Fisheries and Oceans Canada | Pêches et Océans Canada
Ecosystems and Oceans Science | Sciences des écosystèmes et des océans

National Capital Region

Canadian Science Advisory Secretariat
Science Advisory Report 2017/053

A FRAMEWORK FOR THE DEVELOPMENT OF STANDARD METHODS TO EVALUATE THE TOXICITY OF PETROLEUM HYDROCARBONS ON AQUATIC ORGANISMS

Figure 1. The six administrative regions of Fisheries and Oceans Canada's (DFO). The dashed line indicated Canada's Economic Exclusive Zone (EEZ).

Context

With the expansion of oil and gas resource development and transport in Canadian waters, there is an increased potential for aquatic contamination resulting from both routine operations and accidental releases. Given that petroleum hydrocarbons can induce deleterious effects on aquatic biota, it is necessary to develop a framework for standard toxicity tests that will allow for more reproducible results and more valuable comparisons both across test species and the various types of crude oil and refined products (including diluted bitumen) transported within Canada. Comparison of results across toxicological studies is currently hampered by the acceptance of variance within standard procedures used by various researchers to account for differences in the physical –chemical properties of the test material and the application of end-points desired. Factors to consider in a revised standard method for the evaluation of toxic effects in aquatic species to petroleum hydrocarbons, including heavy oils and refined products such as diluted bitumen are described. Consideration of these factors in the development and application of an updated Standard Method for Petroleum Hydrocarbons would enhance the level of comparison of between toxicity results over a wider range of petroleum hydrocarbons, and enable the collection of more environmentally relevant data.

This Science Advisory Report is from the national peer-review on Towards the Development of Toxicity Standard Methods to Evaluate Biological Effects of Heavy Oils on Aquatic Ecosystems, held in Ottawa, from January 31 to February 2, 2017. Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule *as they become available.*

SUMMARY

- This Science Advisory Report (SAR) provides a proposed framework on standard methods to evaluate the toxicity of petroleum on aquatic organisms to produce more comparable results, improve the consistency between studies, and collect more relevant data.
- There is increasing oil and gas resource development and transportation of petroleum products in Canada. This increases the potential for aquatic contamination in more diverse receiving environments. Therefore, there is a requirement for oil toxicity testing to support decision-making and spill preparedness and response.
- Guidance document for preparing test media to assess oil toxicity in sea water (Chemical Response to Oil Spill Ecological Research Forum methods, referred to as CROSERF) exists but has not been updated since 2005. It was not developed for heavy oils or use in freshwater.
- A literature review identified a diversity of toxicity test methods, a lack of detailed reporting of methods, and an inconsistent use of terminology to describe test solutions, all of which hindered comparisons of toxicity among oils, fish species, and environmental conditions.
- CROSERF was often modified to suit specific study objectives without providing sufficient details and rationales for the changes reported. CROSERF recommendations were considered inappropriate for specific research questions or environmental scenarios.
- The proposed framework improves guidance on experimental designs, physical and chemical characterization of test oils, and preparation of physically- and chemically-dispersed oil, characterizing test solutions, toxicity testing and reporting requirements. This will improve transferability, reproducibility and comparability of toxicology data generated by multiple practitioners with diverse expertise.
- Five (5) priority research needs have been identified to further refine the proposed framework.
- The results of this effort will improve comparability and quality of the data to support decision-making related to potential impacts and mitigation of oil spills on aquatic environments.
- This framework will be strengthened by the review of a broader audience including all stakeholders involved in order to reach consensus for global standard.

BACKGROUND

There is increasing oil and gas resource production and development in Canada, a wider array of production and transportation modes for crude oil, emerging petroleum products (e.g. diluted bitumen) and greater volumes of shipped products. These trends have generated concern about the potential impacts on fish and fisheries of spills to marine and freshwater environments.

Case studies (Lee et al. 2015) have shown that the toxicity of petroleum hydrocarbons is affected by differences in species sensitivity, as well as the physico-chemical characteristics and behaviour of the specific contaminant in question, which influences its nature of the exposure (i.e., whether petroleum is dispersed into droplets, coats surfaces underwater, or simply floats on the surface of water) and to differences in sensitivity of the wide-array of marine

and freshwater species that might be exposed. Thus, for diluted bitumen, toxicity maybe quite variable, due to differences in sensitivity of the marine and freshwater species be exposed, and variations in the chemical composition of the oil-gas condensates and or synthetic oils used as diluents, as well as the composition of the bitumen produced during extraction. Measuring toxicity requires the preparation of gradients of petroleum concentrations in water. Published methods have included mechanical and chemical dispersions of oil in water (including mechanical mixing [e.g., Nordtug et al. 2011], addition of chemical dispersants [e.g., Singer et al. 2000], as well as desorption of hydrocarbons from solid substrates [e.g., Marty et al. 1997]). Furthermore, rapid weathering of diluted bitumen during the preparation of test solutions has created a challenge to toxicity test protocols currently used, due to variance in the concentration and composition of toxicants within the test solutions over time. The diversity of protocols for the generation of "standard test" solutions and weathering rates of petroleum hydrocarbons has prevented detailed comparisons between toxicity tests.

The CROSERF (Chemical Response to Oil Spills: Ecological Effects Research Forum**,** Aurand and Coelho, 2005) method, developed in the United States with Canadian participation, is commonly used to evaluate the aquatic toxicity of crude oils. A "standard" method was included for the preparation of a Water Accommodated Fraction (WAF) and a Chemically-Enhanced Water Accommodated Fraction (CEWAF) of oil. Application of this protocol reduced inter-laboratory variability, and provided a means to simulate more 'realistic' exposure conditions in which waterborne hydrocarbons concentrations declined following a single dosing of test solutions. The "method" hasn't been updated since 2005 and while it has been used to evaluate the toxicity of heavy oils and refined products (including diluted bitumen), the original protocol was neither envisioned for this application nor for tests in freshwater.

A recent review of research on dispersants, oil, and dispersed oil toxicity (Adams et al. 2017) demonstrated that many studies did not follow CROSERF test recommendations, as they were often considered inappropriate by the researchers to meet their specific study objectives or specific environmental scenarios (e.g. rapid weathering of diluted bitumen). While the CROSERF guidance document outlined detailed methods for preparing WAF and CEWAF of oil, they were often modified without providing sufficient details and rationales for the modifications reported. As well, the properties of test solutions were rarely measured, limiting the comparability of results among labs, among oils, and among species (Singer et al. 2000, 2001). Comparisons were further confounded by the use of new terms, or modifiers of commonly used terms, to describe the same solutions or methods (see Appendix 1, Glossary). Given the complexity of oil and the number of factors in the design of toxicity tests, variation among toxicity test results is not surprising (Singer et al. 2001).

