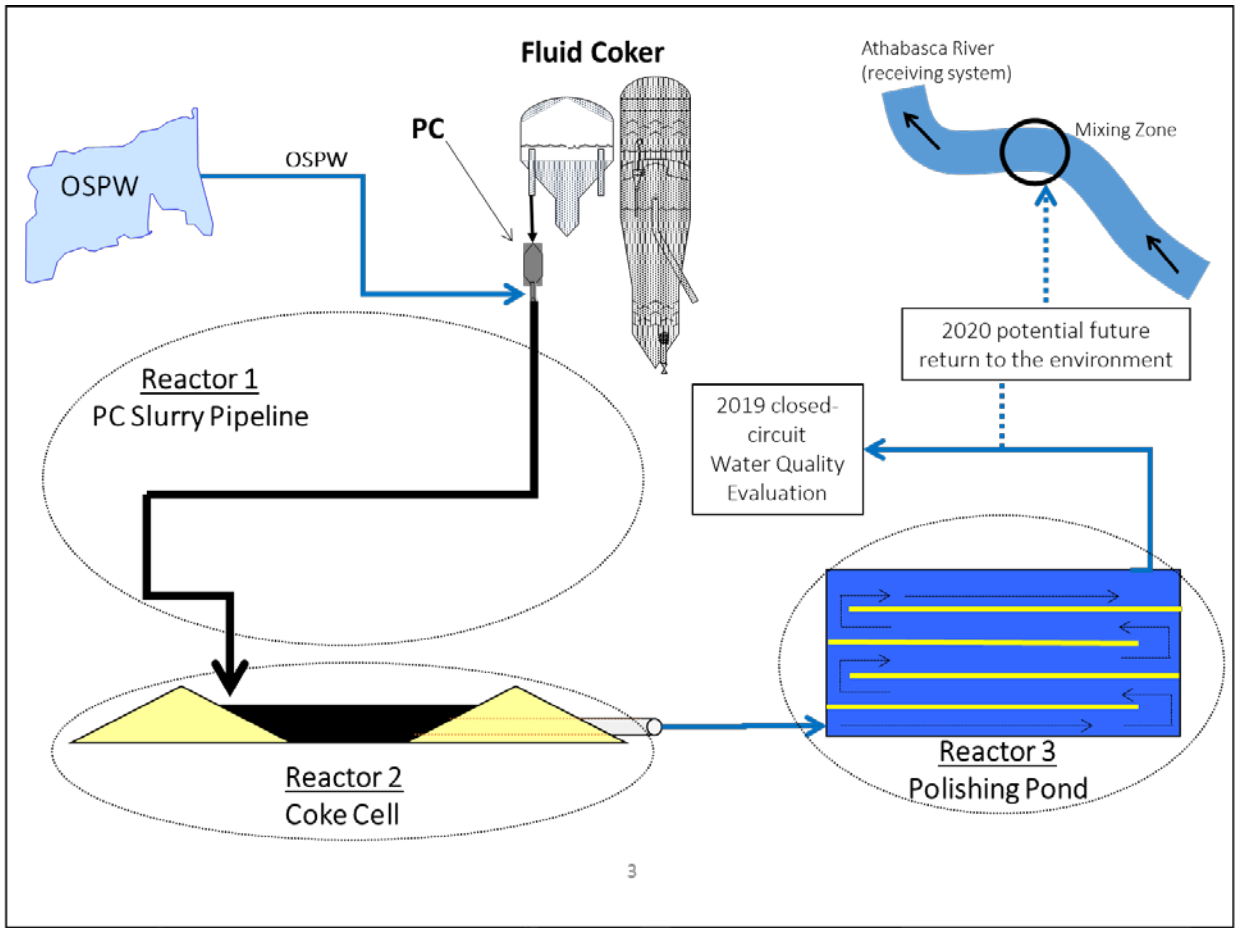
4	Ecotoxicity Assessment of Treated Oil Sands Process-affected Water (OSPW): 2019 Toxicity and Mesocosms Studies	20190507	 Martin Davies Project Director	 Morgan Edwards Project Manager

1.0 INTRODUCTION

Syncrude Canada Ltd. (Syncrude) is developing a pilot-scale treatment facility that uses fluidized petroleum coke to remove chemical constituents from oil sands process-affected water (OSPW) (Zubot et al. 2012). The objective of the treatment process is to treat OSPW to allow for its safe release to the Athabasca River. Treatment occurs as a three-component process, with each step referred to as a "reactor" (Cude et al. 2017). A simplified process flow diagram provided in Figure 1 shows the three reactors that comprise the treatment, which is described in more detail below.

Figure 1 The three-reactor OSPW treatment process.



Firstly, the petroleum coke (i.e., activated carbon) produced from the fluid cokers is mixed with OSPW (i.e., untreated water) that has been sourced from tailings ponds. The water/coke is then transported in a pipeline (Reactor 1) as a slurry and deposited into a large containment cell (Reactor 2) that is equipped with engineered under-drainage to act as a filter bed. The purpose of Reactors 1 and 2 is to reduce concentrations of total suspended solids (e.g., clay particles), free phase hydrocarbons (e.g., bitumen) and dissolved organic constituents (e.g., naphthenic acids). The final stage of treatment is an aerated pond with an eight-day residence time (Reactor 3) to permit biological degradation of ammonia and to serve as a holding facility to allow for final water quality testing. The water from Reactor 3 can be rerouted to the existing OSPW inventory, to facilitate future potential release scenarios, or should confirmatory testing indicate unacceptable water quality. This ensures "off-spec" water is not released to the environment.

The pilot treatment facility was scheduled to be fully commissioned by July 2018, and producing treated OSPW. Unfortunately, a plant-wide power failure at Syncrude in June 2018 resulted in a plant shut-down and required a project delay to June 2019. Comprehensive evaluation of the treated OSPW is scheduled to begin in July 2019. In 2019, the pilot facility will be operated in a closed-circuit configuration, with the treated water retained on-site by returning it to the existing inventory of OSPW.

To evaluate the effectiveness of the treatment facility, particularly as it relates to chemical and toxicological attributes of treated OSPW, Syncrude requested that Hatfield Consultants (Hatfield) design and implement a closed-circuit aquatic toxicity study, building on a study design previously proposed by Cude et al. (2018), and incorporating recommendations provided by the Oil Sands Process-Affected Water Science Team (OSPW Science Team). To accomplish this goal, Hatfield has assembled an experienced team of subject-matter experts that includes Limnotek Research and Development (Limnotek), Nautilus Environmental Company Inc. (Nautilus), and mesocosm experts from Environment and Climate Change Canada (ECCC). The following information summarizes the proposed closed-circuit aquatic toxicity study, including planning, design, and construction activities in 2018, and execution of the study in 2019.

1.1 ASSESSMENT OVERVIEW

The assessment of treated OSPW from the pilot-scale treatment facility will be evaluated using a triad approach (Alberta Environmental Protection 1995) to ensure the treated water presents negligible risk if released to the Athabasca River (Figure 2). Specifically, the study incorporates the following components:

- Chemical characterization of untreated and treated OSPW;
- Toxicological testing of treated OSPW; and
- Assessment of aquatic invertebrate community mesocosm responses to treated OSPW.

Figure 2 The three study components supporting decisions regarding the potential return of treated OSPW to the Athabasca River.

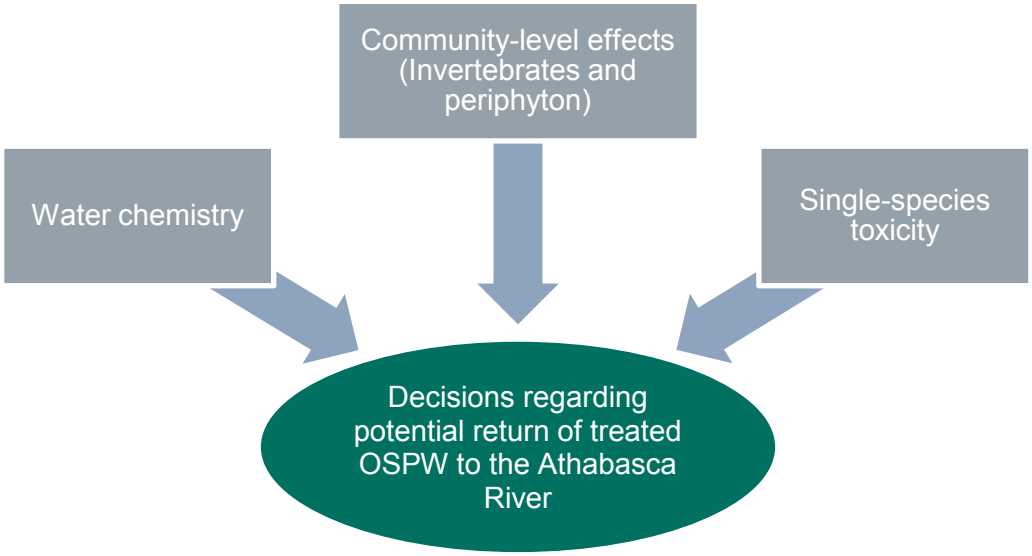


Figure 3 provides a detailed summary of the specific elements of the proposed study proposed, as well as the progression of the study over time and related decision points. The 2019 study will be conducted in two phases: (1) preliminary screening of treated OSPW; and (2) a detailed aquatic toxicity study, incorporating sublethal assessments of treated OSPW at environmentally-relevant concentrations.

The first phase begins following activation of Reactor 3, when the fully treated OSPW will be collected and delivered to the Nautilus laboratory in Calgary, AB for preliminary toxicity screening (see Section 2.0). If the screening tests indicate that the treated OSPW is acutely toxic to rainbow trout or *Daphnia magna*, a preliminary, screening-level Toxicity Identification Evaluation (TIE) process will be conducted to attempt to identify the source of the observed acute toxicity and the results of which will inform the water treatment process. The OSPW Science Team will be consulted if a TIE process is required. If the treatment process is unable to remove acute toxicity, it will be deemed to have failed expectations, and the study will be terminated.

The second phase consists of the detailed aquatic toxicity study ("Detailed Study") designed to test the effectiveness of the Syncrude OSPW treatment process over a six-week period using a large-scale field trial, and a broad suite of toxicity tests. This phase incorporates both laboratory and on-site testing of the treated OSPW. The on-site component includes sublethal toxicity testing using a mobile testing facility (Section 3.2.1.1) and the use of artificial streams (mesocosms) inoculated with periphyton and benthic macroinvertebrate assemblages from the Athabasca River watershed (Section 3.1). This approach combines precise evaluation of toxicity using standard test organisms and recognized protocols, with the evaluation of community responses of river biota to chronic exposure to treated OSPW.

