Evaluation of the Toxicity of the Weathered Crude Oil used at the Newfoundland Offshore Burn Experiment (NOBE) and the Resultant Burn Residue

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Abstract

The purpose of this study was to evaluate the toxicity of the weathered crude oil Alberta Sweet Mixed Blend (ASMB) used at the Newfoundland Offshore Burn Experiment (NOBE) and the resultant burn residue, using the newly developed Environment Canada water-accommodated fraction (WAF) preparation method and exposure protocol. WAF were prepared by gently stirring a range of oil sample loadings for 48 hours in sealed, fluorinated Nalgene carboys. Rainbow trout (*Oncorhynchus mykiss*) were exposed to freshwater WAF prepared from weathered ASMB. Three-spine stickleback (*Gasterosteus aculeatus*), and the gametes of the white sea urchin (*Lytechinus pictus*) were exposed to saltwater WAF prepared from both weathered ASMB and burn residue. The rainbow trout test was of 96 hours duration, was lightly aerated, and the endpoint was lethality. Two sets of threespine stickleback tests were performed. One set was lightly aerated and the other set was not aerated. Both stickleback test sets were of 96 hours duration, and the endpoint was lethality. The sea urchin test was of 20 minutes duration, was not aerated, and the endpoint was inhibition of fertilization. GC/MS headspace analysis of 28 analytes showed low levels of volatile hydrocarbons. The maximum measured concentration was 1.1 μg/mL in 104 samples from all WAF concentrations, in both seawater and freshwater, at all exposure times (0, 24, and 72 hours). All samples were found to be not toxic to all species tested.

1.0 Introduction

The Newfoundland Offshore Burn Experiment (NOBE)(Fingas *et al.*, 1994) was performed in 1993 to look at the feasibility of burning oil offshore as a spill response measure. A toxicity component was built into the NOBE to determine the potential toxic effects to aquatic organisms that could result from in-situ burning and how they compare to the effects of unburned oil. Three sets of samples were collected to address the toxicity issues associated with burning:

(i) Prior to the NOBE, pre-ignition and post-burn water samples were collected from burn chambers used to generate Alberta Sweet Mixed Blend crude oil

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(ASMB) burn residue in the laboratory (Daykin *et al.*, 1995).

(ii) During the NOBE, field water samples were collected 1 m below the water surface, using remote controlled boats which followed the burn and were equipped with Sigma 800SL samplers (Bissonnette *et al.*, 1994).

(iii) During the NOBE, the weathered ASMB crude oil used for the burn, as well as some of the burn residue, was collected for toxicity testing. The ASMB had been weathered in Alberta by pumping the oil from the tank truck through a hot oiler at 60°C, and then into a portable tank where air was blown through it. After this treatment the oil was pumped back into the tank truck (Pat Lambert, pers comm).

As described in Daykin *et al.* (1995), five toxicity tests were conducted on the laboratory-generated water samples: the echinoderm sperm cell test, the echinoderm larvae test, the echinoderm cytogenetics test, the bivalve larvae test, and the inland silverside juvenile fish test. The same suite of toxicity tests, excluding the cytogenetics test, was conducted on the field-burn water samples. Except for the echinoderm sperm cell test results, little or no toxicity was generally found in the laboratory-generated samples. Toxic effects were even lower in the field-burn samples. The chemical analyses and toxicity testing performed on the water samples indicated that in-situ burning did not adversely affect the underlying water column beyond those effects already associated with the unburned oil.

A difficulty arose in testing the weathered oil and burn residue from sample set #3, because Environment Canada did not have a standardized protocol for producing water-accommodated fractions (WAF) from petroleum hydrocarbons for use in aquatic toxicity testing. Literature searches revealed a range of methodologies, but a 'standard method' was not found, nor was a method found which was capable of dealing with a semi-solid oil such as burn residue. As a result, the weathered oil and burn residue from the NOBE were stored in polyethylene containers (Nalgene Co.) in an archival freezer at -20°C until standard methods could be developed. In a study co-funded by Environment Canada and MSRC, a range of variables were investigated and a basic protocol for preparing WAF from liquid and semi-solid oils was developed (Blenkinsopp *et al.*, 1996). Experiments were then performed to determine how to maintain oxygen levels and minimize WAF loss while exposing rainbow trout to WAF (Blenkinsopp *et al.*, 1997). With these methods in hand, toxicity testing of the archived weathered ASMB and burn residue was performed in late 1996, and is the subject of this paper.

2.0 Methods

2.1 Test Oils:

(i) Weathered ASMB (sample #5) collected on August 2, 1993 while loading the oil into the Canadian Coast Guard Ship Sir Wilfred Grenfell.

(ii) Burn Residue (sample #11) collected from the water surface between the fire boom and row boom during Burn 1 (August 12, 1993).

The physical properties of the weathered ASMB and burn residue are listed in Table 1 (see Jokuty *et al.*, 1996). Note that the physical properties are not available for Sample #11 of the burn residue, however data are available for Sample #12, which was collected from the apex of the fire boom after Burn 1. For comparison purposes, the physical properties of the fresh (unweathered) crude oil which was collected in Hughenden, Alberta on July 22, 1993, are also included.