For chemical and toxicological experiments with oils to be more readily comparable among tests and laboratories, more consistent upon repetition, and useful for environmental risk and impact assessments across a wider range of oil types, there is a need to develop standard reference methods for preparing, analyzing, and testing of oil WAF and CEWAF. The method should be applicable to most, if not all, petroleum products. In addition to measuring the aquatic toxicity of 'fresh' petroleum products, the method should also include tests of 'weathered' products to better understand the toxicological implications of weathering over hours, days, and weeks post-spill.

Standardized methods are needed to establish the inherent toxicity of oil, in addition to site-specific tests to assess the influence of the unique environmental conditions at each spill on the

exposure of aquatic organisms to oil and on oil toxicity. As with the original CROSERF recommendations (Aurand and Coelho, 2005), the aim of standardizing toxicity test methods is to minimize the variance in results caused by the method itself; in this regard, variations in toxicity between tests can then be attributed to the different treatments (e.g. concentrations/species).

In consideration of the above, a framework is proposed to develop an updated Toxicity Standard Method to evaluate the toxicity of a wider range of petroleum products on aquatic organisms that is more reproducible, enhances the level of comparison between toxicity studies results, and provides more environmentally relevant data. As the use of WAF/CEWAF formulations remain relevant to the exposure of petroleum hydrocarbons spilled within the aquatic environment, the recommended framework is built upon revision of CROSERF protocols which provided guidelines for toxicity testing, including the preparation of test solutions.

ANALYSIS

Methods for Preparing Test Solutions

In the literature review (Adams et al., 2017), the papers on the standardization of toxicity test protocols by Singer et al. (2000) and Anderson et al. (1974) were identified as the most highly cited for preparation for the preparation of test solutions. Adams et al. (2017) noted that although many publications cited the CROSERF methods (Aurand and Coelho, 2005), most did not report enough detail to enable replication of their studies. While it can be assumed that the CROSERF method was followed as prescribed for most studies, there are a number for which modifications were not justified nor explained.

The toxicity of oil in water depends on the concentrations of hydrocarbons in solution, which is dependent on the amount, size distribution and stability of suspended oil droplets. The importance of the mixing method is the extent to which it promotes the partitioning of hydrocarbons from the oil phase to the dissolved phase. It is where the hydrocarbons are bioavailable to test fish and where it is influenced by the type and efficacy of mixing procedures used. High energy dissipation rate mixing protocols provide higher concentrations of small droplets with high surface-to-volume ratios that remain suspended within the test solution for a longer time and less likely to re-coalesce). As a result, toxicity is more closely related to the measured concentrations of hydrocarbons in test solutions, not the nominal concentrations of dispersed oil added to a test solution (Schein et al. 2009; Wu et al. 2012; Gardiner et al. 2013; Adams et al. 2014b; Martin et al. 2014).

Diluent

The use of fresh water versus sea water as a diluent will affect the behaviour of oils in solution, particularly with regard to the density and buoyancy of droplets. Salinity also affects the accumulation and toxicity of hydrocarbons in aquatic organisms because Polycyclic Aromatic Hydrocarbons (PAHs), the constituents of oil known to cause chronic toxicity, are less soluble in salt water compared to freshwater. CROSERF provides guidance for the type and quality of diluent water for toxicity tests (Aurand and Coelho 2005). The specification of diluent water as sea water indicates that CROSERF protocols were designed for marine species and applications, particularly in response to large volume marine oil spills. Freshwater tests may require modification for optimal results. Furthermore, oil spills in aquatic environments could

also involve aquatic systems that are turbid due, among others, to high levels of primary productivity (algal cells in water) or suspended inorganic particulates due to erosion. Few publications reported suspended solids in diluent water, primarily because most use laboratory supplies of filtered water.

Oil-to-Water Ratio

The mixing volumes of oil and water to prepare WAF are commonly reported as oil-to-water ratios (OWR). The composition and concentration of the test solution is a function of the amount and relative proportions of hydrocarbons in the oil and the unique partitioning rates of each component of oil into water. In the literature reviewed, OWRs ranged from 1:106 667 to 1:1. Given the influence of OWR on the chemical composition of test solutions, it should be included in all test reports.

Chemical Dispersion: Dispersant-to-Oil Ratio

The dispersant-to-oil ratio (DOR) is a ratio of the volume of dispersant applied and the volume of oil in the mixing vessel used for the preparation of CEWAF. It is important to choose an appropriate DOR as concentrations that are too high may lead to surplus dispersant in the water and can be acutely toxic not representing actual conditions encountered in response operations. Concentrations that are too low may reduce dispersant effectiveness. Modification of the DOR can change the interfacial tension of the oil; the highest dispersion occurs with the maximum reduction in interfacial tension (Khelifa et al. 2007). If dispersants are applied to mimic dispersant use at real spill sites, DOR should be consistent with the recommended application rate provided by the manufacturer. The maximum ratio used at spill sites is 1:20, a ratio based on spill response compliance with application rates published on dispersant product labels (ASTM STP 1282). In that context, a DOR of 1:1 would be considered unrealistic and would not be used in a spill response protocol. As they are highly viscous, and thus not likely to be treated with dispersants following a spill, there are no publications to date reporting an optimal DOR or mixing energy needed to generate chemically-dispersed solutions of diluted bitumen for toxicity testing.

The potential for synergism between dispersant and dispersed oil on toxicity has been the topic of debate and highlights the importance of proper controls in oil toxicity testing. In addition to positive controls to ensure toxic effects and negative controls to assess the health of unexposed test organisms, controls for any solvents or methods to enhance the mixing of oil and water should be included. The potential toxicity of the dispersant in the mixture can be estimated by measuring dispersant concentrations in CEWAF stock solutions to compare with an exposure-response dataset of the dispersant alone. However, it is unknown whether existing methods can distinguish the ‘free dispersant’ from dispersant associated with oil droplets in CEWAF samples.

Methods to Characterize Oil and Test Solutions

Quantative detailed analysis of the oil in test solutions will yield information on the actual concentrations of individual hydrocarbons that test organisms were exposed to (Harris et al. 2014). It is essential to characterize test solutions by measuring the concentration of each oil component in test solutions and the parent oil stock to account for weathering. Because most hydrocarbons are hydrophobic, it is essential to measure temporal changes in hydrocarbon concentration, over specified time periods for static or renewal protocols, and over the entire

duration of the test. For studies comparing WAF and CEWAF toxicity, measured concentrations are also needed to support discussions regarding the use of dispersants (Clark et al. 2001).