Mesocosms are regularly used to test hypotheses of change among benthic assemblages exposed to contaminants or other substances (Perrin and Richardson 1997, Culp et al. 2003, Culp and Baird 2006, Alexander et al. 2016), and allow separation and replication of treatments with physical and chemical diagnostics. Tight control of potential confounding variables that are typical in field studies (e.g., flow, depth, habitat and substrate characteristics, water temperature and chemistry, etc.) is a key rationale for undertaking mesocosm studies.

Supporting the 2019 Field Pilot will be on-site acute and sublethal toxicity testing in a customized laboratory trailer, as well as off-site testing by Nautilus in Calgary, AB. Non-standard sublethal toxicity testing using local species (freshwater bivalves and walleye) is also expected to be undertaken to support the trial, by government or academic institutions (see Section 3.2.3). Water quality analyses conducted at each step of the treatment process and during the on-site testing ensure causal inference conclusions will be robust. Various investigations of the potential for partitioning and bioconcentration/bioaccumulation of constituents of treated effluent also will be undertaken, including measurements of metals in exposed organisms and periphyton, use of membrane devices designed to simulate uptake into biological tissues, and exposure of clean sediments to treated waters for the duration of the mesocosm trial.

Results of all these tests will contribute technical information to support decisions for potential future pilot-scale return of treated OSPW to the Athabasca River.

This document is designed to summarize the intended procedure and goals of the study. Each phase and component have been and will continue to be discussed and refined with the OSPW Science Team.

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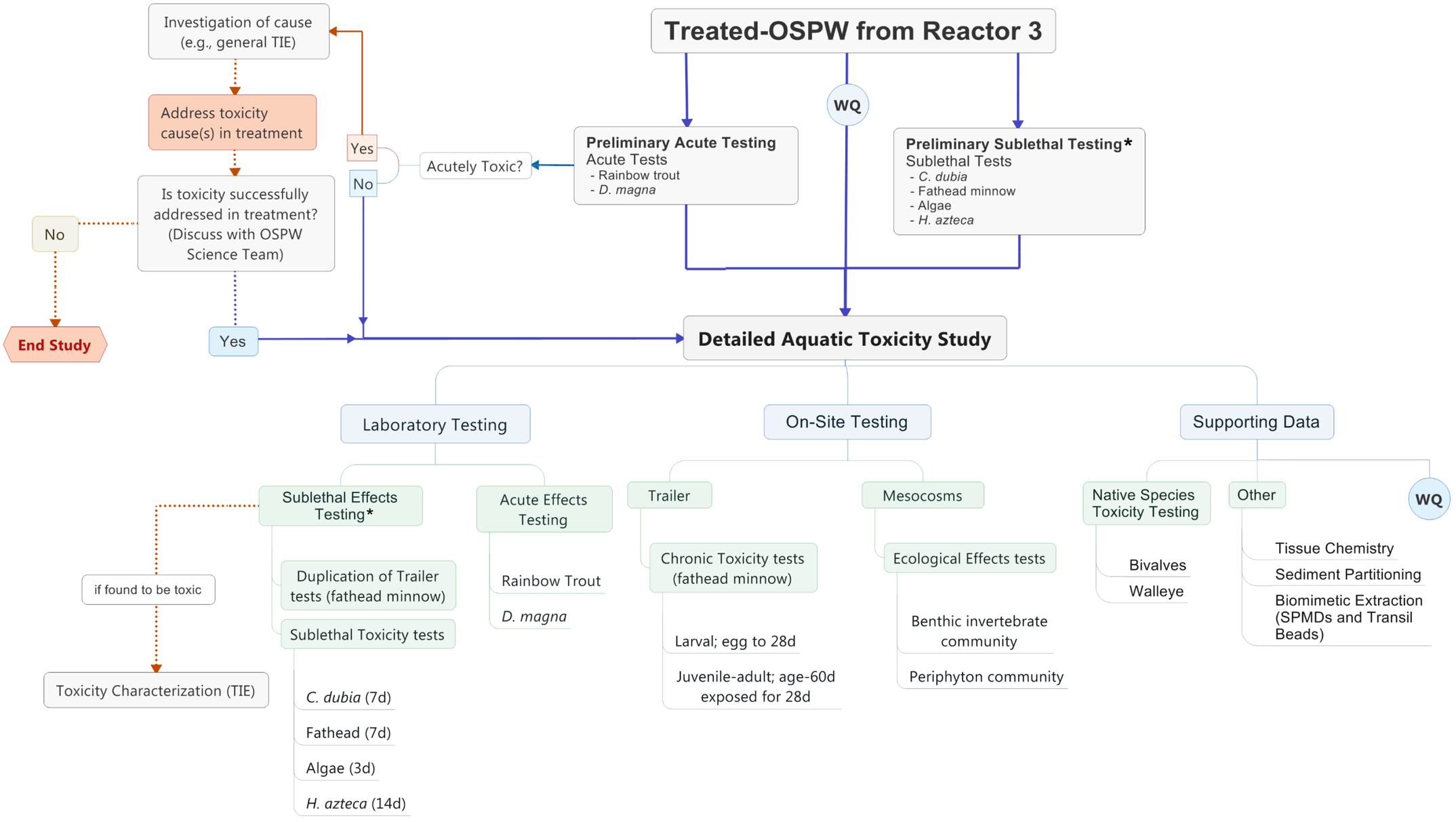
Figure 3 Summary of the 2019 aquatic toxicity study designed to evaluate the chemical and toxicological attributes of treated OSPW.



* **Note:** Sublethal toxicity tests also will provide acute toxicity endpoints for interpretation.

2.0 PHASE 1: PRELIMINARY SCREENING TESTS

A preliminary toxicity screening phase will be used to inform later testing phases (Section 3.2) and the mesocosm study (Section 3.1), including potential refinements to the study plan. The screening will address the following:

- Whether there is acute toxicity in the treated OSPW sample to rainbow trout (*Oncorhynchus mykiss*) and the water flea *Daphnia magna*. If acute toxicity is present, then further evaluation of the effectiveness of the treatment will be necessary before proceeding to sublethal toxicity testing.
- Initial identification of any sublethal toxicity of treated OSPW to test organisms including rainbow trout, *D. magna*, another water flea *Ceriodaphnia dubia*, fathead minnow (*Pimephales promelas*), and the amphipod *Hyallela azteca*. Depending on the extent of any adverse effects, it may be advisable to adjust the test concentrations from those specified in the study plan to more effectively capture the dose-response relationship.
- Whether the site dilution water is suitable to provide acceptable performance of the various test organisms and if amendments may be required to ensure test viability. For example, *H. azteca* requires a suitable concentration of bromide (>0.02 mg/L) and chloride (>15 mg/L) in control and dilution waters to support growth. In addition, microbes that occur naturally in ambient waters (such as fungi and ciliates) can adversely affect fathead minnow and *C. dubia* cultures and may need to be controlled during the extended toxicity tests that are planned.
- Whether there is a risk of testing artifacts compromising the results of the program. For example, upward pH drift during tests involving static exposures can significantly increase the toxicity of ammonia in a manner that is not relevant to site water.

2.1 PHASE 1 TOXICITY IDENTIFICATION EVALUATION

If acute toxicity to rainbow trout or *D. magna* is measured during acute screening tests, a preliminary, screening-level TIE will be conducted to identify the broad class of toxicant that is responsible for the observed toxicity. The OSPW Science Team will be consulted if a TIE process is required. A TIE involves conducting physico-chemical manipulations of the sample, followed by toxicity tests on the treated and untreated samples to establish whether the treatments have effectively reduced toxicity. Each treatment alters the toxicity of a subset of constituents; thus, the results of these procedures can be used to identify the type of constituent that is responsible for the adverse response.

The results of this TIE will be used to inform the treatment process, including potential modifications, by focusing attention on constituents not adequately removed. Additionally, the screening may identify adverse responses occurring due to an unanticipated process or source (e.g., interactions between equipment and treated or dilution water). If modifications to the treatment process are advised and implemented, the treated water would be re-tested for acute toxicity to rainbow trout and *D. magna*. If the revised treatment is found to be successful, the study can move on to Phase 2; if treatment is unsuccessful, the study will be terminated pending further discussions with the OSPW Science Team.

3.0 PHASE 2: DETAILED AQUATIC TOXICITY STUDY

The three components to the second phase will all occur concurrently, using OSPW dilution series of 0%, 0.32%, 3.2%, 32% and 100%. If required, dilutions will be refined by the toxicity range-finding in Phase 1 and through discussions with the OSPW Science Team. The on-site mesocosm testing and mobile toxicity trailer will use identical dilution series and evaluate sublethal toxicity and the effects on benthic assemblages. Off-site laboratory testing will occur at the Nautilus laboratory in Calgary (standard tests), at ECCC in Burlington (bivalves), and at either the University of Saskatchewan or Nautilus in Calgary (walleye). Concurrent with these tests, assessments of the potential for constituents of treated effluent to partition to sediments and to bioconcentrate in tissues will be undertaken.

3.1 MESOCOSM STUDY

Mesocosms allow separation and replication of treatments to unequivocally test hypotheses of change among biological assemblages with physical and chemical diagnostics. Multiple test environments, whether flow-through flumes (Bothwell 1989), or circular streams (Culp et al. 2003) with plumbing to control flows and chemical additions, allow for control of multiple stressors applied to realistic and representative aquatic invertebrate and periphyton assemblages that are derived from the actual river of interest. This capability provides causal inference not achievable in standard toxicity tests that are run in laboratories using non-endemic organisms. Results can also be used to build models of functional response to ranges of doses of chemicals or physical change.

The 2019 mesocosm experiment will evaluate the response of periphyton and benthic invertebrates found in the Athabasca River watershed to a range of dilutions of treated OSPW. The dilutions have been designed through consultation with subject matter experts and the OSPW Science Team to include concentrations of potential toxic chemicals over which a gradient of biological responses may occur. The proposed dilutions will be evaluated before activation of the mesocosms using a screening toxicity study (see Section 2.0, above). The current proposed dilution series includes control-extremes of 100% treated OSPW and 0% treated OSPW. Intermediate dilutions should be sufficient to potentially derive a dose response model or run other statistical tests to show effect of dilutions of treated OSPW on benthic communities: 32% treated OSPW, 10%, 3.2%, 1%, and 0.32%. Consultation with the OSPW Science Team has prompted the inclusion of duplicate 0% treated OSPW control treatments for examining natural variability within the mesocosm. Each dilution treatment will be replicated four times, resulting in a mesocosm running 32 streams (8 treatments x 4 replicates).