Table 1. Physical Properties of the Fresh Alberta Sweet Mixed Blend (ASMB) Crude Oil, Weathered ASMB and Burn Residue from the Newfoundland Offshore Burn Experiment (NOBE)

Physical Property	**Fresh ASMB (Sample #1)**	**Weathered ASMB (Sample #5B)**	**Burn Residue (Sample #12)**
API Gravity	37.2	36.1	17.3
Water Content (%)	0.07	0.54	15
Flash Point (°C)	-11	-13	>90
Density (g/mL) (15°C)	0.8384	0.8440	0.9506
Density (g/mL) (0°C)	0.8524	ND	ND
Pour Point (°C)	-21	-21	31°C
Dynamic Viscosity (cP)(15°C)	8	11	24,230 at a shear rate of 10/s; 98,570 at a shear rate of 1/s
Emulsion Formation (15°C)	no	no	no
Asphaltene Content (weight %)	1	1	2
Wax Content (weight %)	11	10	13
Surface Tension (dynes/cm)(15°C)	23.4	21.2	ND
Oil/Saltwater interfacial tension (dynes/cm) (15°C)	16.6	13.3	ND
Oil/Freshwater interfacial tension (dynes/cm) (15°C)	19.0	13.9	ND
Sulphur Content (weight %)	0.15	0.15	0.4

ND = Not Determined

2.2 WAF Preparation

Prior to removing a sample for WAF preparation, the archived material was brought to room temperature over a 24 hour period. WAF (Table 2) were made from the weathered ASMB in both saltwater (Bedford Basin, collected from 10 m depth) and freshwater (charcoal filtered and U.V. dechlorinated Dartmouth municipal). The burn residue was used only to produce saltwater WAF because the amount of residue was limiting. The protocol followed was as described in Blenkinsopp *et al.* (1996). Briefly, 20 litres of water were added to fluorinated Nalgene carboys. The carboys were then placed on Corning 10" x 10" magnetic stir-plates (Model PC610) and 2-inch long teflon-coated hexagonal stir bars were added. The weathered ASMB was poured onto the water surface, and the amount added determined by weight difference. The burn residue could not be poured, therefore it was packed into a plastic syringe which had the hole in the tip enlarged using a 1/8 inch drill bit. The oil was added by weight difference as 'spaghetti-like strands' to the water surface. The mixing energy was set to 3 to 4 rev/s, which ensures adequate mixing within the carboy but does not produce a vortex. The carboys were then capped, and left to stir in the dark at approximately 20°C for 48 hours.

Table 2. WAF Preparation Summary Table

Oil Sample	Water Type	Oil Loading (mg/L)		
		10,000	1,000	100
Weathered ASMB	Freshwater	X	X	X
	Saltwater	X	X	
Burn Residue	Saltwater	X	X	

2.3 Toxicity Testing

Immediately after preparation, water samples were collected from beneath the oil layer and added to test containers. Freshwater WAF solutions prepared from the weathered ASMB were used for toxicity testing with the fish rainbow trout (*Oncorhynchus mykiss*). Saltwater WAF solutions prepared from both weathered ASMB and burn residue were used for toxicity testing with threespine stickleback (*Gasterosteus aculeatus*), and with gametes of the California white sea urchin (*Lytechinus pictus*).

Rainbow trout were chosen for the freshwater WAF tests, since they are ubiquitous across Canada, and have become the world's standard cool-water fish for research in aquatic toxicology (Environment Canada, 1990a). Rainbow trout are economically important, available in a suitable lifestage for testing throughout the year, and have been found to be sensitive to contaminants. Threespine stickleback are widely distributed in Canadian coastal (Atlantic, Pacific and Arctic) waters, both marine and estuarine. In addition, they are considered a suitable test organism for seawater toxicity tests (Environment Canada, 1990b). The echinoderm fertilization assay was the most sensitive of the assays used in the 1993 NOBE toxicity study by Daykin *et al.* (1995), and was therefore included in this study. The species used in this study does not occur in Canadian waters, but is readily purchased from biological supply houses (Environment Canada, 1992).

The sea urchin test (Environment Canada, 1992) was of 20 minutes duration, was not aerated, and the endpoint was inhibition of fertilization (20 min. IC25). The test vessels were capped glass scintillation vials (20 mL).

The trout (Environment Canada, 1990a) and stickleback tests (Environment Canada, 1990b) were of 96 hour duration, and the endpoint was mortality (96h LC50). The fish tests were modified slightly from the Environment Canada standard methods, as follows, in order to minimize WAF loss. Fish tests were performed in new food-grade polyethylene bags which lined 50-L cylindrical tanks. The lightly aerated bags were loosely sealed (bag gathered up and loosely tied around the air tube and siphon, with as little headspace as possible). The aeration rate in the rainbow trout and threespine stickleback tests was 1/10 of the nominal aeration rate, resulting in 0.65 mL air/L test solution/minute. An additional stickleback test was performed with a saltwater WAF prepared from a 10,000 mg/L loading of weathered ASMB to determine whether aeration was necessary during the test. The stickleback in this case were placed in food-grade bags containing the WAF, and the bags were then sealed without headspace and were not aerated during the test. Controls, which consisted of dilution water added to the test vessels, were used to test the suitability of the dilution water as well as the health of the test organisms. WAF blanks, which consisted of dilution water which had been stored for 48 hours in previously used carboys, were used to determine the suitability of the cleaning procedure, which involved the use of both dichloromethane and acetone (Blenkinsopp *et al.*, 1996).