The main flaw of reporting only nominal loading concentrations is the assumption that the protocol used will result in the same concentration of oil into the WAF or CEWAF stock solution as was delivered in any other independent study where concentrations were measured (or that will be compared). As discussed above, this assumption is highly problematic even when the same oil is used, owing to the many factors that influence oil dispersion into WAF or CEWAF, including weathering factors that cannot be rigorously reproduced. Furthermore, whether it's WAF or CEWAF, oil droplet size would affect availability and the chemical analysis would not be able to differentiate between big and small droplets.

Because oils are such complex mixtures, it is not possible to identify and assign every compound present in crude oils to a designated group (e. g. TPH definitions, Bejarano et al. (2013)). However, with recent improvements in both capability and accessibility of GC-MS analysis, the increased number of resolved analytes and their concentrations could be considered in the interpretation of the collected data. Table 1 specifies a recommended list of analytes to be analyzed.

Table 1: Recommended minimum target analytes list for volatile and semi-volatile analyses by GC-FID or GC-MS to facilitate inter-laboratory comparisons of results (based on Adams et al. (2017).

Analytical method	Minimum Analytes List	
Volatile analysis	Saturates	
	• Pentane • 2-methylpentane • Hexane • Cyclopentane • 2,4-diemthylpentane	• Cyclohexane • Heptane • Cycloheptane • Octane • Nonane
	Unsaturates	
	• Benzene • Toluene • Ethylbenzene • p-xylene • m-xylene	• o-xylene • n-propylbenzene • Cumene • other C_3-benzenes
Semi-volatile analysis (optional)	• Naphthalenes (C_0-C_4) • Biphenyl • Dibenzofuran • Acenaphthylene • Acenaphthene • Fluorenes (C_0-C_3) • Anthracene • Phenanthrene • Phenanthrenes (C_1-C_4) / Anthracenes (C_1-C_4) • Dibenzothiophenes (C_0-C_4)	• Naphthobenzothiophenes (C_0-C_4) • Benzo (a) anthracene • Chrysene + Triphenylene • Chrysenes (C_1-C_4) • Benzo(b)fluoranthene • Benzo (j+k) fluoranthene • Benzo(a)fluoranthene • Benzo(e)pyrene • Benzo (a) pyrene • Perylene

Analytical method	Minimum Analytes List	
	• Benzo(b)fluorene • Fluoranthene • Pyrene • Pyrenes (C_1-C_4)/ Fluoranthenes (C_1-C_4)	• Indeno (1,2,3-cd) pyrene • Dibenzo (a, h) anthracene • Benzo (g, h, i) perylene

[1] Unsaturates TPAH51 list adapted from TPAH50 list from Forth et al. (2016) plus perylene from Aurand and Coelho (2005).

Exposure Regime and Outcome

Species, life stage and duration of exposure of test organisms

While CROSERF recommended the use of temperate species, recent oil exploration and development in the Arctic has expanded the array of relevant test species and test conditions. Consequently, the species selected for standard toxicity tests should be those that are locally available, locally relevant, or specified in standardized toxicity test methods (e.g. Environment Canada 1998).

Sensitivity to oil exposure varies with the life stage of the test organism; the early developmental stages of fish tend to be most sensitive (McIntosh et al. 2010; Binder and Stegeman 1983). Juvenile and adult fish are typically used when the purpose of the test is to assess acute lethality (≤ 4 days) or for tests assessing effects on physiological responses. Chronic tests (>4 days) are typically conducted with embryos to assess sublethal effects. Delayed effects that are expressed beyond the period of the oil exposure could also be considered when developing an experimental design.

Exposure Regime

Test organisms can be exposed to oil solutions with different exposure regimes: static, semi-static, pulse, or continuous flow. The choice of exposure regime is critical in determining the outcome of toxicity tests and their utility. For tests in which there are time-varying concentrations of oil in test solutions, the interactions among time, concentration, and extent of effects should be assessed with statistically based models to determine if there are consistent and predictable relationships that would improve comparisons among test conditions and among test labs. Designs of tests with time-varying concentrations of oil should be guided by observed temporal trends of oil concentrations at sites of actual oil spills. No matter which toxicity test method is selected, chemical characterization of test solutions is essential to describe the change in the composition and concentration of oil over time, particularly for unweathered products.

Effects Measured and Endpoints

The relationship between the magnitude of toxic effects and exposure to oil is used to calculate specific endpoint parameters that can be used to compare results between studies. Depending on the experimental design, lethal and sublethal effects can be measured during exposure, directly after exposure, or at time point(s) following transfer to clean water (i.e., as prolonged or delayed effects). Endpoints may be measured throughout the exposure duration or require sampling or analysis of the organisms at a specific exposure time or stage of development stated above already. The endpoints are expressed as the effect measured after a fixed duration of exposure at a specific concentration.

Effects can also be reported at specific effects levels. The “No Observable Effects Concentration” (NOEC) and “Lowest Observable Effects Concentration” (LOEC) were identified as the most commonly used endpoints in ecotoxicology by Harris et al. (2014), but they have been widely criticized because of potential bias caused by uneven spacing of measured exposure concentrations and high sensitivity to variance at the threshold of toxicity.

For oil toxicity tests, the Median Lethal Concentration (LC50) and Median Effective Concentration (EC50, sublethal effects) were the most common end-points reported. Sublethal effects were assessed most frequently with fish embryos and included, among others, malformations, stunted development, reduced swimming ability, blue sac disease (BSD), altered gene expression, and cardiovascular defects. Fish presenting the most severe effects are less expected to survive to adulthood.

Recommended Framework

CROSERF methods provide guidance for preparing test media to assess oil toxicity in sea water, but were not developed to assess the toxicity of heavy oils or to be used for freshwater exposures. Furthermore, they have not been updated since 2005.

This Science Advisory Report provides a proposed framework for methods to evaluate the toxicity of petroleum to aquatic organisms to produce more comparable results, improve the consistency among studies, collect more relevant data and take into account freshwater environments and the non-temperate species (e.g. Arctic species).

The proposed framework provides guidance on experimental designs, preparation of physically- and chemically-dispersed oil, physical and chemical characterization of test oils and test solutions, toxicity testing, and reporting requirements. This will improve the transferability, reproducibility and comparability of toxicity data generated by multiple practitioners with diverse areas and levels of expertise.