3.1.1 Design

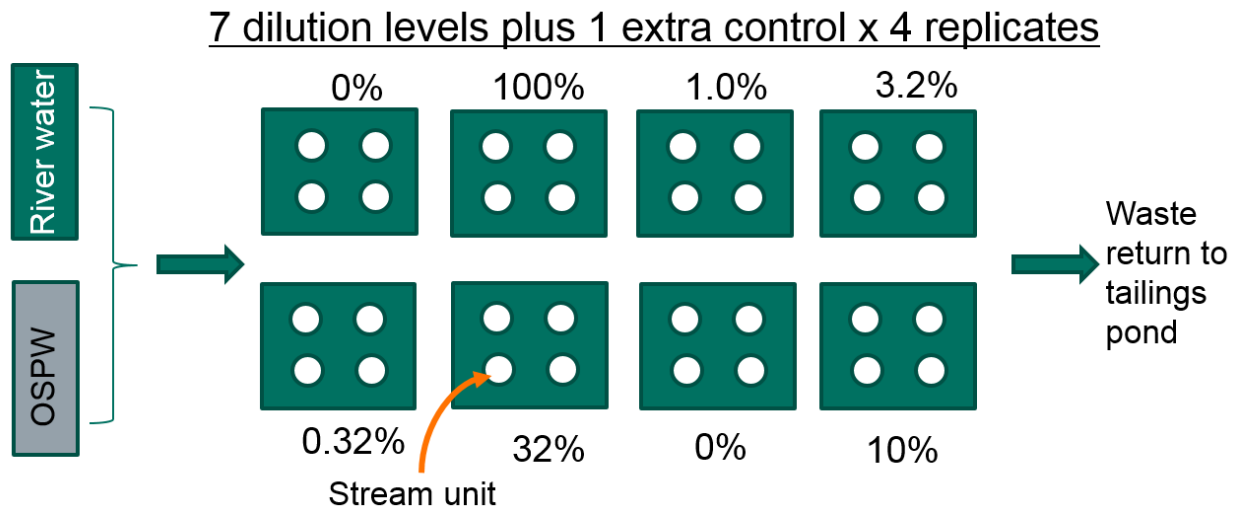
The mesocosm design was selected to suit conditions at the study site and meet study objectives. There are two basic designs available for large river work. One uses flow-through flumes in which each flume is a replicate of a given treatment (e.g., Bothwell 1989, Perrin and Richardson 1997). For studying invertebrates, this design requires a location on or near a river bank incorporating passive or pumped delivery of water and organisms directly from the river. It requires several weeks for colonization of the flumes followed by more weeks of treatment before final sampling. The second design uses circular streams that are inoculated with periphyton and benthic macroinvertebrates (Culp et al. 2003, Alexander et al. 2016). The hardware for this design does not need to be situated on the river bank because continuous recruitment

by the benthic assemblage is not necessary. Using this second design, an experiment can be done within about a month following inoculation.

The circular stream mesocosm design was selected for this experiment due to restrictions on access to water withdrawal from the Athabasca River, and logistical challenges in delivering treated OSPW to a position on the river bank. It will be located on a nearby industrial property (warehouse and office with power) located north of the Syncrude lease, approximately 20-30 minutes from the treatment lagoons and river water supply.

The mesocosm specifications are nearly identical to those reported by Culp et al. (2003), with some study-specific modifications. Briefly, it consists of a series of eight 1.2 x 1.2 m tables (corresponding to the dilution series), each equipped with a polyethylene tray on which four flow-through circular streams are placed (Figure 4). River water and treated OSPW will be delivered to on-site supply tanks by truck, then distributed to each stream by peristaltic pump. Water in the streams will be circulated using a paddlewheel to create and maintain water velocities typical at the water-substratum interface in the Athabasca River.

Figure 4 Proposed mesocosm layout showing stream units, treatment tables, dilutions, and direction of water.



Due to the bespoke nature of many components, fabrication was contracted to specialists with experience constructing the mesocosms. Fabrication of stream components occurred at Quality Molded Plastics and JCM Specialties in Saskatoon, SK, at ECCC facilities in Fredericton, NB, and at Limnotek in Vancouver, BC. Assembly and testing of some key components occurred at Limnotek in Vancouver and ECCC in Fredericton to ensure no crucial parts are missing and the systems are operating successfully. Final assembly occurred at the Hatfield office in Fort McMurray, AB. The intent is for Syncrude to own the mesocosm apparatus, making it available for future experiments or other use.

The mesocosms are designed to mimic erosional habitat conditions (i.e., cobble and gravel substrates) and will be inoculated with benthic assemblages from erosional habitat of the Athabasca River or nearby tributaries. Although it is recognized that the Athabasca River in the vicinity of a future water-return location is dominated by depositional habitat, stony substrata in the mesocosms will host a benthic community that is more sensitive to disturbance (typically sensitive species of Ephemeroptera, Plecoptera and Trichoptera, with chironomids and a host of rarer taxa) than the more robust community that occupies depositional

habitat of fine sediment (typically chironomids and worms). Accordingly, the selection for sensitive taxa using stony substrata will provide a worse-case scenario regarding the potential effects on benthic invertebrate and periphyton communities chronically exposed to treated OSPW. It should be noted that it is not possible to include both stony substrata and fine sediments in the same mesocosm chamber as the fine sediments will embed the courser substrate and greatly impact micro-habitat availability required by the sensitive benthos species colonizing the stony substrata.

3.1.2 Methods

3.1.2.1 Mobilization

Initial construction and fitting of the mesocosm tables occurred at the Hatfield office in Fort McMurray, AB, in August 2018. The apparatuses are currently in storage at Syncrude's warehouse facility at its Mildred Lake operation and will be transported to the study location on Bouchier property for final assembly in July 2019. The Project team has partnered with Bouchier Contracting Ltd. to utilize their industrial property, adjacent to Syncrude property, where a level, graded area is available with power, internet connectivity, and office facilities. Testing of the apparatus will follow the final assembly.

Cobble and large gravels will be collected from either the Athabasca River or a suitable location within the Athabasca watershed and the system will be run for four (4) days to stabilize particle movement. These will provide the substrate for the artificial streams, upon which endemic periphyton will grow and establish – this is the "inoculation" stage of the experiment. Benthic macroinvertebrates will be collected from a suitable location either in the Athabasca River or one of its tributaries and added to the streams following the inoculation stage. All collection, transport, and care of test organisms will follow the method reported by Culp et al. (2003).

3.1.2.2 Operation

The experiment will begin upon addition of the benthic macroinvertebrates and will run for 21 days. During this period, water temperature, dissolved oxygen (DO), and conductivity will be continuously monitored using data sondes to provide supporting data for analyses and indication of any potential malfunction. Water velocities in each stream will be measured every two days to ensure environmental consistency and that the systems are functioning as required. Periodic water samples (every 3-4 days) will be collected from all 32 streams, the raw river water, and the raw treated OSPW. A duplicate sample and field blank will be collected during each water quality sampling event. All water quality samples will be shipped to ALS Laboratories in Fort McMurray, AB.

3.1.2.3 Demobilization

At the end of the 21 days the study is complete, and invertebrate and periphyton assemblages will be collected from each stream, preserved, and sent to Danusia Dolecki at Invertebrates Unlimited in Vancouver, BC for taxonomic analyses. Measurements will be made of periphyton biomass (as chlorophyll-a concentration), periphyton cell counts and biovolume by species, and benthic invertebrate counts and biomass by genus or lowest reliable taxonomic level. Periphyton samples will also be sent to ALS Laboratories in Fort McMurray, AB for chlorophyll-*a* analysis. Disassembly of the mesocosm apparatus will be completed by Hatfield and ECCC and provided to Syncrude following completion.

3.1.3 Mesocosm QA/QC Procedures

Quality Assurance/Quality control procedures that will be incorporated into the mesocosms study include the following:

- Tanker trucks used for transporting river water and treated OSPW will remain the same throughout the study, i.e., one tank will only ever transport one type of water;
- Benthic macroinvertebrates will be sampled from nature using an established method such as kick-netting with a fixed mesh size;
- A random splitting device will be used to add test organisms to each stream;
- Approximately 10 cm of Nytex mesh (or equivalent) will be attached at the brim of all stream units to allow water overflow but prevent escape of invertebrates;
- Specific location decisions and shade cloth will be employed to standardize sunlight and temperature effects as best as possible;
- Periphyton and benthic invertebrate taxonomy will be completed by an experience, certified taxonomist, and follow the standard EEM QA/QC procedures for taxonomic data; and
- All data will receive QA/QC review for transcription and analytical errors by a qualified biologist that was not directly involved in the original testing.

3.2 TOXICITY TESTING

Nautilus Environmental in Calgary, AB, will test the toxicity of treated OSPW from Reactor 3 through both off-site laboratory testing for acute and sublethal toxicity, and on-site sublethal toxicity testing (the off-site and on-site programs will occur simultaneously). Additional tests on non-standard organisms (freshwater bivalves and walleye) will be completed by government and academic partners. A summary of the proposed toxicity tests is presented in Table 1. Detailed information on test conditions and procedures is summarized in Appendix A1.

Table 1 Proposed toxicity testing of treated OSPW to be conducted during the aquatic toxicity study, 2019.

Species	Test	Test Method	Frequency
Standard Tests			
Rainbow trout (*O. mykiss*)	Acute toxicity	ECCC EPS 1/RM/13	Weekly
Water flea (*D. magna*)	Acute toxicity	ECCC EPS 1/RM/14	Weekly
Water flea (*C. dubia*)	Sublethal toxicity	ECCC EPS 1/RM/21	Weekly
Fathead minnow (*P. promelas*)	Sublethal toxicity	ECCC EPS 1/RM/22	Weekly
Green alga (*P. subcapitata*)	Sublethal toxicity	ECCC EPS 1/RM/25	Weekly
Amphipod (*H. azteca*)	Sublethal toxicity	ECCC EPS 1/RM/33	Weekly
Fathead minnow (*P. promelas*)	Sublethal toxicity, 0-30d	USEPA (1996); ASTM (2013)	Once
Fathead minnow (*P. promelas*)	Sublethal toxicity, 60-88d	Adapted from ASTM (2013)	Once
Additional Native Species Tests			
Bivalve (*L. cardium*) glochidia	Acute toxicity, 48h	Dr. Patricia Gillis (ECCC)	Once
Bivalve (*L. cardium*) juveniles	Sublethal toxicity, 28d	Dr. Patricia Gillis (ECCC)	Once
Walleye (*S. vitreus*) embryo*	Sublethal toxicity, 30d	Raine et al. (2017)	Once (2020)*
Walleye (*S. vitreus*) juveniles	Sublethal toxicity, 30d	Modified from Raine et al. (2017) and ASTM (2013)	Once (2019)

* Walleye embryo test to be conducted in spring 2020 using frozen effluent from 2019 trial, depending on results of 2019 walleye juvenile test.

