AQUATIC TOXICITY RESULTING FROM *IN SITU* BURNING OF OIL-ON-WATER

By

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ABSTRACT *In situ* burning is emerging as an effective response technique to counteract oil spills. However, its implementation still raises many concerns regarding its risks to public health and safety and its effects on the environment. The Newfoundland Oil Burn Experiment (NOBE) was conducted in August of 1994 to address issues relating to oil combustion. One component study of NOBE was designed to determine the potential aquatic toxicity to the water column and the subsequent effects to aquatic organisms. To assess the effects of *in situ* burning on aquatic toxicity, both chemical analysis and toxicity testing were performed on laboratory-generated burn samples and on full-scale field samples. Results from the testing performed in this study generally found that *in situ* burning did not adversely affect the underlying water column beyond those effects already associated with the unburned oil. Lethal and sublethal toxicity and concentrations of petroleum hydrocarbons from the water in the vicinity of unburned and burned crude oil slicks in the open sea were extremely low with no significant differences found between the unburned and burned oil samples. Toxicity and petroleum hydrocarbon concentrations in water samples from a closed-system small-scale laboratory burn unit were higher than those found in the field. The data did indicate a weak trend of increasing toxicity and PAH

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concentrations from samples beneath unburned oil and those beneath burned oil; however, values were still very low and the conditions of sample generation skewed to the worst-case.

INTRODUCTION

In situ burning of marine oil spills, has been a controversial issue since it was first tried over 25 years ago. This countermeasure response technique involves the intentional ignition and combustion of spilled oil on the water surface very shortly after its accidental release. Burning has distinct advantages over other countermeasures, namely, the rapid reduction in the quantity of oil before it has spread out of control and/or contaminated shorelines. In addition, it requires little supporting infrastructure or equipment and therefore, can be executed quickly and in remote areas. Conversely there are disadvantages and concerns about burning, such as those related to emissions, ignition and efficacy. Questions remain on health and safety risks, environmental quality and operational constraints.

Both laboratory and meso-scale experimentation had produced substantial information about burning, however, comparable experiments conducted under realistic full scale conditions were lacking. Consequently, in August of 1994, a group of 25 agencies from Canada and the United States conducted a major offshore burn near Newfoundland, Canada, which involved over 20 vessels, 7 aircraft and 230 field personnel. The field experiment involved the release of two repeat oil spills of about 50 tons each into a fire-proof boom. Each burn was monitored for emissions and other physical parameters, yielding data on over 2000 parameters or substances.

Since little was known regarding the impact of oil combustion on the underlying water column, one component study of the overall burn project was designed to specifically address this issue. This component study involved the chemical analysis and biological effects (toxicity) testing of both laboratory-generated burn samples and full scale field-generated burn samples.

The primary objective of this study was to compare background (seawater only), pre-ignition (seawater and unburned oil) and post-burn (seawater and burned oil) sample chemistry and toxicity. Testing of the field-burn samples also included analysis of two additional samples collected during the early and late stages of the burn-period. An additional study objective was to evaluate the burn residue remaining after combustion which will be addressed in a future publication.

METHODS

Initial testing was conducted on samples generated in the laboratory using six identical stainless steel burn units (Figure 1). Each unit supported a burn from a 1-cm layer (100 mL) of standard light crude oil (Alberta Sweet Mix Blend) and generated 1-L water samples for testing. The water used for sample generation was unfiltered, unsterilized seawater obtained from Burrard Inlet, Vancouver, B.C., at a depth of 12 m. Test samples were collected from the bottom of each crucible into pre-cleaned glass containers. Three different sample types were generated; background, pre-ignition and post-burn. The background samples were generated by adding 1050 mL of test seawater to the crucible and holding for 30 minutes. No oil was added. Pre-ignition samples were generated by adding a 100-mL aliquot of crude oil to the top of the seawater prior to the 30-min holding period. Post-burn samples were prepared in the same manner except that the oil was ignited following the 30-min holding time using a propane torch. All water samples were immediately chilled following collection and kept cool (4 °C) prior to analysis. In order to assess variability in the performance of the lab burn unit, five replicate samples of each water type (i.e., background, pre-ignition and post-burn) were generated and submitted for chemical analyses. To obtain the larger volumes required for toxicity testing, replicate 1-L samples were composited to generate three separate samples: background, pre-ignition and post-burn. Sufficient sample of each water type was prepared to test immediately (T = 0 h) and again 48 h later (T = 48 h) to determine if the toxicity of the original sample changed after a 48 h holding time. Holding time effects were of concern because samples generated in the field could not be tested immediately. Before initiating the toxicity tests at T = 0 h and T = 48 h, a 1-L sample was removed and sent for chemical analysis. Additional 1-L samples were sent for chemical analysis at the completion of the toxicity tests. Remaining burn residue, a thick liquid material found in the interface between the seawater and remaining unburned oil, was collected in pre-cleaned glass jars in the same manner used for the water samples. The residue sample have been archived at -20 °C and will be analyzed in the second phase of this study. All sample generation was undertaken by EVS Consultants in Vancouver, British Columbia.