The proposed framework references the CROSERF protocols for toxicity testing, highlights widely accepted changes to the original methods, including changes recommended by Adams et al. (2017), and provides specific advice for tests with diluted bitumen products.

CROSERF aimed to develop standardized test methods while at the same time providing test scenarios that were more ‘realistic’ models of a marine spill. CROSERF methods have been described as useful in comparing oil toxicity but limited in their representation of real world environmental scenarios (Bejarano et al. 2014; Coelho et al. 2013). This is particularly the case given the new reality of constant and prolonged discharges of oil from deep sea well blow-outs (2010 Deepwater Horizon Spill), including the prolonged application of dispersants and the widespread distribution of deep water plumes of dissolved and particulate oil (Beyer et al. 2016). This scenario is of obvious concern in Canada as frontier offshore oil and gas development is anticipated to move into deeper waters off the shelf in both the Arctic and the East Coast. Other emerging scenarios include spills of oil and diluted bitumen to freshwater and Arctic ecosystems.

The proposed framework is not meant to be “realistic” because it is impossible for lab tests to include every combination of conditions encountered at different spill sites. Not all species, exposure regimes/duration, and environmental conditions can be assessed and not all test methods and measurements are practical. Careful consideration of the time, personnel, and cost for parameters recommended is required in the development of test protocols. There is a

trade-off between the amount of information required by regulators and the quality of research that can be conducted within time and budget constraints. Therefore protocol recommendations should be flexible to allow for tests of different environmental conditions typical of site-specific spills.

In response to these issues, the proposed framework of recommended test methods summarizes the CROSERF protocols for marine oil toxicity tests with WAF and CEWAF and suggests amendments to respond to testing issues (Table 2). The summary makes a distinction between acute and chronic toxicity tests, and is divided into sections corresponding to the requirements for:

- Explicit statements of the objectives of the test
- Experimental design
- Toxicity testing
- Physically and chemically characterizing the test oil
- Chemically characterizing test solutions
- Measuring responses and calculating endpoints; and
- Guidance on reporting the results.

For each method, criteria are presented that should be considered when testing any oil product. Nevertheless, test methods should be sufficiently flexible to allow their application to site-specific conditions or to oils with unique characteristics, particularly if tests are backed-up by sufficient chemical analyses to understand the relationships between exposure and effect common to all oils. Many of the recommendations for test methods in Table 2 are unchanged from original CROSERF guidelines (Aurand and Coelho 2005).

In Table 2, where changes have been made, or there is a change in emphasis, [the text is blue and in square brackets], and the rationale for these changes is explained by Adams et al. (2017). These modifications also apply to other petroleum products with extreme or unique characteristics, such as heavy fuel oil or Bakken light crude.

This framework will allow for some flexibility (e.g., test species), but will set basic standards or minimum requirements (i.e., source oil, DOR, analytes, data forms, endpoints) for inter-laboratory calibration and validation of the framework.

Table 2: A proposed framework for toxicity tests of physically- and chemically-dispersed oil to fish to support regulatory assessments and comparisons of toxicity among oils, species, and test conditions, based on the revision of the original CROSERF protocol.[1]

1. **Experimental Design**

	Nature of test solution	
	Physically-dispersed (WAF) [2]	**Chemically-dispersed (marine, CEWAF)** [2]
1.1. Test species, life stage	• [If possible, ECCC, EPA, OECD standard test species and life stages or commonly used 'model' species] • [If not, justify]	
1.2. Concentration range	• Encompass range from non-toxic to toxic to ensure that end-points can be calculated	
1.3. Exposure time	• Acute – [Follow standard test protocol (See 1.1), typically 96 h] • Chronic – [Follow standard test protocol (See 1.1)] • [If end-points are needed for shorter exposure times (e.g., 12 h LC50s), make observations of responses at short intervals (e.g., 1,2,4,8,16 h from start)]	
1.4. Exposure regime options	• Acceptable test regimes include: static non-renewal (SNR), static renewal (SR), and continuous-flow to recognize the objectives of the test.	
1.5. Water type/quality	• [Fresh water to] marine, characterized by chemical analysis • Fresh water: characterize [pH, alkalinity, conductivity, hardness, TOC] • Salt water and brackish water: characterize [salinity or conductivity, pH, TOC]	
1.6. Oil state	• Weathered or unweathered oil	• Dispersed weathered or unweathered oil
1.7. Controls	• Water only • [Positive controls are recommended, where appropriate] • [For example, when testing a previously untested oil, include a well-tested oil as a reference toxicant (e.g., Prudhoe Bay Crude Oil (PBCO), Alberta Sweet Mixed Blend (ASMB))]	• Water only • [Positive controls are recommended, where appropriate] • [For example, when testing a previously untested oil, include a well-tested oil as a reference toxicant (e.g., PBCO, ASMB)] • [Dispersant control (e.g., Nujol CEWAF at same OWR and DOR as oil test)]

2. Preparing Test solutions

	Nature of test solution	
	Physically-dispersed (WAF)	**Chemically-dispersed (marine, CEWAF)**
2.1. Oil and dispersant storage and handling	• Fresh stocks of oil should be thoroughly mixed in a closed container and stored in tightly sealed glass or metal containers with minimal headspace. [Refer to ASTM D4057 for volatile petroleum handling recommendations.] • [Oil should be characterized physically and chemically when received, when first used if stored longer than 1 month, and at the end of experiments if they last for more than 1 month.] • [Aliquot fresh oil into volumes needed for each days' use, and store in sealed containers with minimum head-space at 4º C; re-mix if stored longer than 1 month.] • [Store as briefly as possible before testing and open bottles as infrequently as possible] • [The same procedures should be used for fresh stocks of dispersant, but without physical or chemical characterization.]	
2.2. Oil weathering	• [ASTM Method D2892 or D86 methods can be used to artificially weather oils, but temperatures above 130˚ C must not be used to avoid chemically changing the oil (including dehydration of cycloalkanes and breaking of chemical bonds). Under vacuum distillation conditions (ASTM Method D1160), maximum recommended temperature is 200˚ C.] • [Weather to a constant weight or target loss, not to exceed 48 h.]	
2.3. Dilution water	• Use the same water source for holding and acclimating stocks of fish, for preparing WAF/CEWAF, and for preparing test solutions • [Fresh water: characterize pH, alkalinity, conductivity, hardness, TOC] • Salt water and brackish water: characterize [salinity or conductivity, pH, TOC] Salt water tests: • Minimum filtration 0.45 μm • Local sea water is recommended; dilute with deionized water as needed to achieve a target salinity. • Reconstituted sea water is acceptable if it supports good survival and health of test organisms Fresh water tests: • [Minimum filtration 0.45 μm if derived from an untreated surface water] • [For municipal water sources, treat to reduce total chlorine to <10 μg/L]	