Laboratory toxicity tests will be conducted according to standard ECCC or ASTM biological test methods. The Nautilus Laboratory is accredited by the Canadian Association for Laboratory Accreditation (CALA) to ISO 17025 standards for tests listed in the scope of accreditation.

During the 2019 trial, laboratory-based acute and sublethal toxicity tests will be conducted on a weekly basis. The same geometric dilution series will be used for the on-site testing and laboratory testing, except for an additional test concentration of 56% treated OSPW and a laboratory control water test evaluated in the laboratory exposures.

3.2.1 On-site Testing

On-site sublethal toxicity testing with fathead minnow will be conducted in the mobile testing facility concurrent with the mesocosm study and consists of two tests: a ~30-day embryo-larval test and a 28-day juvenile-adult test.

If on-site testing indicates sublethal toxicity of treated OSPW, another TIE may be triggered to characterize the source(s), likely using short-term (≤7-day) laboratory tests to fit within the testing period. Results of this TIE will help inform the OSPW treatment process.

3.2.1.1 Toxicity Trailer

The purpose of the mobile testing facility tests is to identify potential sublethal effects associated with long-term exposure to treated OSPW on fish. Fathead minnow is a standard species used for long-term testing, is known to be sensitive to OSPW and is endemic to the lower Athabasca River. The embryo-larval stage tests will provide characterization of a potential effect at early life-stages, with endpoints including percent of hatched eggs, post-hatch survival, growth rate, and deformities. The juvenile-adult test will further characterize effects on survival, length, weight, and body condition factor. Tissue samples will be collected from the adult fish and used for other assessments such as gill histology, gonad development, and tissue concentrations of metals. The trailer will be located at the same site as the mesocosm study and the tests will incorporate the identical OSPW dilution series as used for the mesocosm study.

The embryo-larval test will start with eggs <24-hours post-fertilization. The juvenile-adult test will be initiated on approximately 60-day old fish. The duration of the tests will be 28 days following hatch for the embryo-larval test, and 28 days for the juvenile-adult exposure.

The trailer was equipped and tested by Nautilus in Calgary and is currently in storage. Relocation to the Bouchier property will occur prior to study execution in July 2019. Test organisms will either be supplied from a laboratory culture established at the Nautilus laboratory in Calgary, AB, or directly from Aquatic Research Organisms in Hampton, New Hampshire. In either case, permits required for transportation and housing of the fish will be obtained from Canadian Food Inspection Agency and the Alberta Department of Environment and Parks.

It should be noted that temperature control in the mobile testing facility may be affected by daily and seasonal fluctuations in external temperature. Timing of the experiments in mid-late summer is designed to mitigate seasonal fluctuations as best as possible. However, the goal will be to maintain the exposures at 25°C, to provide a comparable exposure to the laboratory-based tests.

3.2.2 Laboratory-Based Testing

Laboratory test organisms used for evaluating the toxicity of treated OSPW are listed in Table 1. These standard tests extend for ≤7 days and will be undertaken weekly throughout the trial.

In addition to these standard weekly tests, the long-term (28d) on-site toxicity trailer tests using fathead minnow will be duplicated concurrently at the Nautilus laboratory, to provide a backup in the event of an on-site test failure, as well as to assess whether tests conducted on-site provide a higher level of sensitivity than laboratory tests (for example, if toxicity were to dissipate while the samples are in transit to the laboratory).

3.2.3 Additional Toxicity Testing using Native Species

Based on discussions with the OSPW Science Team, additional laboratory testing will be conducted on species that do not have standardized testing protocols but are locally important, namely the bivalve *Lampsilis sp.* (freshwater mussel) and walleye (*Sander vitreus*). All non-standard species testing will use the same dilution series of treated-OSPW as the standard laboratory tests and mesocosms.

3.2.3.1 Bivalves

Native bivalves are known to have heightened sensitivity to some ubiquitous contaminants such as chloride and ammonia and are often the most sensitive group used to develop some water quality regulations in Canada and the United States (Augspurger et al. 2007; CCME 2011). Additionally, bivalves have been identified as an important indicator by Indigenous communities.

Bivalve testing will be completed by Dr. Patricia Gillis' laboratory at ECCC in Burlington, ON, concurrently with other toxicity testing during the trial so that results are comparable with all other toxicity testing using standard species. Two tests will be undertaken: one focusing on survival of developing mussel glochidia (larvae); and one focusing on survival and growth of juveniles.

Glochidia will be wild-sourced from gravid females and exposed to 10-12 treatments (refinements will be made through discussion with the OSPW Science Team), including two controls and varying concentrations of treated-OSPW and a reference toxicant (NaCl). Replicates will contain 500-1000 individuals. Samples of glochidia (100 individuals) will be collected at 24h and 48h, visually assessed for viability (ability to close valves) and enumerated according to ASTM (2013).

Laboratory-cultured juvenile mussels will be obtained from Dr. Chris Barnhart at Missouri State University and exposed to the same 10-12 treatments as glochidia, in replicates of 10 individuals. Permits required for transportation and housing of the mussels will be obtained from Canadian Food Inspection Agency and the Alberta Department of Environment and Parks. The test will run for 28 days, changing to fresh exposure solution at 7 and 21 days and holding vessels at 14 days. Survival and attempts-to-bury will be assessed at day 14 and day 28.

Further details regarding native bivalve testing can be found in Appendix A1.

3.2.3.2 Walleye

Walleye is a top-level piscivorous fish species and important food resource for Indigenous people in the lower Athabasca River basin. Walley has been identified as a Key Indicator Resource for the Athabasca River for monitoring and management purposes (CEMA 2001) and continues to be a key species for long-term monitoring under the Joint Oil Sands Monitoring Plan for the Athabasca oil sands region.

Walleye spawn in the spring soon after ice break-up, with eggs hatching approximately two weeks later (Scott and Crossman 1973). As such, egg-to-embryo exposures to treated OSPW will not be possible in 2019 as the trial will not be producing treated OSPW until June/July. However, exposure of juvenile walleye is possible in 2019, using a modified protocol analogous to the fathead minnow juvenile tests being performed in this study. Juvenile testing in 2019 would provide information regarding walleye sensitivity to treated OSPW, which would be directly comparable with fathead minnow results and allow for comparison of the sensitivity of these species. To conduct the juvenile test, fertilized walleye eggs or juvenile walleye

would be required; these could potentially be sourced from a hatchery in Fort Qu'Appelle, SK (although intra-provincial import permits may be difficult to obtain), or they could be grown from gametes collected from local broodstock in spring 2019. There are various locations to potentially obtain wild walleye broodstock locally, including from an abundant and isolated population of walleye in Mildred Lake on the Syncrude lease. Sublethal tests using juvenile walleye (including pre-exposure rearing of juveniles from eggs, if necessary) would be undertaken at the Nautilus laboratory in Calgary, and would use a dilution series consistent with extended tests conducted using fathead minnow.

Based on the 2019 results of the early life stage test using juvenile walleye (and other testing), a decision could be made by the OSPW Science Team to conduct early-life-stage testing using walleye gametes/embryos collected in spring 2020. This test would be conducted using treated OSPW collected during the 2019 trial and frozen until spring 2020 when walleye eggs are available. Such testing on walleye embryos in 2020 would be undertaken by either the Nautilus laboratory or by the laboratory of Dr. Jason Raine of the University of Saskatchewan. Further details regarding the proposed testing of walleye eggs exposed to treated OSPW can be found in Appendix A1. It should be noted that freezing effluent samples can result in changes in water quality, such as dissolution of effluent constituents, that could potentially affect test results.

3.2.4 Sample Collection, Delivery, and Holding Times

Water samples used for all laboratory toxicity tests will be collected on-site in clean 20-L containers from the same supply as used for the trailer and mesocosm tests and delivered to Calgary by overnight courier on a weekly basis. Both treated OSPW and site control water will be provided; the site control water will be used in the laboratories for dilution of the treated OSPW, as well as a secondary control (i.e., in addition to the usual control water used in the laboratory for each of the test species). Water samples will be stored at each laboratory in the dark at 4 ± 2°C prior to use in the tests. Toxicity tests will be initiated as soon as feasible following receipt at the laboratory; a holding time of no more than three days will be used prior to initiation of the tests.

3.2.5 Toxicity Testing QA/QC Procedures

Quality Assurance/Quality control procedures that will be incorporated into the toxicity study include the following:

- Organisms will be obtained from reliable suppliers and will meet the health history requirements specified in the test method;
- The tests will be conducted according to standardized protocols and this work plan;
- The tests will be conducted by staff who have appropriate training and experience to conduct these tests;
- A negative control will be conducted with the test to provide data against which to evaluate control performance criteria for the test methods;
- A positive control (reference toxicant test) will be conducted to evaluate the sensitivity of the test organisms compared with results obtained for these species previously in the laboratory;
- Instruments will be calibrated daily, or as recommended by the manufacturer; and
- The data will receive QA/QC review and approval by a qualified biologist that was not directly involved in conducting the tests.

3.3 SUPPORTING DATA

3.3.1 Water Chemistry

Water chemistry of the OSPW will be analyzed at the source (i.e., untreated), and after each Reactor stage throughout the treatment process. Continuous *in-situ* monitoring of the mesocosm water as discussed above, including velocity measurements every 2 days, will ensure the apparatus is functioning correctly and support conclusions regarding toxicity. Water samples collected every 3-4 days from each mesocosm stream unit will be tested for the same analytes as the OSPW during treatment. All chemistry analyses will be completed by ALS Laboratories in Fort McMurray, AB.

Table 2 summarizes supporting water quality data to be collected during this study.

Table 2 Supporting water chemistry analyses to be conducted during the aquatic toxicity study of treated OSPW, 2019.

Test or Analyte	Frequency
Temperature, DO, Conductivity	Continuous
Water velocity	Every 2 days
pH, conductivity, TSS, TDS, F, NO_3-N, NH_4-N, SO_4, Cl, TP, TDP, SRP, DIC, DOC, TOC, dissolved metals, total metals, PAH's, naphthenic acids, BTEX (benzene, toluene, ethylbenzene, xylene), phenols	Every 3-4 days

3.3.2 Tissue Chemistry

Fathead minnows used for sublethal toxicity testing, as well as bulk samples of benthic invertebrate communities and periphyton scrapings from the mesocosms, will be retained for tissue chemistry analyses of metals accumulation. Samples from each treatment concentration will be tested separately, with individual treatment replicates analyzed separately if sufficient tissue is available (otherwise, samples will be pooled within treatments).