The *in situ* field experiment consisted of two separate burns conducted sequentially on August 12, 1993 off the coast of Newfoundland. Samples intended to be collected from each burn included background, pre-ignition, early-burn, late-burn and post-burn samples. Two background grab samples were collected before the first burn. Two remote-controlled boats, one in front of the other, were used to collect the remaining samples for each burn. Unfortunately, technical difficulties were encountered with some of the sampling equipment and not all of the samples could be collected. The majority of the intended samples (i.e., pre-ignition, early-burn, late-burn and post-burn) were collected by the two boats (Boats

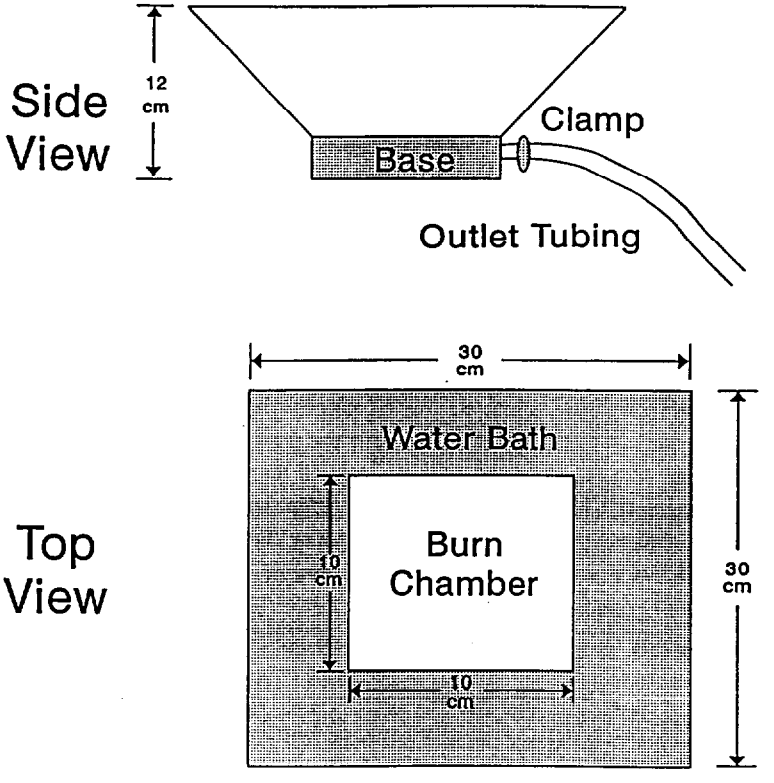

Figure 1 Oil burn crucible chamber.

2 and 4) used in the first burn (Burn I) and by one of the two boats (Boat 1) used in the second (Burn II). Due to volume restrictions, sub-samples could not be removed from the toxicity samples before test initiation for chemical analyses. Four samples, the background as well as the pre-ignition, early-burn and late-burn samples collected by Boat 2 in Burn I, were submitted for chemical analysis following completion of the toxicity testing. All samples were kept cool until analyzed.

Water samples generated in the laboratory or collected in the field were submitted for chemical and biological effects testing. All chemistry samples were sent to the Environmental Technology Centre of Environment Canada in Gloucester, Ontario and analyzed for 24 target polycyclic aromatic hydrocarbons (PAH) as well as total petroleum hydrocarbons (TPH).

Biological effects testing of the water samples was undertaken by EVS Consultants, in Vancouver and Dick Kocan, at the University of Washington. Toxicity was determined using three standard marine organisms: echinoderm (*Dendraster excentricus*), bivalve (*Crassostrea gigas*) and inland silverside (*Menidia beryllina*). The tests performed were the echinoderm sperm cell fertilization test, the echinoderm larvae test, the echinoderm cytogenetic test, the bivalve larvae test and inland silverside juvenile fish test. All five tests were conducted on the laboratory-generated samples. Toxicity tests were conducted immediately upon sample generation and again 48 h later to determine potential holding time effects.

Unfortunately, due to the technical difficulties encountered in the sampling boats, many of the field samples collected did not contain sufficient volumes to conduct the full suite of toxicity tests. None of the field samples could be tested using the echinoderm cytogenetics test and various combinations of the remaining four test were used depending on sample volumes. The toxicity tests performed on each field sample are summarized in Table 1. All toxicity tests were initiated upon sample receipt, on August 13, 1993. Unlike the laboratory-generated sample, these field samples were not re-tested 48 h later.

Natural seawater, used for conducting all toxicity tests and holding test organisms, was obtained from the same source as the water used to generate the laboratory samples. However, this water was filtered through a 5-μm Cuno filter and passed through an ultraviolet sterilizing unit prior to use. A positive (toxic) control was conducted with each toxicity test using a selected reference toxicant. A series of five test concentrations, with two replicates each, was prepared. The reference toxicant test was set up at the time of sample testing and treated in the same manner. The reference toxicant test is used to assess the relative sensitivity of the test organisms by comparing the results with the range of

acceptable values (mean ± 2SD) obtained by the laboratory in previous testing. Negative (clean) controls consisting of natural sterilized seawater were also tested concurrently with each sample. Detailed methods for each of the five toxicity tests are as follows.

Table 1. Toxicity tests performed on field-burn samples.

Sample		Toxicity Test				
		Echinoderm Sperm Cell	Echinoderm Larvae	Echinoderm Cytogenetics	Bivalve Larvae	Inland Silverside
Burn I						
	Background	✓	✓	-	✓	✓
Boat 2	Pre-ignition	✓	✓	-	✓	✓
	Early-burn	✓	✓	-	✓	✓
	Late-burn	✓	✓	-	✓	✓
	Post-burn	✓	-	-	✓	-
Boat 4	Pre-ignition	✓	-	-	✓	-
	Early-burn	✓	-	-	✓	-
	Late-burn	✓	-	-	✓	-
Burn II						
Boat 1	Pre-ignition	✓	-	-	✓	-
	Early-burn	✓	-	-	✓	-
	Late-burn	✓	-	-	✓	-
	Post-burn	✓	-	-	✓	-

Echinoderm Sperm Cell Fertilization Test

The echinoderm sperm cell toxicity tests using the sand dollar, *Dendraster excentricus*, were conducted according to procedures described in Environment Canada (1992). Tests were conducted at 15 ± 1°C in a constant environment room and were completed within 3-5 h. Local sand dollars, collected from Semiahmoo Spit, Washington, were used for testing.