	Nature of test solution	
	Physically-dispersed (WAF)	**Chemically-dispersed (marine, CEWAF)**
2.4. Dissolving oil in water	• Batch: Stirring, mixing • [Continuous-flow: pumps, oil desorption columns]	• Batch: Stirring, mixing with chemical dispersants • [Continuous-flow: pumps]
2.5. Oil-water ratios (OWR)	• [Acute and chronic toxicity tests: 25 g/L or sufficient oil that concentrations of oil in WAF and CEWAF are not limited by the amount of oil added, as indicated by residual floating oil after mixing, and by chemical analysis of stock solutions.]	
2.6. Mixing temperature	• [Mix at room temperature (20-22˚ C)]	
2.7. Mixing light	• Mixing should be carried out in darkness or in [low UV] laboratory fluorescent lighting	
2.8. Addition of oil	• [Add known quantity of oil at the centre of the water surface during mixing] • Calculate delivery mass by weight difference	
2.9. Mixing vessel size	• [No restrictions]	
2.10. Headspace	• [Acute toxicity tests: 20-25% headspace in a sealed mixing vessel] • [Chronic toxicity tests: open mixing vessel]	
2.11. Mixing energy	• When using a magnetic stir bar the amount of energy applied is judged solely by the depth of the mixing vortex • [For both WAF and CEWAF, adjust stirring to give a mixing vortex equivalent to 20-25% of water depth (Clark et al. 2001)] • [Mixing methods with higher energy and chemical dispersion may be beneficial when mixing oils with higher viscosity] • [For HEWAF and HECEWAF, use vortex mixer and sonication to make a thorough mix of oil and water] • Conduct a pre-test on oil to determine how the oil and dispersant should be added to the water, and whether oil-in-water emulsions form. If the latter, reduce the mixing energy or try a different OWR • [When available, use standard protocol/apparatus with known energy dissipation rate]	
2.12. Mixing duration	• Should be [18 h to suit the 24 h cycle of daily solution preparation and to avoid bias by microbial growth]	• Total stirring time should be 18 h to [suit the 24 h cycle of daily solution preparation] and to avoid bias by microbial growth. [Add dispersant immediately after oil vortex is established.]

	Nature of test solution	
	Physically-dispersed (WAF)	**Chemically-dispersed (marine, CEWAF)**
2.13. Dispersant- oil ratio (DOR)	• N/A	• [1:20]
2.14. Addition of dispersant	• N/A	• Add known quantity of dispersant to oil at the centre of vortex after a vortex has been established • Calculate delivery mass by weight difference • Do not pre-mix oil and dispersant
2.15. Settling times	• [Settling for 6 h prior to extraction of aqueous layer to allow resurfacing of oil droplets] • [Use immediately – do not store prior to use]	
2.16. Dilution gradients	• Prepare gradients with variable dilution of WAF or CEWAF stock solutions, [or by preparing a series of oil desorption columns with gradients of oil concentrations in gravel, or by variable dilutions of a continuous flow of oil-contaminated water produced by oil-pumping and mixing systems] • Serial dilutions of a stock solution are not recommended because of a potential bias in the relative proportions of hydrocarbons with different water solubilities	
2.17. Storage of test solutions	• [Do not store test solutions]	

3. Toxicity Testing

	Nature of test solution	
	Physically-dispersed (WAF)	**Chemically-dispersed (marine, CEWAF)**
3.1. Test species	• Guidelines for fish culture and handling should follow those published by one of ECCC, ASTM, OECD, EPA, etc., and local animal care protocols • [When fish are fed, daily solution renewals should immediately follow feeding, preferably by transferring the fish to a fresh test solution in a clean tank. The previous tank should be thoroughly cleaned to remove debris and organic films that absorb hydrocarbons or harbour bacteria that can create metabolites of hydrocarbons] • Remove dead animals daily to avoid accumulation of biological oxygen demand (bacteria) and organic matter that could absorb hydrocarbons]	

	Nature of test solution	
	Physically-dispersed (WAF)	**Chemically-dispersed (marine, CEWAF)**
3.2. Biomass loading	• [Follow the standard protocol for each test organism]	
3.3. Test solution volume and container size	• [Follow the standard protocol for each test organism]	
3.4. Test container material	• [All glass (seamless, i.e., no aquaria with plate glass assembled with silicone seal) or stainless steel] • [Avoid plastics, with the exception of Teflon]	
3.5. Aeration	• [Acute - Aeration of acute lethality tests should be minimized to reduce volatilization and weathering of compounds causing acute narcosis, but sufficient to sustain > 60% saturation (EPA 850.1075)] • [Chronic - Low rates of aeration sufficient to maintain oxygen concentrations within the optimal range for each test species]	
3.6. Test containers	• Acute - Closed container to retain volatiles (covered but not air-tight seal) • [Chronic - Open container]	
3.7. Endpoints	• Lethality (i.e., 96 h LC50) • “Initial effect” narcosis/moribundity (0.5 to 1 h EC50) • [Sublethal toxicity (EC50; e.g., embryo toxicity, growth, survival, behaviour, reproduction, molecular, physiological responses)]	

4. Oil Characterization

	Nature of test solution	
	Physically-dispersed (WAF)	**Chemically-dispersed (marine, CEWAF)**
4.1. Chemical composition	• [Saturates, aromatics, resins, and asphaltenes (SARA)] • [VOCs including BTEX, TPH (CCME fractions) (CCME 2001)] • [PAH and TPAH (See minimum analytes list in Table 1)] • [If possible, characterize oil hydrocarbons by high-temperature simulated distillation (e.g. ASTM D7169)]	
4.2. Physical characteristics	• [Viscosity, density]	

	Nature of test solution	
	Physically-dispersed (WAF)	**Chemically-dispersed (marine, CEWAF)**
4.3. Storage and Handling	• Store in tightly sealed glass or metal containers with minimal headspace • Open containers as infrequently as possible (See Section 2.1, Oil and dispersant storage and handling) • Store in darkness at [4˚ C] • Recommended chemical characterization of stored oil to monitor changes in composition over time	