2.4 WAF Chemical Analysis

Water samples were collected from all bags at time 0, 24, and 72 hours, in vials without headspace, and were couriered on ice to the Environmental Technology Centre in Ottawa, for analysis the following day. GC/MS headspace analysis of 28 analytes (2-methylbutane, n-pentane, 2-methylpentane, hexane, 2,4-dimethylpentane, benzene, cyclohexane, heptane, cycloheptane, toluene, n-octane, ethylbenzene, p-xylene, o-xylene, n-propylbenzene, C3-benzenes, decane, n-butylbenzene, naphthalene, dodecane, tridecane, tetradecane, pentadecane) was performed as described in Li *et al.* (1996).

3.0 Results

All samples were found to be not toxic to all species tested (Table 3). The hydrocarbon analysis of 28 analytes by GC/MS headspace analysis showed low levels of volatile hydrocarbons (104 samples; maximum measured concentration 1.1 ug/mL) in samples from all WAF concentrations in both seawater and freshwater, at all sampling times (Table 3). There were very low levels of hydrocarbons present in the controls and WAF blanks. There was no toxicity (no mortality) noted in the control blanks, or in the WAF blanks, indicating that the dilution water and cleaning protocol were suitable.

Table 3. Toxicity of Oil Samples

Oil Sample	Test Species	Test Endpoint	Toxicity (mg/L)	Maximum Measured Petroleum Hydrocarbon Concentration (μg/mL)
NOBE Weathered ASMB Oil	Rainbow Trout	96 hour LC50	>10,000*	≤0.79
	Threespine Stickleback	96 hour LC50	>10,000*	≤1.10
	Threespine Stickleback	96 hour LC50	>10,000*+	≤1.10
	White Sea Urchin Fertilization	20 min. IC25	>10,000	≤1.10
NOBE Burn Residue	Threespine Stickleback	96 hour LC50	>10,000*	≤0.13
	White Sea Urchin Fertilization	20 min. IC25	>10,000	≤0.13

* No obvious adverse effects at 10,000 mg/L.
+ Unaerated test for comparison purposes.

4.0 Discussion

In an oil spill situation, it is likely that the oil will have had time to weather before the slick is reached by responders and the decision to burn is made. The results of this study demonstrate that freshwater and saltwater WAF prepared from weathered ASMB have a low toxicity. The saltwater WAF prepared from the NOBE burn residue are also of low toxicity. In other words, this study found that ASMB burn residue is not more toxic to the test species than the weathered oil.

We have not yet performed experiments comparing the relative toxicity of fresh oils to their burn residue. It is anticipated that the toxicity of the resultant burn residue would be less than that of the fresh oil. Recent experiments on freshwater WAF prepared from fresh ASMB (reference oil #4, see Jokuty *et al.*, 1996 for properties) at a loading of 10,000 mg/L using the same WAF preparation and exposure protocols as in this study, resulted in 100% rainbow trout mortality within 48 hours (Blenkinsopp *et al.*, 1997). Note that this differs from the toxicity results of the laboratory-generated pre-ignition samples (Daykin *et al.*, 1995) where, with the exception of the echinoderm sperm cell test results, little or no toxicity was generally found, even though the fresh ASMB (reference oil #3; see Jokuty *et al.*, 1996 for properties) loading in the crucibles was quite high (approx. 95,000 mg/L). One probable explanation is that all of the water-soluble components of the fresh

ASMB did not have time to solubilize in the study by Daykin *et al.* (1995), because the pre-ignition water samples were only in contact with the fresh ASMB (1 cm thick layer) in the open burn crucible for 30 minutes, without mixing, prior to removal for toxicity testing.

During the stickleback study, chemical analysis showed low levels of hydrocarbons in both aerated and unaerated WAF prepared from the weathered ASMB, and toxicity results were identical (no adverse effects noted). It is not necessary to supply aeration in future WAF tests using threespine stickleback because of their small size and low oxygen requirements.

The operational implication of the research in this paper and by Daykin *et al.* (1995), is that *in-situ* burning of ASMB does not produce a burn residue that is more toxic than the weathered oil itself. All three sets of water samples collected to address the aquatic toxicity issues associated with burning (described in the introduction) support this conclusion. It is appealing that a large weathered oil slick could potentially be reduced down to a relatively small amount of burn residue without causing an increase in aquatic toxicity. Nevertheless, our data set to date is limited in that it is representative of only one oil, ASMB. Additional work should be performed which includes other oils, to determine if the same trend exists.

5.0 Acknowledgments

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6.0 References

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