Tests were conducted in 15-mL glass test tubes with 10-mL test volumes. The test treatments were prepared simultaneously for both the echinoderm sperm cell test and the echinoderm larvae test. Three replicates were prepared for each test concentration and negative control. The positive control was prepared using the reference toxicant cadmium chloride ($CdCl_2$). Initial water quality parameters (temperature, salinity, dissolved oxygen and pH) were measured in each treatment. Due to the small test volumes and short test duration, water quality parameters were not measured at the end of the test.

Spawning of the sand dollars was induced by injecting 0.5M KCl into the coelomic cavity. Female sand dollars were wet spawned by placing them aboral side down on top of glass beakers filled with seawater. Male sand dollars were wet spawned by placing them aboral side down in small Petri dishes with about 5 mL of seawater. The animals were allowed to spawn for 15-30 min. Sperm were collected with a glass pipette, transferred to small beakers and stored on ice until used. The gametes were examined microscopically to assess their quality and their densities were adjusted to appropriate levels. Prior to beginning the test, the sperm-to-egg ratio yielding closest to 90% fertilization in control seawater was determined.

Each test tube was inoculated with a 0.1-mL aliquot of sperm suspension and allowed to incubate for 10 min. A 1-mL aliquot of egg suspension was then added to each test tube and, after a 10-min fertilization period, the test solutions were preserved by adding 1 mL of 50% buffered formalin.

Toxicity of the sample was based on fertilization success. Percent fertilization was determined by examining subsamples of 100 eggs per replicate for the presence or absence of a normal fertilization membrane. Mean percent unfertilization was calculated as follows:

$$\textit{Mean \% Unfertilized} = \frac{\textit{sum of unfertilized eggs}}{\textit{sum of total eggs}} (100)$$

Echinoderm Larvae Test

The echinoderm larvae tests using the sand dollar, *Dendraster excentricus*, were conducted using a modification of procedures described in ASTM (1989) and PSEP (1991). Tests were conducted at 15 $\pm$ 1°C in a constant environment room. A 14:10 h light:dark photoperiod was maintained during the 48-h exposure. The sand dollars used for testing were from the same supply used for the echinoderm sperm cell tests.

The tests were conducted in clean 250-mL polyethylene beakers containing 100 mL of test volume. Two sets of negative controls were tested concurrently with samples for each test which included seawater alone as well as additional "zero-time" seawater controls to confirm embryo inoculation density and monitor larval development.

Three replicates were prepared for each control and each test concentration. The positive control was prepared using the reference toxicant cadmium chloride ($CdCl_2$). Initial water quality parameters (temperature, salinity, dissolved oxygen and pH) were measured in the

test solutions used for both the echinoderm larvae and the echinoderm sperm cell tests. Water quality measurements for the same parameters were also recorded in each container at the termination of the test.

Spawning of adult sand dollars was induced according to methods described for the echinoderm sperm cell tests. The animals were allowed to spawn for 15-60 min. Fertilization was accomplished by combining the eggs from several females and 20-25 mL of sperm from several male sand dollars in a 1-L beaker. The embryos were kept in suspension by aeration and gentle agitation with a perforated plunger. Embryo density was determined by making triplicate counts of the number of embryos in 1.0-mL samples of a 1:99 dilution of homogeneous embryo suspension.

Within 2 h of fertilization, each container was inoculated with approximately 2,000 - 3,000 developing embryos using an automatic pipette. The embryo suspension was gently stirred during the inoculation. The number of embryos introduced was confirmed by removing duplicate 10-mL subsamples from three "zero-time" controls immediately after inoculation, preserving them in 5% formalin and counting the number of embryos under a microscope. The larvae were not fed during the testing period.

After 48 h, the contents of each container were re-suspended using a perforated plunger. A 10-mL subsample of larvae was quantitatively transferred to a screw-cap vial using an automatic pipette, and preserved in 5% buffered formalin. A second subsample was also collected and archived as a back-up to confirm results if needed. The preserved samples were later examined in Sedgewick-Rafter counting chambers under 40X magnification. Samples collected from the "zero-time" controls on Day 0 were counted to confirm the number of embryos introduced.

Toxicity in the echinoderm larvae toxicity test was based on abnormal development and mortality. Larvae which failed to transform to the echinopluteus stage, with well-developed arms, were considered abnormal. Normal and abnormal echinopluteus larvae were enumerated for each replicate, and mean percent abnormality was calculated using the following equation:

$$\textit{Mean Abnormality (\%)} = \frac{\textit{sum of abnormal larvae}}{\textit{sum of total larvae}} \times 100$$

Mortality was calculated using the following equation:

$$\textit{Mean Mortality (\%)} = 100 - \frac{(\textit{mean no. of total larvae})\left(\frac{100\ \textit{mL test volume}}{10\ \textit{mL subsample}}\right)}{\textit{no. of embryos introduced}} \times 100$$

Echinoderm Cytogenetics Test

Echinoderm cytogenetics tests using the sand dollar, *Dendraster excentricus*, were conducted based on procedures described in ASTM (1989), PSEP (1991), Liquori and Landolt (1984), Kocan et al.(1982), and Nichols et al. (1979). Embryo exposure was conducted at 15 ± 1°C in a constant environment room and a 14:10 h light:dark photoperiod was maintained during the 14-h exposure period. Sand dollars used for these tests were from the same supply used for the echinoderm sperm cell and echinoderm larvae tests.