5. Characterizing test solutions

	Nature of test solution	
	Physically-dispersed (WAF)	**Chemically-dispersed (marine, CEWAF)**
5.1. Sampling, Storage and Handling	• [Water samples for hydrocarbon analyses must be collected from fresh WAF and CEWAF stock solutions prior to toxicity testing.] • [Water samples for hydrocarbon analyses should be collected from each test solution at the start and end of a toxicity test (preferably more frequently), to compare measured concentrations of oil to nominal dilutions.] • [The highest concentrations of test solutions should be sampled frequently (N≥3 at reasonable intervals throughout the test) to describe temporal trends of oil concentrations and to calculate mean concentrations with a measure of variance.] • [Pooling should be considered where test solution volumes are restricted.] • [For static, or static daily renewal exposures, the highest concentration should be sampled repeatedly over a 24-hour cycle of solution renewals to describe the time-varying exposure regime.] • [If samples are analyzed externally by a qualified oil chemistry lab (recommended), consult with the lab in advance about sampling, pooling sample volume, bottles, preservatives, storage conditions, storage time, shipping, and selection of internal standards and recovery standards.] • [Samples should be processed (e.g. extracted) at the laboratory within 14 days of collection, and analyzed within 14 days of processing. Samples should be held at 4 ^{0} C during shipping and storage.]	
5.2. TPH (C_{10} – C_{36})	• [Analyze TPH from CCME fractions (CCME 2001)] • Do not correct for analytical recovery • Detection limit of at least 10 ppb for any specific n-alkane that is in the standard	

	Nature of test solution	
	Physically-dispersed (WAF)	**Chemically-dispersed (marine, CEWAF)**
5.3. VOC including BTEX (C_6 < C_{10})	• [See Table 1 for list of minimum target analytes] • [VOCs should be measured following the USEPA method EPA SW-846]	
5.4. Semi-volatiles and PAHs	• [See Table 1 for a minimum list of PAH, including alkylated homologs] • [TPAH as defined as the sum of PAH from the recommended list] • Do not correct for analytical recovery • Detection limit of at least 10 ppb for any specific PAH that is in the standard	
5.5. Rapid oil analysis (e.g., fluorescence and UV detection)	• [Methods for semi-quantitative rapid analyses can supplement other quantitative analytical methods for repeated characterizations of WAF, CEWAF and test solutions provided that a sub-set of parallel samples are analyzed by the quantitative methods.] • [Perform calibration with standard solutions as available, using the same matrix as the test solutions.]	

6. Measuring Responses, calculating end-points

	Nature of test solution	
	Physically-dispersed (WAF)	**Chemically-dispersed (marine, CEWAF)**
6.1. Statistics	• [Must include statistical analysis of data, including variability (e.g., LC50s and EC50s including confidence limits by statistical tests appropriate for binomial or continuous data)] • [Express endpoints in terms of measured TPH and TPAH, or other analytes as appropriate]	

7. Guidelines for Reporting; where methods deviate from the standard, these changes should be reported (See Appendix 4 in Adams et al. (2017) for additional guidance)

	Nature of test solution	
	Physically- dispersed (WAF)	**Chemically- dispersed (marine, CEWAF)**
7.1. Objectives of the test	• [Explicitly state the objectives of the test]	

	Nature of test solution	
	Physically- dispersed (WAF)	**Chemically- dispersed (marine, CEWAF)**
7.2. Rationale and details for the experimental design	• [Explain how the experimental design addresses the test objectives] • [Identify all controls and a rationale for including each] • [Provide details on the ranges of test concentrations, the intended pattern of exposure (e.g., constant or declining concentrations), responses measured, and sample sizes] • [Identify assumptions inherent in methods applied for testing toxicity and characterizing test solutions, and discuss the importance of any violations of these assumptions]	
7.3. Rationale and details for methods	• [Outline all methods for preparing test solutions, toxicity testing, chemical analyses of oil and test solutions, data manipulation, and statistical analyses] • [Provide a citation to any published protocols that were followed, identify any deviations from the published protocol, and provide a rationale for any changes] • [Provide sufficient details of the test methods that the experiment can be replicated by another laboratory]	
7.4. Characteristics of the test oil	• [Results of physical and chemical characterization of the oil when received, when tested, and when testing was complete, with an emphasis on any loss of volatiles due to weathering, concentrations of low molecular weight compounds (<500) for acute lethality tests, and TPH and TPAH concentrations for chronic toxicity tests] • [For artificially weathered oils, the maximum temperature of the method used should be reported]	
7.5. Characteristics of test solutions	• [Report the sampling design] • [Compare measured concentrations (with sample size and a measure of variance) to nominal concentrations of oil] • [Report other water quality characteristics, including average (variance, *N*) of salinity, alkalinity, temperature, pH, oxygen]	

	Nature of test solution	
	Physically- dispersed (WAF)	**Chemically- dispersed (marine, CEWAF)**
7.6. Variability in test solution concentration	• [Measured oil concentrations over the duration of the experiment, including changes in concentrations over the intervals between solution renewals for SR protocols. Graph the decay rate of measured concentrations in test solutions over 24 h, and over the course of the entire experiment, with a measure of variance and *N*] • [For acute lethality tests at T=0 and at the end of the test, report measures of volatiles and low molecular weight compounds (<500) to demonstrate the extent of losses due to weathering] • [For chronic toxicity tests, report the average (variance, *N*) of measured concentrations of oil over time of the highest, mid-level and control concentrations] • [Compare measured concentrations to nominal dilutions]	
7.7. Characteristics of test organisms	• [Source, age, and life stage of test organisms and whether they were from an aquaculture facility or an in-house culture] • [Range and average weight of test organisms and biomass loading rate in test solutions (i.e., g biomass/L of test solution)] • [Mortality rates of the stock population in the week prior to testing] • [Mortality rates in each oil concentration, including controls, throughout the test] • [Any unusual behaviour or evidence of stress or poor health]	
7.8. Statistics	• [Report statistical methods for estimating LC50, EC50, or other toxicity data] • [Show all results using measured concentrations of hydrocarbons as the metric of exposure] • [Include sample size and a measure of variance (preferably 95% confidence limits)] • [Note and justify any data manipulations, including identification and removal of outliers and calculation of ratios, and note any potential for statistical bias]	

1 [Where changes have been made to the CROSERF methods, or there is a change in emphasis, the text is blue and in square brackets.] The rationale for these changes is explained in Adams et al. (2017). If the properties of test oils (e.g., viscosity) might interact with test methods (e.g., oil dispersion) and affect the efficiency of mixing or the outcome of toxicity tests, preliminary experiments are recommended to indicate whether changes to methods are needed to avoid bias. It is also recommended that these changes be described in detail and justified.