All chemistry analyses will be completed by ALS Laboratories in Fort McMurray, AB. Table 3 summarizes supporting tissue chemistry data to be collected during this study.

Table 3 Supporting tissue residue tests (performed for all exposure concentrations) to be conducted during the aquatic toxicity study of treated OSPW, 2019.

Test or Analyte	Frequency
Fathead minnow tissue chemistry (metals)	At end of on-site and laboratory sublethal exposures (28d)
Walleye tissue chemistry (metals)	At end of juvenile study (28d / ~30d)
Benthic invertebrate tissue chemistry (metals)	At end of study (28d / ~30d)
Periphyton tissue chemistry (metals)	At end of study (28d / ~30d)

Separately from the aquatic toxicity study, additional tissue samples from fish and benthic invertebrates will be collected from the Athabasca River as part of the complimentary AEP baseline sampling program. These data will provide baseline data for future in-stream monitoring and will also facilitate interpretation of data from the aquatic toxicity study of treated OSPW.

3.3.3 Additional Supporting Data – Bioconcentration Studies

Discussions with the OSPW Science Team have identified additional areas of monitoring that would expand or complement the scope of this assessment, either in 2019 or subsequent to the 2019 study. The addition of these components to the 2019 scope was discussed in the September 2018 at the Toxicity Testing subgroup meeting of the OSPW Science Team.

These additions relate to the potential for partitioning of metals from water to sediment, and the use of passive bioaccumulation surrogates and are highlighted below.

3.3.3.1 Partitioning of Metals into Sediment

Concern has been raised regarding the future potential for effluent constituents, particularly dissolved metals, to partition from the water column into sediments in the Athabasca River upon discharge, potentially creating a risk of bioconcentration of these metals in downstream biota. The 2019 trial proposes tissue-residue testing of fathead minnow from the extended laboratory test and of invertebrates from mesocosms, which will provide estimates of potential for bioconcentration from the water column to biota.

Laboratory Exposures

A screening-level assessment of partitioning of metals from water to sediments will be undertaken in parallel with laboratory toxicity testing. Sediments would be collected from the Athabasca River downstream of the potential water return location, homogenized, and split for use in multiple exposures to different overlying waters (i.e., pre-exposure baseline, laboratory control water, river water, and 100% treated OSPW). These exposures would occur in the Nautilus laboratory in Calgary, AB to ensure proper control (particularly, to avoid the potential for test-chamber contamination by dust or natural river sediments contributed with dilution water that could occur in field exposures) and include replicated beakers per treatment. These exposures would be set up at the beginning of the extended testing period for fathead minnows, left to equilibrate and allow partitioning over the duration of these toxicity tests, and taken down at the end of these tests. Metals analyses on sediments would be performed on all exposures, to provide a preliminary assessment of the likelihood of metals to partition from treated OSPW to sediments. Because the partitioning of some metals to sediments is highly dependent on the pH of test waters, some pH control among trials (to a representative pH for the Athabasca River) likely would be required.

Reactor 3 Field Exposures

The final stage of the treatment process, Reactor 3, is intended to reduce ammonia in the treated-OSPW to non-toxic levels. As a second assessment of potential for metals partitioning, samples of clean Athabasca River sediment (identical to those used in laboratory exposures above) will be placed in Reactor 3 (the polishing pond) at the beginning of the trial and left in place over the operating period, with metals concentrations measured in pre-exposure and post-exposure samples. Before/after comparisons will be used to identify potential for metals partitioning. Three replicate samples of homogenized Athabasca River sediment will be placed in Reactor 3, then collected and analyzed at the end of the trial. Sediment containers placed in Reactor 3 will be loosely covered during the exposure period to prevent potential contamination of sediments by dust or blowing sand/soil into the polishing pond from the surrounding work site.

In addition to before/after comparisons of sediments placed in Reactor 3, scraping of periphyton growing in Reactor 3 will be sampled at specific times over the trial period (i.e., 7, 14, 28 and 56 days) to assess metals concentrations and identify any accumulation of metals in these primary producers. A single, composite sample of periphyton will be collected and analyzed for each exposure time period.

3.3.3.2 Quantifying Bioavailable Trace Organic Chemicals using Passive Samplers

Through a process broadly known as biomimetic extraction (BE), passive exposure of plastics or lipids such as triolein-impregnated polyethylene or polydimethylsiloxane (PDMS) to test solutions containing trace concentrations of bioavailable organic chemicals such as PAHs or organic acids can provide valuable, time-integrated data that precisely describe ultra-trace concentrations of these chemicals in test solutions. Additionally, these biological analogs can provide an estimate of sublethal toxicity and potential for organic chemicals to bioconcentrate from water to biota.

ECCC successfully used semi-permeable membrane devices (SPMDs) in the JOSM program to quantify ultra-trace concentrations of PAHs in regional rivers. Recent studies by Redman et al. (2018) used solid phase microextraction (SPME) fibres coated with PDMS to measure bioavailable organics in OSPW. Using OSPW as a test solution, Redman et al. (2018) demonstrated that target-lipid-model (TLM)-derived estimates of sublethal toxicity using SPME data corresponded closely with published sublethal toxicity curves, suggesting that this BE-based analysis has potential to be used as a surrogate for, or to support, classical toxicity tests. Furthermore, Zhang et al. (2016) demonstrated that classes of dissolved organics (notably naphthenic acids) from untreated Base Mine Lake OSPW bioconcentrate in Japanese medaka (*Oryzias latipes*) and Transil^{XL}® beads.

With collaboration and support from relevant researchers, such passive samplers will be integrated into the 2019 treated-OSPW trial. This includes placing SPMDs in treated-OSPW and river water holding tanks (or in Reactor 3 and the Athabasca River water holding pond) for the duration of the mesocosm/trailer trials. These data will facilitate an integrated, precise estimate of PAH concentrations in these trials and constituent treatments. Additionally, SPMDs will be placed at select locations in the Athabasca River for concurrent collection of *in-situ* baseline data as part of AEP's baseline sampling program.

Direct placement of SPMDs in mesocosm treatments is not advised because of the theoretical potential for SPMDs to adsorb potential toxicants of concern. The circular mesocosms are small enough that any adsorption could affect practical doses of these chemicals to test organisms; the same concerns are not applicable in a very large vessel like a holding tank or pond. Support will be required from ECCC to provide appropriate samplers and undertake subsequent chemical analyses. The experiment implementation crews will place and monitor the devices over the duration of the trial.

Based on the work by Redman et al. (2018) and Dr. Steve Wiseman of the University of Lethbridge, fine-scale BE tests will be conducted with treated OSPW using Transil^{XL}® beads, which mimic cell membranes, and with PDMS-coated fibers, which mimic storage lipids. The PDMS-coated fibers will be placed in untreated and treated OSPW, to measure uptake of bioavailable organic chemicals to storage lipids before and after treatment.

A Transil^{XL}® bead study will be conducted off-site at the University of Lethbridge by Dr. Wiseman. The study will compare treated OSPW from Reactor 3 to untreated OSPW from Syncrude and other Alberta Oil Sands operators, at pH ~8.5 (similar to OSPW) and pH ~8.0 (similar to Athabasca River), given the bioconcentration potential of OSPW compounds appears to be pH-dependent. Information from these exposures will indicate potential for transfer of organic chemicals from effluent to aquatic organisms. Further details regarding the use of PDMS-coated fibers and Transil^{XL}® beads as part of the aquatic toxicity study of treated OSPW has been provided Appendix A1.

4.0 DATA ANALYSIS AND TECHNICAL REPORT

Data analysis and reporting are anticipated to occur during November 2019 through February 2020. Primary analysis of the mesocosm results will be completed by Alexa Alexander-Trusiak of ECCC with support from Hatfield. The final technical report will be synthesized and delivered by Hatfield no later than Spring 2020 to Syncrude and the OSPW Science Team.

Mesocosm data will support a variety of analytical options. Replicating each treatment four times allows an analysis of variance followed by paired comparisons on key endpoints like algal biomass or benthic invertebrate diversity (Table 4) to test treatment effect. Regression modeling will be used to show dose-response curves for benthic invertebrate and periphyton metrics to dilutions of the treated OSPW. Multivariate statistics such as ordinations will be used to examine changes in whole-assemblage patterns to dilutions of treated OSPW. Interpretation of the toxicity analyses will be completed by Nautilus and will be appended to the mesocosms study to be used to compliment conclusions. Water chemistry, temperature, velocity and other descriptive variables will be also used to assist with interpretations of the biological responses to dilution of the treated OSPW.

Table 4 Analytical endpoints for each biotic assemblage sampled from mesocosms used for the aquatic toxicity study of treated OSPW, 2019.

Assemblage	Endpoint
Periphyton	Chlorophyll *a* concentration, species biomass and biovolume
Benthic Macroinvertebrates	Abundance, taxa richness, species diversity, biomass (by taxon and community)

There are numerous factors to consider in the interpretation and synthesis of the large amount of complementary toxicology data that will be generated by this project. The OSPW Science Team has committed to developing a discussion document to guide interpretation of these data, with a preliminary test of guiding principles or questions including criteria for reference-organism survival, environmentally relevant exposures and concentrations, and various questions regarding decision-making for potential effluent release to the receiving environment. We expect this interpretive guidance document to be developed through the first half of 2019 by the OSPW Science Team.

Acute and sublethal toxicity tests will be used to determine the IC25 and LC50 endpoints (Table 5) using probit regression analyses. The IC25 endpoint estimates the concentration of effluent that causes 25% inhibition of a quantitative biological function, such as reproduction or growth. The LC50 endpoint defines the “lethal concentration” of effluent that causes 50% or more mortality of the test organisms.

Table 5 Endpoints for standard acute and sublethal toxicity tests conducted during the aquatic toxicity study of treated OSPW, 2019.

Species	Endpoint
Rainbow Trout (*O. mykiss*)	Survival; LC50
Water flea (*D. magna*)	Survival and immobility; LC50
Water flea (*C. dubia*)	Survival and reproduction; LC50 and IC25
Fathead Minnow (*P. promelas*)	Survival and growth; LC50 and IC25
Green alga (*P. subcapitata*)	Growth; IC25
Amphipod (*H. azteca*)	Survival and growth; LC50 and IC25

The endpoints reported for the 28- and 30-day fathead minnow sublethal toxicity tests will include hatch success, survival, length, biomass, post-hatch survival, and normal development (which assesses incidence of deformities) over the test period.