The test set-up and spawning of the sand dollars were conducted according to methods described for the echinoderm larvae test. It was not possible to obtain the standard reference toxicant, benzo-a-pyrene, used in this test. In order to simulate the effects of benzo-a-pyrene (i.e., show increased levels of genotoxicity), a small amount of burn residue (2-3 mL) was added to 100 mL of clean seawater and treated in the same manner as the test containers.

Within 2 h of fertilization test containers were inoculated with approximately 3,000 embryos. Embryo inoculation was conducted in the same manner as for the echinoderm larvae test. When the embryos had developed to the blastula stage, approximately 14 h after fertilization, the contents of each container were re-suspended using a perforated plunger. Two 10-mL aliquots from each test container were transferred to screw-cap glass vials, and preserved in 10% buffered formalin. When samples were held for more than 48 h after preservation, the seawater/formalin mixture was replaced with a freshwater/formalin mixture of the same strength to prevent crystallization of salts which could interfere with the cytogenetic analysis.

Upon analysis, the 10% formalin in the vials was replaced with a 3:1 mixture of methanol and acetic acid. Embryos were then transferred to 45% acetic acid for 5 min and stained with aceto-orcein stain. The embryos remained in the stain until their chromosomes became visible. Twenty to fifty embryos were then randomly selected from each replicate and

transferred to microscope slides for observation. Embryos were examined for cytogenetic damage at 600-1000X magnification.

The two endpoints measured in the echinoderm cytogenetics test were the mean number of mitoses and percentage of abnormal anaphase nuclei. Toxic effects were indicated by a decrease in mitotic activity or by an increase in anaphase aberrations.

Bivalve Larvae Test

The bivalve larvae tests using the Pacific oyster, *Crassostrea gigas*, were conducted according to procedures described by ASTM (1989). The test organisms were obtained from a commercial supplier in California and maintained in spawning condition by thermal conditioning, increased photoperiod and increased feeding. Tests were conducted at 20 $\pm$ 1°C in a constant environment room and a 14:10 h light:dark photoperiod was maintained during the 48-h exposure.

The tests were conducted in 15-mL glass test tubes with 10-mL test volumes. Two sets of negative controls were tested concurrently with samples for each test which included a seawater alone as well as additional "zero-time" seawater controls to confirm embryo inoculation density and monitor larval development.

Three replicates were prepared for each negative control and test concentration. A positive (toxic) control was conducted using the reference toxicant cadmium chloride ($CdCl_2$). Measurements of water quality parameters (pH, dissolved oxygen, salinity and temperature) were recorded at test initiation and termination.

Spawning of conditioned oysters was induced by thermal and biological stimulation. Oysters were placed in Pyrex baking dishes filled with UV-sterilized, filtered 20°C seawater in a water bath. The temperature was increased to 28°C over a 30-min period. Once oysters had begun to spawn, they were removed from the water bath and spawning was allowed to continue for approximately 60 min. Fertilization was accomplished by combining all the eggs from one female and 10 - 15 mL of sperm from a male oyster in a 2-L beaker. The fertilized eggs were washed through a 250-μm Nitex screen to remove excess gonadal material, and suspended in filtered seawater at test temperature. The embryos were kept in suspension by aeration and gentle agitation with a perforated plunger. Embryo density was determined by making triplicate counts of the number of embryos in 1.0-mL samples of a 1:99 dilution of homogeneous embryo suspension.

Within 2 h of fertilization, each test tube was inoculated with approximately 300 developing embryos using an automatic pipette. The number of embryos introduced was confirmed by removing duplicate 10-mL subsamples from the three "zero-time" controls immediately after inoculation, preserving them in formalin and counting the number of embryos under a microscope. The larvae were not fed during the testing period.

After 48 h the contents of each test tube were preserved in 5% buffered formalin. The preserved samples were later examined in Sedgewick-Rafter counting chambers under 40X magnification. Toxicity in the oyster larvae toxicity test was based on abnormal development and mortality. Larvae which failed to transform to the fully shelled, straight hinged "D"-shaped prodissoconch I stage, were considered abnormal. Normal and abnormal prodissoconch I larvae were enumerated for each replicate, and mean percent abnormality was calculated using the following equation:

$$\textit{Mean Abnormality (\%)} = \frac{\textit{sum of abnormal larvae}}{\textit{sum of total larvae}} \times 100$$

Mortality was calculated using the following equation:

$$\textit{Mean Mortality (\%)} = 100 - \left(\frac{\textit{mean no. of total larvae}}{\textit{no. of embryos introduced}}\right) \times 100$$

Inland Silverside Test

Acute lethality tests with inland silverside (*Menidia beryllina*) were conducted as described in EPA (1991). The juvenile fish were obtained from a commercial supplier in California. Tests were conducted in a constant environment room at 25 ± 2°C. A 16:8 h light:dark photoperiod was maintained for the 96-h exposure.

The test containers were 1-L glass beakers. Three replicates were prepared for each negative control and test concentration. A positive (toxic) control was conducted using the reference toxicant cadmium chloride ($CdCl_2$). Because inland silversides are extremely sensitive to handling, the test containers were filled with seawater and pre-seeded with fish the day before test initiation. The following day, any mortalities were removed and replaced with new fish. To begin the test, 90% of the seawater was removed and replaced with test solution.

Daily water quality parameters (pH, dissolved oxygen, salinity and temperature) were measured in one replicate for each test concentration and control. Observations were made each day and the number of surviving fish were recorded. At the conclusion of the test, final counts of surviving juveniles were recorded and mean percent survival was calculated for each test concentration. Control survival failed to meet acceptable criteria ($\geq$ 90%) after the 96-h exposure for both the laboratory-generated samples and the field-burn samples. Control survival however, did meet the necessary criteria at 48-h. Therefore, in order to gain some valuable information, the 48-h results were used in data interpretation for all tests.