2 WAF refers to the water accommodated fraction of oil produced by low, medium, or high energy mixing of oil and water, modified from the CROSERF methods. Similarly, CEWAF refers to chemically-enhanced WAF produced by low, medium or high energy mixing.

Sources of Uncertainty

Multiple modifications to the CROSERF method have been reported since it was first published. Because the method has not been updated since 2005, these modifications have made comparisons among studies difficult, and contributed to inconsistencies in data analyses. The proposed framework for oil toxicity tests will reduce the uncertainties and inconsistencies in toxicity tests, and improve the understanding of the effects of heavy oils on aquatic ecosystems.

CONCLUSIONS

This Science Advisory Report proposes a framework to improve the guidance on experimental designs of oil toxicity tests, preparation of physically- and chemically-dispersed oil, physical and chemical characterization of test oils and test solutions, toxicity testing, and reporting requirements. While reducing the sources of uncertainties, the framework will improve transferability, reproducibility and comparability of toxicology data generated by multiple practitioners with diverse expertise.

The results of this effort will improve comparability and quality of the data to support decision-making related to potential impacts and mitigation of oil spills on aquatic environments.

This framework will be strengthened by engaging a broader audience of stakeholders to reach consensus on a global standard.

OTHER CONSIDERATIONS

Priority Research Needs

While recent reports on oil pollution have identified research needs related to understanding the fate, behaviour and effects of oil in Canada's aquatic ecosystems, particularly in fresh and Arctic waters (Lee et al. 2015; NAS 2016), this review identified research needs focused on methods for oil toxicity testing and assessment of factors affecting the outcomes of toxicity tests.

The following five (5) priority research needs have been identified to further refine the proposed framework:

- Experimental assessment of the effect of i) mixing vessel headspace, ii) oil to water ratio, and iii) test volume on the chemical composition of test solutions to improve correlations with toxicity;
- Developing routine methods (or systems) for determining mixing energy or provide a protocol to produce test solutions over a range of known energy dissipation rates;
- Identify reference standard compounds and reference oils that can be used to standardize test methods;
- Develop rapid analytical methods for chemical characterization of test oils and oil solutions; and
- Develop standard methods for SARA analysis for oil characterization.

Path moving forward

- Given that the original CROSERF process was consensus-based, involving practitioners from industry, government and academia, including round-robin testing among participating laboratories, a similar process should be followed to ensure that any recommendations will meet the needs of those who use the methods. Key international players, including the original CROSERF members, and lead scientific and technical representatives from industry, academia and government, commercial laboratories and regulators, should be engaged in the development of a revised standard method for the evaluation of biological effects of petroleum hydrocarbons on aquatic ecosystems that includes heavy oils and commercial products such as diluted bitumen.

SOURCES OF INFORMATION

This Science Advisory Report summarizes the proceedings of a workshop titled Towards the Development of Toxicity Standard Methods to Evaluate Biological Effects of Heavy Oils on Aquatic Ecosystems. The workshop was held in Ottawa, Ontario, January 31 to February 2, 2017, as part of the National Science Advisory Process. Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

Adams, J., Bornstein, J.M., Munno, K., Hollebone, B., King, R.T., Brown, S., and Hodson, P.V. 2014. Identification of compounds in heavy fuel oil that are chronically toxic to rainbow trout embryos by effects-driven chemical fractionation. Environ. Toxicol. Chem. 33: 825–835.

Adams, J., Charbonneau, K., Tuori, D., Brown, R.S. and Hodson, P.V. 2017. Review of Methods for Measuring the Toxicity to Aquatic Organisms of the Water Accommodated Fraction (WAF) and Chemically-Enhanced Water Accommodated Fraction (CEWAF) of petroleum. DFO Can. Sci. Advis. Sec. Res. Doc. 2017/9316. x + 108 p.

Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E., and Hightower, G.M. 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. 27: 75–88.

Aurand, D. and Coelho, G. 2005. Cooperative aquatic toxicity testing of dispersed oil and the Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF). Ecosystem Management & Associates, Inc. Technical Report. 105 p.

Bejarano, A.C., Levine, E., and Mearns, A.J. 2013. Effectiveness and potential ecological effects of offshore surface dispersant use during the Deepwater Horizon Oil Spill: A retrospective analysis of monitoring data. Environ. Monit. Assess. 185: 10281–10295.

Bejarano, A.C., Clark, J.R., and Coelho, G.M. 2014. Issues and challenges with oil toxicity data and implications for their use in decision-making: A quantitative review. Environ. Toxicol. Chem. 33: 732–742.

Beyer, J., Trannum, H.C., Bakke, T., Hodson, P.V, and Collier, T. 2016. Environmental effects of the Deepwater Horizon oil spill: a review. Marine Poll. Bull. 110:28–51.

Binder, R.L. and J.J. Stegeman. 1983. Basal levels and induction of hepatic aryl hydrocarbon hydroxylase activity during the embryonic period of development in brook trout. Biochem. Pharmacol. 32: 1324–1327.

CCME. 2001. Reference Method for the Canada-Wide Standard for Petroleum Hydrocarbons in Soil – Tier 1 Method. Canadian Council of Ministers of the Environment, Winnipeg, MN. (accessed February 27, 2017).

Clark, J.R., Bragin, G.E., Febbo, E.J., and Letinski, D.J. 2001. Toxicity of physically and chemically dispersed oils under continuous and environmentally realistic exposure conditions : Applicability to dispersant use decisions in spill response planning. *In* International Oil Spill Conference Proceedings: 1249–55.

Coelho, G., Clark, J., and Aurand, D. 2013. Toxicity testing of dispersed oil requires adherence to standardized protocols to assess potential real-world effects. Environ. Pollut. 177: 185–188.

Environment Canada (EC). 1998. Biological test method; Toxicity tests using early life stages of salmonid fish (rainbow trout) EPS1/RM/28 Second Edition. Method Development and Applications Section (MDAS), Biological Methods Division, Environment Canada, Ottawa, ON.

Forth, H. P., Mitchelmore, C. L., Morris, J. M., & Lipton, J. 2016. Characterization of oil and water accommodated fractions used to conduct aquatic toxicity testing in support of the Deepwater Horizon Oil Spill Natural Resource Damage Assessment. Environ Toxicol Chem. doi.org/10.1002/etc.3672.