Given that this experiment is unique and will contribute new science, the study team is expected to pursue publication of the findings in a scientific journal pending approval from Syncrude. Both the technical report and the journal publication will focus on effects of a gradient of dilutions of treated OSPW on bioassay organisms and benthic assemblages and will not give an opinion on whether treated OSPW may be returned to the Athabasca River.

5.0 PROJECT SCHEDULE

A summary project schedule is presented in Table 6 below. A detailed Gantt chart showing specific milestones related to the implementation of the preliminary screening tests and detailed aquatic toxicity study is provided in Figure 5.

Table 6 Anticipated Project schedule for the aquatic toxicity study of treated OSPW, 2018-2020.

Task	Anticipated Timeline	Status
Design and planning, including meetings with Syncrude and OSPW Science Team, other stakeholders	Spring 2018-2019, completed by end of June 2019	Ongoing
Equipment procurement, mobile toxicity testing trailer inspection and setup, any required safety training	May 2018 to end of July 2018	Complete
Construction of mesocosms	July and August 2018	Complete
Preliminary toxicity screening tests, range-finding, TIE (if necessary)	Early-mid July 2019	Planned
Assembly and testing of mesocosms on-site	July 2019	Planned
Inoculate mesocosms	Late July / early August 2019	Planned
Run experiments	Mid-August 2019 to early September 2019	Planned
Laboratory analyses (water quality, biota, etc.)	Late August 2019 to end of November 2019	Planned
Data QA/QC, analysis, and interpretation	November 2019 to end of January 2020	Planned
Technical report	January 2020 to Spring 2020	Planned
Deadline for Technical Report Deliverable	End of April 2020	Planned

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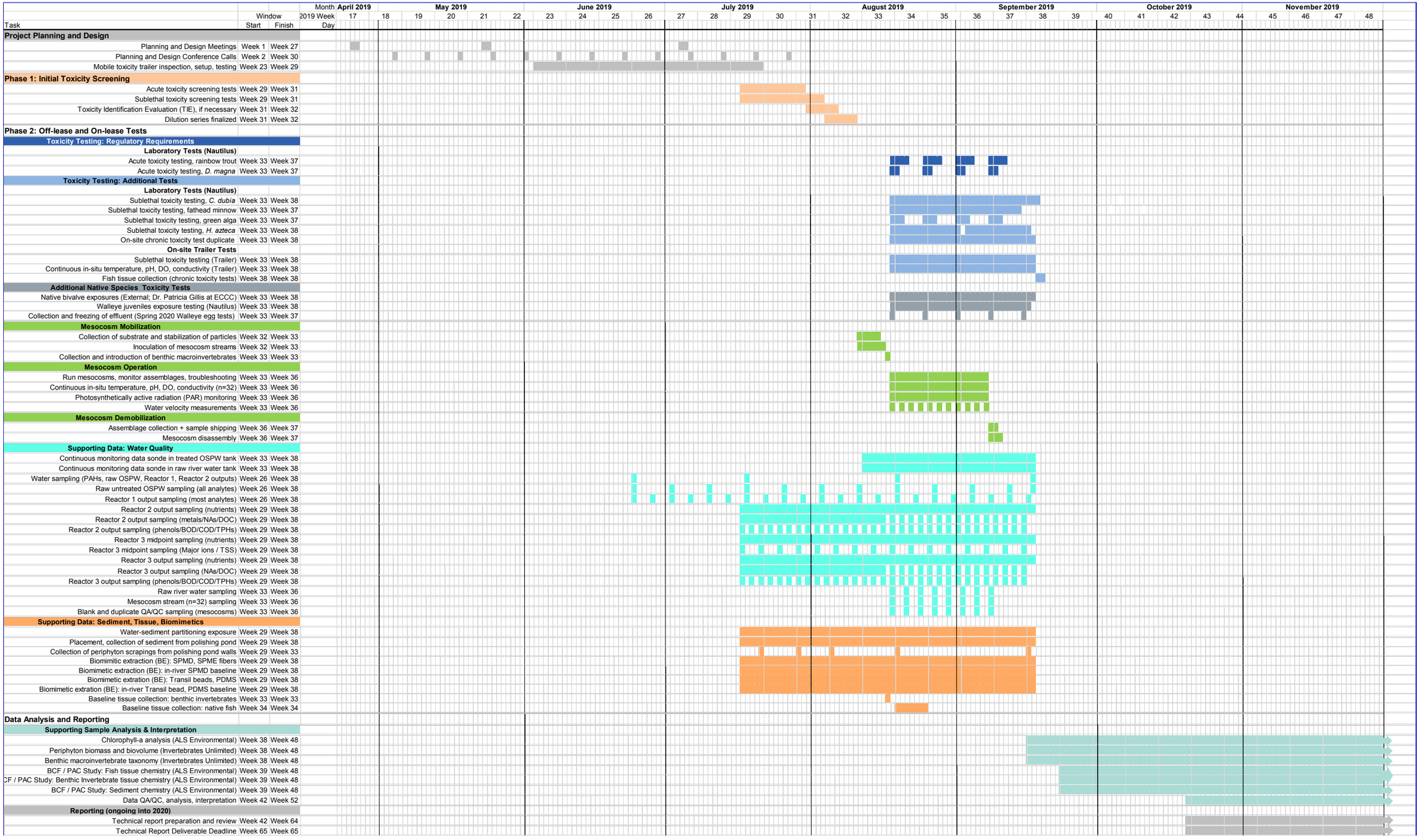
Figure 5 Gantt chart showing specific milestones related to the implementation of the 2019 phases of the treated-OSPW aquatic toxicity study.



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APPENDICES

Appendix A1

Toxicity Test Summaries

A1.1 SUMMARY TABLES – NAUTILUS LABORATORY TESTS

Table A1.1 Summary of test conditions: 96-h Rainbow Trout (*Oncorhynchus mykiss*) survival test.

Test species	*Oncorhynchus mykiss*
Organism source	Fish hatchery
Organism age	Juvenile
Test type	Static
Test duration	96 hours
Test vessel	20-L glass aquaria
Test volume	10-20 L depending on size of fish
Test concentrations	Five concentrations (100, 50, 25, 12.5 and 6.25%), plus laboratory and site water control
Test replicates	1 per treatment
Number of organisms	10 per replicate
Control water	Dechlorinated City of Calgary tap water
Test solution renewal	None
Test temperature	15 ± 1°C
Feeding	None
Light intensity	100 to 500 lux
Photoperiod	16 hours light/8 hours dark
Aeration	6.5 ±1 mL/min/L
Test Measurements	pH, conductivity, dissolved oxygen and temperature were measured at test initiation and test completion; salinity measured at test initiation; evaluated for survival daily
Test protocol	Environment Canada (2000a), EPS 1/RM/13, with 2007 &2016 amendments
Statistical software	CETIS
Test endpoints	96-hour LC50
Test acceptability criterion for controls	Survival ≥ 90%
Reference toxicant	Potassium chloride (KCl)

Table A1.2 Summary of test conditions: 48-h zooplankton (*Daphnia magna*) survival test.

Test species	*Daphnia magna*
Organism source	In-house culture
Organism age	<24 hours
Test type	Static
Test duration	48 hours
Test vessel	385 mL plastic vessels
Test volume	150 mL
Test concentrations	Five concentrations (100, 50, 25, 12.5 and 6.25%), plus laboratory and site water controls
Test replicates	1 per treatment
Number of organisms	10 per replicate
Control water	Dechlorinated City of Calgary tap water amended with 4 mg/L KCl and with B12 (2 µg/L) and Na_2SeO_4 (2 µg Se/L)
Test solution renewal	None
Test temperature	20 ± 2°C
Feeding	None
Light intensity	400 to 800 lux
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test measurements	pH, conductivity, dissolved oxygen and temperature measured at test initiation and completion; salinity and hardness measured at test initiation in undiluted sample; evaluated daily for survival
Test protocol	Environment Canada (2000b), EPS 1/RM/14 with 2016 amendments
Statistical software	CETIS
Test endpoints	48-h LC50
Test acceptability criterion for controls	Survival ≥ 90%
Reference toxicant	Sodium chloride (NaCl)

Table A1.3 Summary of test conditions: zooplankton (*Ceriodaphnia dubia*) survival and reproduction test.

Test species	*Ceriodaphnia dubia*
Organism source	In-house culture
Organism age	<24 hour old neonates, produced within a 12-hour window
Test type	Static-renewal
Test duration	6 – 8 days
Test vessel	16 x 135 mm glass test tube
Test volume	15 mL
Test concentrations	Seven concentrations (100, 50, 25, 12.5 and 6.25, 3.13, 1.56%), plus laboratory and site water controls
10 per treatment	10 per treatment
Number of organisms	1 per replicate
Control water	20% Perrier water and 80% deionized water supplemented with vitamin B12 (2µg/L) and Na_2SeO_4 (5µg Se/L)
Test solution renewal	Daily (100% renewal)
Test temperature	25 ± 1°C
Feeding	Daily with *Pseudokirchneriella subcapitata* and YCT (3:1 ratio)
Light intensity	100 to 600 lux at water surface
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test measurements	pH, conductivity, dissolved oxygen and temperature measured daily; evaluated for survival and reproduction daily
Test protocol	Environment Canada (2007a), EPS 1/RM/21
Statistical software	CETIS
Test endpoints	Survival and reproduction
Test acceptability criteria for controls	≥80% survival; ≥15 young per surviving control producing three broods; ≥60% of controls producing three or more broods, no ephippia present
Reference toxicant	Sodium chloride (NaCl)

Table A1.4 Summary of test conditions: Fathead Minnow (*Pimephales promelas*) survival and growth test.

Test species	*Pimephales promelas*
Organism source	Aquatox Inc., Hot Springs, Arkansas
Organism age	<24 hours post hatch
Test type	Static-renewal
Test duration	7 days
Test vessel	385 mL plastic containers
Test volume	250 mL
Test concentrations	Seven concentrations (100, 50, 25, 12.5 and 6.25, 3.13, 1.56%), plus laboratory and site water controls
Test replicates	4 per treatment
Number of organisms	10 per replicate
Control water	Dechlorinated City of Calgary tap water amended with 4 mg/L KCl
Test solution renewal	Daily (80% renewal)
Test temperature	25 ± 1°C
Feeding	Twice each day with approximately 1500-2250 newly hatched brine shrimp *(Artemia nauplii)* per 10 fish.
Light intensity	100 to 500 lux
Photoperiod	16 hours light / 8 hours dark
Aeration	None
Test measurements	pH, conductivity, dissolved oxygen and temperature were measured daily; evaluated for survival daily
Test protocol	Environment Canada (2011), EPS 1/RM/22
Statistical software	CETIS
Test endpoints	Survival and biomass
Test acceptability criteria for controls	≥80% survival, ≥0.25 mg mean dry weight
Reference toxicant	Sodium chloride (NaCl)

Table A1.5 Summary of test conditions: Green algae (*Pseudokirchneriella subcapitata*) growth inhibition test.