Statistical analyses were performed on the toxicity test results to determine significant differences between the test treatments. Results from the echinoderm cytogenetics test were evaluated by an analysis of variance (ANOVA) and Fisher's Exact Test, and significant differences determined at $P<0.05$ or $P<0.01$. A two-sample t-test was performed on results from the other five toxicity tests using the STATISTIX computer program (NH Analytical Software, 1986) to compare the background samples to the negative controls. Statistical analyses of the test samples (i.e., pre-ignition, burn-period and post-burn) were performed using the TOXSTAT computer program (Gulley et all., 1990) to calculate the NOEC (no observed effect concentration) values. The NOEC value is the highest concentration of toxicant to which organisms are exposed in a full life-cycle or partial life-cycle test which causes no statistically significant adverse effect on the observed parameters (usually hatchability, survival, growth and reproduction). The data were transformed prior to statistical analysis using an arcsine square root transformation as recommended for binomial data expressed as percentages (Steel and Torrie, 1960). Each data set was tested for normality and homogeneity of variance prior to detailed analysis. Significant differences ($P < 0.05$) in test results for each sample were determined using an analysis of variance (ANOVA) and Dunnett's t-test. Statistical comparisons were made against the negative control as well as against the background sample. The EC50 values were calculated using the EFFL program (version 1.0) as detailed by Stephan (1977). The EC50 value is the concentration that results in 50% abnormal development or unfertilized eggs in the test population.

RESULTS

Chemistry

Results from PAH and TPH analyses are summarized in Tables 2, 3 and 4. Seven of the 1-L laboratory generated (replicate chemistry) samples were judged to be contaminated and

not included in the data analysis. Results from the analysis of the remaining replicate chemistry samples generated in the laboratory did indicate a possible trend of increasing polycyclic aromatic hydrocarbon (PAH) composition as well as total PAH concentrations from the background samples through the pre-ignition and post-burn samples. The total average PAH concentration ranged from <0.10 μg/L in background samples to 2.03 μg/L in the pre-ignition samples and 3.78 μg/L in post-burn water samples. While the background seawater showed none of the target PAH compounds above the analytical detection limit, both the pre-ignition samples contained four of the target PAH, and the three post-burn samples contained between five and ten of the target compounds. Naphthalene and alkyl homologues of naphthalene are the main components in the water samples. This is likely due to their low molecular weight and relatively higher solubilities in water compared to the other PAH target compounds. The highest ring number PAH was phenanthrene, found in the post-burn sample.

Table 2. Total PAH Results.

Sample	Background (μg/L)	Pre-ignition (μg/L)	Early-burn (μg/L)	Late-burn (μg/L)	Post-burn (μg/L)
LAB					
Replicate Chemistry[1]	ND	2.03	-	-	3.78
Toxicity					
T=0 h					
initial	ND	1.83	-	-	4.27
final	ND	ND	-	-	ND
T=48 h					
initial	ND	0.67	-	-	5.05
final	ND	ND	-	-	ND
FIELD					
Chemistry					
Burn I[2]	<0.10	0.14	< 0.10	0.10	0.12
Burn II[3]	-	ND	0.15	< 0.10	< 0.10
Toxicity					
initial	-	-	-	-	-
final	ND	ND	ND	ND	-

1 The values listed for the background, pre-ignition and post-burns samples are averages from five, two and three measurements, respectively

2 The values listed for the background, pre-ignition, early-burn, late-burn and post-burn samples are averages from two, four, four, four and two measurements, respectively

3 The values listed for the pre-ignition, early-burn, late-burn and post-burn samples are all averages of two measurements

ND Not detected

- Sample not generated and/or not tested

Table 3. Results of target PAH analysis for laboratory-generated samples

Compound	Background	Replicate Chemistry Samples		Composite Toxicity Samples 0-h		Composite Toxicity Samples 48-h	
	Background - average[1] (μg/L H_2O)	Pre-ignition[1] (μg/L H_2O)	Post-burn[1] (μg/L H_2O)	Pre-ignition initial (μg/L H_2O)	Post-burn initial (μg/L H_2O)	Pre-ignition initial (μg/L H_2O)	Post-burn initial (μg/L H_2O)
Sampling Date	June 17, 1993	June 1, 1993	June 1, 1993	June 15, 1993	June 15, 1993	June 17, 1993	June 17, 1993
naphthalene	0.00	0.96	1.70	0.86	1.60	0.26	2.11
2-methyl-naphthalene	0.00	0.53	0.89	0.43	0.85	0.15	1.10
1-methyl-naphthalene	0.00	0.43	0.64	0.32	0.64	0.26	0.79
biphenyl	0.00	0.00	0.03	0.00	0.11	0.00	0.00
2,6-dimethyl-naphthalene	0.00	0.11	0.22	0.11	0.21	0.00	0.53
acenaphthylene	0.00	0.00	0.14	0.00	0.21	0.00	0.26
acenaphthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,3,5-trimethyl-naphthalene	0.00	0.00	0.03	0.00	0.11	0.00	0.00
fluorene	0.00	0.00	0.03	0.00	0.11	0.00	0.00
debenzothiophene	0.00	0.00	0.03	0.00	0.11	0.00	0.00
phenanthrene	0.00	0.00	0.07	0.11	0.32	0.00	0.26
1-methyl-phenanthrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benz(a)anthracene/chrysene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benz(b)fluoranthene/b(k)fluor.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo(e)pyrene/benzo(a)pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
perylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
indeno(1,2,3-cd)pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
debenz (a,h) anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo (g,h,i) perylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total PAH	0.00	2.03	3.78	1.83	4.27	0.67	5.05

[1] The values listed for the background, pre-ignition and post-burn samples are averages from four, two and three measurements respectively.