Gardiner, W.W., Word, J.Q., Word, J.D., Perkins, R.A., McFarlin, K.M., Hester, B.W., Word, L.S., & Ray, C.M. 2013. The acute toxicity of chemically and physically dispersed crude oil to key Arctic species under Arctic conditions during the open-water season. Environ. Toxicol. Chem. 32 (10): 2284–2300.

Harris, C.A., Scott, A.P., Johnson, A.C., Panter, G.H., Sheahan, D., Roberts, M., and Sumpter, J.P. 2014. Principles of sound ecotoxicology. Ecotox. Environ. Sci. Tech. 48: 3100–3111.

Khelifa, A., Fingas, M., Hollebone, B.P., and Brown, C.E. 2007. Effects of chemical dispersants on oil physical properties and dispersion. *In* 13th Arctic and Marine Oil Spill Program Technical Seminar (AMOP), Environment Canada, Ottawa, Ontario, 105–116.

Lee, K., Boufadel, M., Chen, B., Foght, J., Hodson, P.V., Swanson, S., Venosa, A. 2015. Expert panel report on the behaviour and environmental impacts of crude oil released into aqueous environments. Royal Society of Canada, Ottawa, ON. ISBN: 978-1—928140-02-3

Martin, J.D., Adams, J., Hollebone, B.P., King, T., Brown, R.S., Hodson, P.V. 2014. Chronic toxicity of heavy fuel oils to fish embryos using multiple exposure scenarios. Environ. Toxicol. Chem. 33: 677–687.

Marty, G.D., Heintz, R.A., and Hinton, D.E. 1997. Histology and teratology of pink salmon larvae near the time of emergence from gravel substrate in the laboratory. Can. J. Zool. 75: 978–988.

McIntosh, S., King, T., Wu, D., and Hodson, P.V. 2010. Toxicity of dispersed weathered crude oil to early life stages of Atlantic herring (*Clupea harengus*). Environ. Toxicol. Chem. 29: 1160–1167.

Nordtug, T., Olsen, A.J., Altin, D., Meier, S., Overrein, I., Hansen, B.H., and Johansen, O. 2011. Method for generating parameterized ecotoxicity data of dispersed oil for use in environmental modelling. Mar. Pollut. Bull. 62: 2106–2113.

Schein, A., Scott, J.A., Mos, L., and Hodson, P.V. 2009. Oil dispersion increases the apparent bioavailability and toxicity of diesel to rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 28: 595–602.

Singer, M.M., Aurand, D., Bragin, G.E., Clark, J.R., Coelho, G.M., Sowby, M.L., and Tjeerdema, R.S. 2000. Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. Mar. Pollut. Bull. 40: 1007–1016.

Singer, M.M., Aurand, D.V., Coelho, G.M., Bragin, G.E., Clark, J.R., Sowby, M., and Tjeerdema, R.S. 2001. Making, measuring, and using water-accommodated fractions of petroleum for toxicity testing. *In* International Oil Spill Conference 2001 2: 1269–1274.

Wu, D., Wang, Z., Hollebone, B., McIntosh, S., King, T., and Hodson, P.V. 2012. Comparative toxicity of four chemically dispersed and undispersed crude oils to rainbow trout embryos. Environ. Toxicol. Chem. 31: 754–765.

APPENDIX 1—GLOSSARY

Word	Definition
ANSC	Alaska North Slope crude oil
CEWAF	Chemically-Enhanced Water Accommodated Fraction. A solution of hydrocarbons and a suspension of oil droplets created when a chemical dispersant is added to oil and water with stirring.
Continuous flow systems	Refers to a constant exposure in which the organisms are placed in full-strength test solution in a flow-through test chamber. The pumps are turned on and the chambers are pumped with a constant concentration test solution (i.e., no dilution of the test solution occurs). When performing this type of exposure, care must be taken to assure that components of the test solution are not being lost through the tubing walls.
Dispersant	A chemical or mixture of chemicals applied, for example, to an oil spill to disperse the oil phase into small droplets in the water phase.
Fracking	Also known as hydraulic fracturing; an unconventional method for recovering liquid petroleum from shale oil deposits that otherwise would not release the gas and/or fluid in the reservoir.
HECEWAF	High Energy Chemically-Enhanced Water Accommodated Fraction.

Word	Definition
	A solution of hydrocarbons and a suspension of oil droplets created when oil and water are mixed by high energy agitation.
HEWAF	High Energy Water Accommodated Fraction
LC50	LC stands for "Lethal Concentration", referring to the concentration of a chemical that kills 50% of the test animals during the observation period.
LOEC	Lowest observable effect concentration
MESA	Medium South American crude oil
NOEC	No observable effects concentration
PAH	Polycyclic Aromatic Hydrocarbons
PBCO	Prudhoe Bay crude oil
Pulse exposure	Refers to an exposure where organisms are placed in a closed, static chamber with no head space above the test solution. The organisms are left in the solution for a certain period of time, then removed and placed in clean water.
SARA	Saturates, aromatics, resins, and asphaltenes
TPAH	Total Polycyclic Aromatic Hydrocarbons
WAF	Water-accommodated fraction of oil (WAF): hydrocarbons that will partition from oil to water during gentle stirring or mixing; may contain droplets, in contrast to water-soluble fractions.
Weathering	A suite of changes in spilled oil composition and properties brought about by a variety of environmental processes including spreading, evaporation, photo oxidation, dissolution, emulsification and biodegradation, among others

THIS REPORT IS AVAILABLE FROM THE:

Canadian Science Advisory Secretariat (CSAS)
National Capital Region
Fisheries and Oceans Canada
200 Kent Street
Ottawa, ON K1A 0E6

Telephone: 613-990-0293
E-Mail: csas-sccs@dfo-mpo.gc.ca
Internet address: www.dfo-mpo.gc.ca/csas-sccs/

ISSN 1919–5087
© Her Majesty the Queen in Right of Canada, 2017

Correct Citation for this Publication:

DFO. 2017. A Framework for the Development of Standard Methods to Evaluate the Toxicity of Petroleum Hydrocarbons on Aquatic Organisms. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2017/053.

Aussi disponible en français :

MPO. 2017. Cadre pour l'élaboration de méthodes normalisées afin d'évaluer la toxicité des hydrocarbures pétroliers sur les organismes aquatiques. Secr. can. de consult. sci. du MPO, Avis. Sci. 2017/053.