Test species	*Pseudokirchneriella subcapitata*, strain UTCC# 37
Organism source	In-house axenic culture, obtained from Canadian Phycological Culture Centre
Organism age	3-to 7-day old culture in logarithmic growth phase
Test type	Static
Test duration	72 hours
Test vessel	Microplate
Test volume	220 µL
Test concentrations	Seven concentrations (100, 50, 25, 12.5 and 6.25, 3.13, 1.56%), plus laboratory and site water control
Test replicates	5 per treatment, at least 3 enumerated; 8 for control
Number of organisms	10,000 +/- 1000 cells/mL
Control water	85% deionized water; 15% City of Calgary dechlorinated tap water supplemented with nutrients
Test solution renewal	None
Test temperature	24 ± 2°C
Feeding	None
Light intensity	3600 to 4400 lux
Photoperiod	24 hours light
Aeration	None
Test measurements	pH of 91% and control at test initiation and test completion; light levels and temperature measured daily
Test protocol	Environment Canada (2007b), EPS 1/RM/25
Statistical software	CETIS
Test endpoints	Algal cell growth inhibition
Test acceptability criteria for controls	>16-fold increase in number of algal cells; CV ≤ 20%; no trend when analyzed using Mann-Kendall test
Reference toxicant	Zinc (added as $ZnSO_4$)

Table A1.6 Summary of test conditions: Amphipod crustacean (*Hyalella azteca*) survival and growth test.

Test organism	*Hyalella azteca*
Test organism age	2 - 9 days old, and produced within a 3 day period
Test type	Static-renewal
Test duration	14 days
Test vessel	375 mL glass container with a 5 cm disc of Nitex for substrate
Test volume	275 mL
Test concentrations	Seven concentrations (100, 50, 25, 12.5 and 6.25, 3.13, 1.56%), plus site water and laboratory controls
Test replicates	5
Number of organisms	10 per replicate
Control water	SAM-5S water (Environment Canada 2017) containing 73 mg/L Cl and 0.02 mg/L Br.
Test solution renewal	3 times per week
Test temperature	23 ± 1°C
Feeding	6.3 mg of YCT three times per week
Light intensity	500 to 1000 lux at water surface
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test protocol	Environment Canada (2017); EPA 1/RM/33
Test endpoint	Survival, dry weight
Test acceptability criteria for controls	Mean control survival of ≥80% survival; dry weight of at least 0.1 mg per amphipod
Reference toxicant	Copper

Table A1.7 Summary of test conditions: Fathead Minnow embryo-larval test.

Test organism	*Pimephales promelas*
Test organism age	<24-h old fertilized eggs
Test type	Static-renewal
Test duration	~ 32 days (28 days post-hatch)
Test chamber	1-L glass jars
Test solution volume	1 L
Test concentrations	Six concentrations (100, 32, 10, 3.2, 1.0, 0.32%), plus site water controls
Number of replicates	4
Control water	Site water
Test solution renewal	Daily (~80%)
Test temperature	~25°C
Number of organisms/chamber	15
Feeding	Twice daily, with *Artemia* nauplii
Light intensity	100 to 600 lux
Photoperiod	16 hours light/8 hours dark
Aeration	None unless required to maintain DO >50% saturation
Test protocol	USEPA (1996); ASTM (2013)
Test endpoints	Survival, hatch, growth, deformities
Test acceptability criterion for controls	>66% hatch; ≥70% post-hatch survival

Table A1.8 Summary of test conditions: Fathead Minnow juvenile-adult test.

Test organism	*Pimephales promelas*
Test organism age	60 days post hatch
Test type	Static-renewal
Test duration	28 days
Test chamber	6-L glass aquaria
Test solution volume	4 L
Test concentrations	Six concentrations (100, 32, 10, 3.2, 1.0, 0.32%), plus site water controls
Number of replicates	4
Control water	Site water
Test solution renewal	Daily (~80%)
Test temperature	~25°C
Number of organisms/chamber	10
Feeding	Twice daily, with *Artemia* nauplii
Light intensity	100 to 600 lux
Photoperiod	16 hours light/8 hours dark
Aeration	Continuous gentle aeration
Test protocol	Adapted from ASTM (2013)
Test endpoints	Survival, length, growth, body condition factor
Test acceptability criterion for controls	≥70% survival

A1.2 NON-STANDARD TEST DESCRIPTIONS

Evaluation of the toxicity of treated oil sands process water using early life stage freshwater mussels

PL Gillis, Environment and Climate Change Canada, Burlington, ON. June 2018

Study Design and Methods

The toxicity of treated oil sands process water (OSPW) will be assessed using two freshwater mussel early life stages. The glochidia (larvae) exposure will be an acute (48 h) static exposure and the juvenile mussel exposure will be a sub-chronic (48 d) static exposure with water renewals. Both tests will be conducted with serial dilutions of treated OSPW.

Glochidia

Glochidia will be wild-sourced from gravid freshwater mussels collected from either Ontario's Maitland River (*Lampsilis siliquoidea*, fatmucket) or the Speed River (*Lampsilis fasciola*, wavy-rayed lampmussel). The species ultimately used in the exposures will depend on the viability of glochidia at the time exposures are initiated. Because *L. siliquoidea* are found in northern Alberta, they are the first choice for this study. However, *L. siliquoidea* in ON rivers are only gravid in early spring, and the gravid *L. siliquoidea* collected in anticipation of this study (May 2018) may no longer have glochidia that meet the American Society for Testing Materials (ASTM) required pre-test 90% viability at the time the OSPW is to be tested (ASTM, 2013). The viability of spring-collected *L. siliquoidea* held in Environment and Climate Change Canada's (ECCC) Aquatic Life Research Facility (ALRF) using flow through conditions (12^{o}C) typically declines to 60-85% between September and October. Therefore as a backup for the likely outcome of unacceptable *L. siliquoidea* glochidia viability in September, wild mussels from the same genus (*L. fasciola*) that are gravid in late summer (with glochidia viability >90%) will be collected and used in this study if required. The required (2018) ON Ministry of Natural Resources and Forestry permit for collecting and holding mussels has been obtained.

Glochidia Exposure Details:

- The experiment will use 6 or 7 concentrations of treated OSPW, 2 or 3 concentrations of a reference toxicant (sodium chloride, NaCl) and 2 control treatments (a river water and a reconstituted water control).
- Glochidia are removed from gravid females as per ATM (2013). The viability (ability to close valves) of each mussel's glochidia are determined prior to pooling glochidia from three to five mussels which meet the minimum 90% viability. Glochidia are acclimated to the 21^{o}C test temperature (gravid mussel holding temperature, 12-14^{o}C) over 2-3 hours. To begin the test, 500-1000 glochidia from the pooled sample will be added to each (replicate) test vessel (250 mL glass beakers) each containing 100 mL of the appropriate test solution. Four replicates will be run for each treatment and the control will be conducted with 6 replicates.
- Basic water quality (dissolved oxygen, pH, conductivity, ammonia) will determined with benchtop meters at initiation of the exposure (t=0).

- After 24 and 48 hours of exposure approximately 100 glochidia from each test vessel will be removed and (destructively) assessed for viability (surrogate for survival). All open and closed glochidia will be enumerated before and after the addition of a saturated salt (NaCl) solution as per ASTM (2013).
- Samples of the exposure water will be prepared as required (i.e., filtered, preserved) and submitted to the National Laboratory for Environmental Testing (NLET) for analysis the exposure water.
- Exposure vessels will not be aerated or fed during the exposure. Vessels are held at 21^{o}C at a photoperiod of 16 h light: 8 h dark.

Juvenile Mussels

Laboratory-cultured, juvenile *L. siliquoidea* will be purchased from Professor Chris Barnhart at Missouri State University. Cultured juvenile mussels can be used in toxicity exposures at various ages; each with its own advantages and disadvantages. ECCC's mussel ecotoxicology lab has most frequently used 6-8 month old mussels (length 0.5 to 1 cm) in sub-chronic (28 d) exposures and have outlined below an exposure that will employ these 'older' juvenile mussels. However we have also conducted some studies with the younger (typically referred to as 'newly released') < 2 wk old (length ~0.5 mm) mussels and this proposal can be changed to use this life stage if desired. One of the advantages of 'older' juvenile mussels is that they are easier to work with (i.e., including locating after exposure) and because of this survival can be assessed at the mid-point as well as the end of an exposure therefore there is less risk if the test fails between days 14 and 28. Also, in addition to survival which is assessed for both ages, in tests employing older mussels a behavioral endpoint can be employed (@ 14 and 28 d) to assess the exposed mussel's ability to bury in sand within 24 h (burial ability). The key disadvantages of older juvenile mussels is that they are typically less sensitive to waterborne contaminants than younger life stages, growth alterations are not as evident after a 28 day exposure, and the supplier requires a longer lead time to produce the organisms.

Juvenile Exposure Details:

- The experiment will use 6 or 7 concentrations of treated OSPW, 2 or 3 reference toxicant (NaCl) concentrations, and 2 control treatments.
- Juvenile mussels will be assessed for activity (foot movement, siphoning) prior to use in exposures. Ten mussels will be added to each replicate vessel (1 L glass beakers) each containing 800 mL of the appropriate test solution and about 1 cm of clean aquarium sand. Four replicates will be run for each treatment and the control will be conducted with 6 replicates.
- Basic water quality (DO, pH, conductivity, ammonia) will determined with benchtop meters at initiation of the exposure, at days 7, 14, 21, and upon test completion (28 d).
- After 7 and 21 days of exposure 80% of the exposure solution will be replaced with fresh solution. A vessel change (clean beaker with fresh solution and sand) will occur after 14 days of exposure.
- Samples of the exposure water at test initiation, and on days 14 (before and after vessel change) and 28 will be prepared as required (i.e. filtered, preserved) and submitted to NLET for analysis (dissolved organic carbon, metals, major cations and anions, alkalinity, hardness, ammonia (Note: no organics)) of the exposure water.

- Exposure vessels will be aerated and mussels will be fed according to established (twice daily weekdays, and daily weekend days). Vessels are held at 21°C at a photoperiod of 16 h light: 8 h dark in an environmental chamber.
- After 14 and 28 days of exposure the surviving mussels in each replicate vessel will be transferred to fresh vessels and any attempt to bury in clean sand will be recorded. Survival of mussels will be assessed at vessel change (day 14) and after 28 days of exposure (prior to the burial assessment).