Table 4. Total TPH Results.

Sample	Background (μg/L)	Pre-ignition (μg/L)	Early-burn (μg/L)	Late-burn (μg/L)	Post-burn (μg/L)
LAB					
Replicate Chemistry[1]	40	41	-	-	44
Toxicity					
T=0 h					
initial	30	31	-	-	47
final	129	146	-	-	112
T=48 h					
initial	100	77	-	-	109
final	62	80	-	-	86
FIELD					
Chemistry					
Burn I[2]	15	6	6	4	8
Burn II[3]	-	6	4	4	6
Toxicity					
initial	-	-	-	-	-
final	7	18	22	4	-

[1] The values listed for the background, pre-ignition and post-burns samples are averages from five, two and three measurements, respectively

[2] The values listed for the background, pre-ignition, early-burn, late-burn and post-burn samples are averages from two, four, four, four and two measurements, respectively

[3] The values listed for the pre-ignition, early-burn, late-burn and post-burn samples are all averages of two measurements

\- Sample not generated and/or not tested

Unlike the laboratory-generated samples, the analysis of samples form the full-scale field burn revealed very low levels of PAHs (< 1.00 μg/L) with many of the samples having non-detectable levels. No discernable differences were apparent between water types pre-ignition through to post-burn. Where PAHs were present, the compound detected was naphthalene, except in one sample (Burn I, pre-ignition) which also contained methyl-naphthalene.

Total PAH concentrations measured in the T = 0 h water samples generated for toxicity testing were 1.83 μg/L in the pre-ignition sample and 4.27 μg/L in the post-burn sample. As for the replicate chemistry samples, none of the target PAH were detected in the background water. Water samples sent to the ETC laboratory after the 48-h holding period time showed similar total PAH levels, <0.01 μg/L in the background, 0.67 μg/L in pre-ignition and 5.05 μg/L in post-burn samples. All samples collected at the termination

of the toxicity tests (i.e., laboratory-generated and field-burn samples) showed no detectable target PAH compounds. This decrease in PAH levels may have resulted from uptake by the juvenile fish, adsorption to the fish, or adsorption to the test containers.

Total petroleum hydrocarbon (TPH) analysis of the laboratory-generated samples did not detect substantial differences between the three water types. Average TPH levels were 40 μg/L in the background seawater, 41 μg/L in the pre-ignition samples, and 44 μg/L in the post-burn samples. As for the PAH levels, the total average TPH concentrations were much lower in the field-burn chemistry sample with all reporting values of $<$ 16 μg/L.

Total TPH concentrations in the initial T = 0 h toxicity test samples were 30 μg/L in the background seawater, 31 μg/L in the pre-ignition sample and 47 μg/L in the post-burn sample. The initial T = 48 h toxicity test samples showed higher variability and a less uniform trend: 100 μg/L in the background sample, 77 μg/L in the pre-ignition sample and 109 μg/L in the post-burn sample. Levels in samples collected at the termination of the T = 0 h test increased substantially compared to initial levels. Concentrations were 129 μg/L, 146 μg/L and 112 μg/L in the background, pre-ignition and post-burn samples, respectively. Conversely, TPH levels in the samples submitted at the termination of the T = 48 h test, decreased from initial levels. Concentrations found in the background, pre-ignition and post-burn samples were 62 μg/L, 80 μg/L and 86 μg/L, respectively. The TPH levels were much lower in the field-burn samples at the completion of the toxicity tests ranging from only 4 - 22 μg/L.

Toxicity

Results of the toxicity tests conducted at T = 0 h and T = 48 h on the laboratory-generated samples are summarized in Tables 5 and 6. Toxicity test results of the field-burn samples are summarized in Tables 7 and 8.

Echinoderm Sperm Cell Fertilization

Mean percent unfertilized eggs in the laboratory-generated samples ranged from 23.7 to 73.3%. The mean percent unfertilized eggs was much higher in the T = 0 h post-burn sample, 73.3%, than that observed in the other laboratory samples, including the T = 48 h post-burn sample. An unexpected result was that a two-sample t-test indicated that the background sample was significantly ($P < 0.05$) different from the negative control.

Table 5. Toxicity test results: laboratory-generated samples.

Sample	Echinoderm Sperm Cell Mean % Unfertilized	Echinoderm Larvae Development Mean % Abnormal	Echinoderm Cytogenetics Mean # Mitoses/Embryo	Mean % Abnormal Anaphase	Bivalve Larvae Development Mean % Abnormal	Inland Silverside Mean % Survival at 48 h
T = 0 h						
Background	38.0*	23.6*	20.5	2.7	5.7*	100
Control	4.0	5.1	19.0	1.7	3.1	100
Pre-ignition	36.7*	16.2*	20.4*	4.3*	30.7*	100
Control	4.0	7.2	26.6	1.7	3.9	100
Post-burn	73.3*	15.4*	27.1*	5.0	19.1*	93.3
Control	11.7	5.5	21.5	7.0	3.7	100
T = 48 h						
Background	23.7*	8.4*	26.3	8.1	4.9*	100
Control	10.0	3.5	30.2	6.5	1.6	100
Pre-ignition	24.3	12.1*	23.4	11.8	34.0*	100
Control	17.0	2.7	19.9	12.2	3.1	100
Post-burn	26.0	14.1*	31.8[1]	12.5*[1]	32.6*	100
Control	16.3	3.6	-	-	3.4	100

Only values for the 100% (v/v) test concentrations are reported.

* Asterisks denote values significantly different ($P < 0.05$) from the control.

[1] Reliable data could not be obtained for either the full-strength (i.e., 100%) sample or its associated negative control; for evaluation purposes, the 50% post-burn sample test endpoints were compared to those obtained from the negative control sent with the 48 h background sample.

Table 6. Summary of toxicity endpoints: laboratory- generated samples.

Sample	Echinoderm Sperm Cell				Echinoderm Larvae				Bivalve Larvae			
	NOEC[1]		EC50[1]		NOEC[1]		EC50[1]		NOEC[1]		EC50[1]	
	vs Ctrl	vs Back	s Ctrl	vs Back	vs Ctrl	vs Back	vs Ctrl	vs Back	vs Ctrl	vs Back	vs Ctrl	vs Back
T = 0 h												
Pre-ignition	<6.25	100	>100	>100	6.25	100	>100	>100	6.25	6.25	>100	>100
Post-burn	<6.25	50	76.4	94.2	12.5	100	>100	>100	6.25	6.25	>100	>100
T = 48 h												
Pre-ignition	100	100	>100	>100	<6.25	25	>100	>100	<6.25	6.25	>100	>100
Post-burn	6.25	100	>100	>100	<6.25	6.25	>100	>100	6.25	6.25	>100	

[1] All endpoint values are percent volume by volume (%v/v).

Ctrl Control

Back Background

Table 7. Toxicity test results: field-burn samples.

Sample		Echinoderm Sperm Cell Mean % Unfertilized	Echinoderm Larvae Development Mean % Abnormal	Bivalve Larvae Development Mean % Abnormal	Inland Silverside Mean % Survival at 48 h
Background		15.7	6.1	6.3	95
Control		14.7	6.9	1.8	85
Pre-ignition	Burn I, Boat 2	29.3*	10.9*	6.0*	95
	Control	10.7	5.2	2.4	100
	Burn I, Boat 4	45.3*	-	8.9*	-
	Control	18.0	-	1.8	-
	Burn II, Boat 1	62.3*	-	11.1*	-
	Control	16.3	-	1.3	-
Early-burn	Burn I, Boat 2	30.0*	18.7*	9.8*	100
	Control	9.3	5.4	1.6	85
	Burn I, Boat 4	43.3*	-	12.2*	-
	Control	21.7	-	1.6	-
	Burn II, Boat 1	55.0	-	18.0*	-
	Control	23.3	-	1.5	-
Late-burn	Burn I, Boat 2	28.7*	17.0*	10.9*	100
	Control	16.7	4.4	2.3	95
	Burn I, Boat 4	53.3*	-	7.4*	-
	Control	27.0	-	1.8	-
	Burn II, Boat 1	55.7*	-	13.1*	-
	Control	24.3	-	2.1	-
Post-burn	Burn I, Boat 2	30.7*	-	8.0*	-
	Control	12.7	-	1.2	-
	Burn I, Boat 4	-	-	-	-
	Control	-	-	-	-
	Burn II, Boat 1	24.7	-	7.9*	-
	Control	17.0	-	2.1	-

Only values for the 100% (v/v) test concentrations are reported.

* Asterisks denote values significantly different ($P < 0.05$) from the control.

Table 8. Summary of toxicity endpoints: field-burn samples.

Sample	Echinoderm Sperm Cell				Echinoderm Larvae				Bivalve Larvae			
	NOEC[1]		EC50[1]		NOEC[1]		EC50[1]		NOEC[1]		EC50[1]	
	vs Ctrl	vs Back	vs Ctrl	vs Back	vs Ctrl	vs Back	vs Ctrl	vs Back	vs Ctrl	vs Back	vs Ctrl	vs Back
Burn I, Boat 2												
Pre-ignition	<6.25	<6.25	>100	>100	12.5	50	>100	>100	25	100	>100	>100
Early-burn	6.25	50	>100	>100	12.5	50	>100	>100	<6.25	100	>100	>100
Late-burn	<6.25	<6.25	>100	>100	<6.25	12.5	>100	>100	6.25	100	>100	>100
Post-burn	6.25	6.25	>100	>100	-	-	-	-	<6.25	100	>100	>100
Burn I, Boat 4												
Pre-ignition	<6.25	<6.25	>100	>100	-	-	-	-	6.25	100	>100	>100
Early-burn	<6.25	<6.25	>100	>100	-	-	-	-	12.5	100	>100	>100
Late-burn	25	<6.25	>100	>100	-	-	-	-	12.5	100	>100	>100
Burn II, Boat 1												
Pre-ignition	<6.25	<6.25	86.7	85.9	-	-	-	-	<6.25	100	>100	>100
Early-burn	12.5	6.25	>100	>100	-	-	-	-	<6.25	50	>100	>100
Late-burn	6.25	6.25	>100	>100	-	-	-	-	6.25	50	>100	>100
Post-burn	25	6.25	>100	>100	-	-	-	-	<6.25	100	>100	>100

[1] All endpoint values are percent volume by volume (%v/v).
Ctrl Control
Back Background

Data sets for all the pre-ignition and post-burn samples passed Shapiro-Wilk's test for normality and Bartlett's test for homogeneity of variance. Results of the analysis of variance indicated that there were some significant differences ($P < 0.05$) in fertilization between these treatments and the negative controls. The NOEC values for the pre-ignition samples were <6.25% (v/v) and 100% (v/v). This apparent disparity is a result of the higher number of unfertilized eggs in the negative control associated with the T = 48 h sample. A conservative estimate of the NOEC values for both the post-burn samples would be <6.25% (v/v). The EC50 value for the T = 0 h post-burn sample was 76.4% (v/v). The EC50 values for both the pre-ignition samples and the T = 48 h post-burn samples were >100% (v/v). Only the T = 0 h post-burn sample was found to be significantly different than the background. In all cases, higher NOEC and EC50 values were obtained when the pre-ignition and post-burn samples were compared to the background samples instead of the negative controls.