Endpoints

Glochidia:

- Viability (ASTM accepted surrogate for survival) at 24 and 48 hours of exposure
- Exposure water quality including dilution water characterization

Juvenile Mussels:

- Survival after 14 and 28 days of exposure
- Burial ability (surviving mussels) after 14 and 28 days of exposure
- Exposure water quality including dilution water characterization
- If desired, bioaccumulation in composite samples of exposed mussels can be assessed, however addition funds (not budgeted here) or external analysis will be required for this endpoint.

Possible Risks

- The locally (AB) relevant species, *L. siliquoidea* was collected from an Ontario river in May 2018, but based on previous experience, there is a good chance that glochidia viability of those mussels will not meet minimum pre-test requirement in Sept/Oct, therefore gravid mussels of an alternate species of the same genus, *L. fasciola* will be collected in Sept. 2018 as a backup for the OSPW exposures.
- There is a risk that adequate numbers of *L. fasciola* will not be available in Sept 2018 and therefore the required numbers of glochidia will not be available for testing. This risk is considered minimal as this has not happened in the past 5 years.
- There is a risk that the climate controlled and flow through conditions required by the gravid mussels will fail and this will cause the gravid mussels to release glochidia prior to use in exposures. ECCC's ALRF is monitored 24/7 by an experienced facility manger to prevent this from happening and there are backup/emergency power supplies in place, but there is still the potential for catastrophic power failure of all systems that could lead to the loss of test organisms.
- There is a good chance that the supplier of juvenile mussels will not be given enough lead time to produce the mussels for the OSPW exposures (Sept/Oct 2018) and the tests will need to be delayed until the mussels can be grown to desired age/size. This can be avoided if the supplier happens to have an adequate supply of 1-cm'ish *L. siliquoidea* on hand for purchase. Note: It is very important to inform supplier (Dr. Barnhart) as soon as possible if this project is going ahead to avoid or reduce the length of a delay.

- There is a chance that there may be any range of unforeseen problems (e.g., facility problems, host fish deaths) in the juvenile mussel supplier's lab and therefore juvenile mussels would not be available for purchase. This is a risk as there is no alternate supplier that could produce juvenile mussels of this species within the time frame of this project. In the last 10 years there have been no major problems obtaining test organisms, but we have experienced some delays until adequate size and numbers of mussels became available.

Timeline

Month 1 (and prior)

- Collect (May 2018) gravid *L. siliquoidea* and maintain in the ALRF.
- Pre-order juvenile mussels from supplier (mussels are 6-8 months old at test initiation).
- Hire and train new personnel.
- Lab supplies and husbandry consumables ordered.
- Prepare (ALRF and brokerage) for arrival of juvenile mussels and additional gravid mussels.
- Collect gravid *L. fasciola* (Sept 2018) and maintain in the ALRF.

Month 2

- Plan timelines and prepare for glochidia and juvenile mussel exposures.
- Initiation of glochidia and juvenile exposures. Note: timing of the juvenile test will ultimately be dependent upon availability/arrival of juvenile mussels.
- Completion of glochidia exposure, viability assessments and post-test cleaning.
- Prepare and submit water samples from glochidia test for NLET analysis.
- Compile glochidia benchtop water quality data.

Month 3

- Continuation of juvenile mussel exposure, assess survival and burial ability at test-mid point (14 d).
- Water sampling, submit water samples for NLET analysis (throughout and end of exposure).
- Completion of juvenile mussel exposure, post-test survival and burial assessment at take down (28 d) and post-test cleaning.
- Compile benchtop water quality data.

Month 4

- Compile NLET-derived water quality data.
- Data analysis and report writing.

Reference: ASTM, 2013. Standard Guide for Conducting Toxicity Tests with Freshwater Mussels. E2455-06. ASTM International, West Conshohocken, PA.

Evaluation of the toxicity of treated oil sands process water using early life stage walleye

J. C. Raine, Toxicology Centre, University of Saskatchewan. May 15, 2018

Design of the study

- Walleye eggs and milt will be obtained from either Lake Diefenbaker or the Qu'Appelle River in the spring of 2019. We will partner with the Saskatchewan Ministry of the Environment who collect walleye and other fish species each year from one of these Saskatchewan water bodies for the provincial stocking program. By collaborating with the Saskatchewan Ministry of the Environment to collect eggs, we will dramatically reduce costs associated with collection of the wild fish.
- Walleye eggs will be fertilized, transferred to the University of Saskatchewan and placed in the exposure.
- The experiment will use 5 concentrations of the treated OSPW, 2 concentrations of OSPW prior to treatment (if available) and 2 control treatments.
- 150 walleye eggs will be allocated to each of 4 replicate egg containment vessels within 1 litre beakers for each treatment. Use of these vessels previously likely contributed to very high survival of walleye, which are notoriously difficult to rear in the lab.
- Walleye spawn at water temperatures of approximately 8°C and therefore the toxicity test would begin at this same temperature. In a previous walleye exposure, we gradually increased water temperature during the exposure by 2 °C each week to simulate the natural increase in summer water temperature and to provide the optimal temperature suggested in aquaculture for walleye rearing.
- A photoperiod of 16 h light: 8 h dark would be used to simulate summer light conditions.
- The exposure would run until the swim up stage of development, approximately 1 month. This stage of development is a significant bottle neck for rearing walleye and mortality is generally very high. Larvae would be fed brine shrimp approximately 1 week after hatch to ensure food is available when exogenous feeding begins.
- The exposure would be monitored daily for mortalities, deformities and hatched larvae.
- Additional walleye eggs will be reared separately under culture conditions to provide additional back up animals as a safety net
- Analytical measurement of treatment solution components will be required. If this could be supplied by the committee or a collaborator it would reduce the cost associated with sending it to a commercial lab.

Concurrent Exposure

- To provide a direct comparison under similar conditions, a concurrent exposure using early life stage fathead minnows would also be run. A stock of fathead minnows is maintained in the Aquatic Toxicology Research Facility and eggs would be readily available for this experiment.
- Methods would change slightly to reflect the different species and holding conditions.

Endpoints

- Survival
- Hatching success and time to hatch
- Time to eye up stage
- Time to swim up stage and proportion swimming up
- Percentage developmental abnormalities and types of developmental abnormalities
- Larval length and weight
- Swim bladder inflation
- If desired, additional histological, biochemical or molecular endpoints could be included

Possible Risks

Walleye spawning is affected by many environmental factors and this can make it difficult to obtain eggs in a given year. Northern pike (and several other species) spawn at the same time as walleye and could potentially be substituted if ripe walleye are not obtainable.

Emergency generator, dechlorinated water storage and redundant water treatment and delivery systems will mitigate most potentially catastrophic issues that could affect the exposures.

Timeline

Month 1

- Hire and train new personnel, U of S regulatory courses completed
- Set up of culture and exposure systems, water temperature and conditions stabilized.
- Egg containment vessels made
- Lab supplies and husbandry consumables ordered

Month 2

- Field collections will occur over at least 2 weeks. Fish have to be captured daily and checked for ripe eggs/milt
- Coordination of fathead minnow and walleye exposure start times
- Initiation of exposures

Month 3

- Continuation of exposures
- Sampling, exposure take down and cleaning
- Initiation of sample analysis

Month 4

- Continuation of sample analysis
- Data analysis and report writing

BIOAVAILABILITY AND BIOACCUMULATION OF ORGANICS FROM COKE TREATED OSPW

Steve Wiseman, PhD. Associate professor, Department of Biological Sciences, University of Lethbridge

Markus Brinkmann, PhD. Assistant Professor, School of Environment and Sustainability, University Saskatchewan

BACKGROUND

Bioaccumulation of OSPW Organics

Zhang et al. (2016) demonstrated that several heteroatom classes of dissolved organics from Base Mine Lake OSPSW have the potential to bioaccumulate. In a bioaccumulation/depuration study with Japanese medaka, it was found that SO^+, NO^+, O_2^- heteroatoms bioaccumulate. In the same study, using TRANSIL^{XL} beads*, species of SO^+, NO^+, O_2^+, O^+, O_2^-, and SO_2^- heteroatom classes were found to have potential to bioaccumulate.

We propose to use TRANSIL^{XL} beads to identify whether bioaccumulative heteroatom classes are present in OSPW that has been treated with Coke. Samples of OSPW should be collected before, during, and after treatment, and should represent multiple treatment times.

Bioavailability of OSPW Organics

Uptake and effects of ionizable organic chemicals (IOCs) in fish can significantly differ as a function of pH. We have studied the pH-dependent permeation of chemicals from oil sands process-affected water (OSPW) using a rapid in vitro screening assay based on the permanent rainbow trout gill cell line RTgill-W1. Cells were cultured in transwell tissue-culture inserts, and the movement of chemicals from the apical chamber (corresponding to ambient water with varying pH) to the basal chamber (corresponding to the fish circulatory system) was quantified Orbitrap mass spectrometer operated in ESI^- and ESI^+ mode. We observed a significant pH-dependency of chemical permeability across the epithelium, with lesser pHs generally leading to greater permeability. These results were in agreement with bioconcentration data from a 96 h static renewal exposure of juvenile rainbow trout. Additional data suggest that active transport of some compounds present in OSPW might contribute to their uptake across the fish gill.

We propose to repeat these experiments at various ambient pHs that are representative of the Athabasca river to determine whether organics present in discharged OSPW might be bioavailable to fishes.

Supplemental Information

Supplemental Information on responses of embryo-larval walleye to oil sands tailings pond sediments is provided by:

Raine, J.C., Turcotte, D., Tumber, V., Peru, K.M., Wang, Z., Yang, C., Headley, J.V., Parrott, J.L. 2017. The effect of oil sands tailings pond sediments on embryo-larval walleye (Sander vitreus). Environmental Pollution 229: 798-809.

Supplemental information on the use of the target lipid model to characterize the relative sensitivity and modes of toxic action of organic acids and to validate the biomimetic extraction method for application to organic acids in oil sands process affected water application of the be method to organic acids is provided by:

Redman, A.D., Parkerton, T.F., Butler, J.D., Letinski, D.L., Frank, R.A., Hewitt, L.M., Bartlett, A.J., Gillis, P.L., Marentette, J.R., Parrott, J.L., Hughes, S.A., Guest, R., Bekele, A., Zhang, K., Morandi, G., Wiseman, S., Giesy, J.P. 2018. Application of the target lipid model and passive samplers to characterize the toxicity of bioavailable organics in oil sands process-affected water. Environmental Science and Technology 52: 8039-8049