Mean percent unfertilized eggs in the field-burn samples ranged from 15.7 to 62.3%. The mean percent unfertilized eggs was similar between the four test samples (pre-ignition, early-burn, late-burn and post-burn) collected by the lead boat (Boat 2) in Burn I and ranged in the 100% test concentrations from 28.7 to 30.7%. Levels of unfertilization were higher in the three samples (i.e., pre-ignition, early-burn and late-burn) collected by the second boat (Boat 4), ranging from 43.3 to 53.3%. Except for the post-burn sample, the mean percent unfertilization was even higher for the samples collected during Burn II. These values ranged from 55.0 to 62.3%. In contrast, the percent unfertilized eggs in the post-burn sample was lower at 24.7%. The mean percent unfertilization in the background sample, 15.7%, was very similar to that obtained for the control seawater, 14.7%. A two-sample t-test confirmed that the background sample was not significantly ($P < 0.05$) different from the negative control.

Data sets for all the field samples passed Shapiro-Wilk's test for normality and Bartlett's test for homogeneity of variance. Results of the analysis of variance indicated that there were significant differences ($P < 0.05$) in fertilization between the test samples and the control. In Burn I, Boat 2, the NOEC values for the pre-ignition, early-burn, late-burn and post-burn samples were <6.25, 6.25, <6.25 and 6.25% (v/v) respectively. In Burn I, Boat 4, NOEC values were <6.25% (v/v) for the pre-ignition and early-burn samples and 25% (v/v) for the late-burn sample. In Burn II, Boat 1, the NOEC values for the pre-ignition, early-burn and late-burn samples were <6.25, 12.5 and 6.25% (v/v), respectively. A conservative estimate of the NOEC value for the post-burn sample would be 25% (v/v). The EC50 values were >100% (v/v) for all samples in both burns except for the pre-ignition sample collected during the second burn. The EC50 value for this sample was 86.7% (v/v).

Statistical comparisons between the test samples and the background generally showed similar NOEC values with those obtained when the treatments were compared to the negative controls. The EC50 values were all >100% except for the pre-ignition sample collected in the second burn which had an EC50 value of 85.9%.

Echinoderm Larvae Test

Mean percent abnormalities in all the laboratory-generated samples were relatively low, ranging from 8.4 to 23.6%. All the pre-ignition and post-burn samples had relatively similar mean percent abnormalities. The highest percent abnormality was found in the T = 0 h background sample and the lowest value found in the T = 48 h background sample. A two-sample t-test indicated that both background samples were significantly ($P<0.05$) different from the negative controls.

Data sets for both the pre-ignition and post-burn samples passed Shapiro-Wilk's test for normality and Bartlett's test for homogeneity of variance. Results of the analysis of variance indicated that there were significant differences ($P<0.05$) between all the pre-ignition and post-burn samples, and the negative controls. A conservative estimate of the NOEC value for the pre-ignition sample tested immediately upon generation would be 6.25% (v/v). The NOEC value for the second pre-ignition sample was <6.25% (v/v). The NOEC value for the two post-burn samples were 12.5% (v/v) and <6.25 % (v/v). Although the NOEC values were low, the data showed that mean abnormality was approximately 16% or less at all concentrations. These relatively low abnormalities are not considered to be biologically significant. The EC50 values were >100% (v/v) for all samples.

Statistical comparisons between the pre-ignition and post-burn samples with the background sample yielded higher NOEC values than those obtained when the treatments were compared to their respective negative control. The EC50 values were the same at >100% (v/v).

Mean percent abnormality in the four field-burn samples tested (background, pre-ignition, early-burn and late-burn from Burn I) were relatively low, ranging from 6.1% to 18.7%. The mean percent abnormality was the lowest in the background sample, 6.1%, which was very similar to that obtained for the negative control, 6.9%. A two-sample t-test confirmed that the background sample was not significantly ($P<0.05$) different from the negative control.

Data sets for the other three test samples passed Shapiro-Wilk's test for normality and Bartlett's test for homogeneity of variance. Results of the analysis of variance indicated that there were significant differences ($P < 0.05$) in larval abnormality between these treatments and the negative controls. A conservative estimate of the NOEC value for the pre-ignition sample would be 12.5% (v/v). The NOEC values for the early-burn and late-burn samples were 12.5 and <6.25% (v/v) respectively. As was the case with the laboratory-generated samples, the low mean abnormalities are not considered to be biologically significant even though the low NOEC values indicate statistical significance. The EC50 values were >100% (v/v) for all three samples.

Statistical comparisons between the test samples and the background yielded slightly higher NOEC values than those obtained when comparisons were made against the negative controls. The NOEC values for the pre-ignition, early-burn and late-burn samples were 50, 50 and 12.5% (v/v) respectively. The EC50 values were all >100% (v/v).

There was no adverse effect on echinoderm larvae survival as larvae mortality in the laboratory-generated samples and field samples were relatively low.

Echinoderm Cytogenetics Test

All results refer to those obtained from the analysis of the laboratory-generated samples. No toxic response was found in either mitotic activity or in the number of anaphase aberrations in the two background samples. A genotoxic effect was found in the T = 0 h pre-ignition sample since the mean number of mitoses per embryo was significantly lower than the negative control. The data for the anaphase aberration also found a genotoxic effect since the pre-ignition sample produced a significant increase in the number of aberrations. In contrast, neither mitotic activity or the number of anaphase aberrations indicated a genotoxic effect in the T = 48 h pre-ignition sample.

The T = 0 h post-burn sample showed a significant increase in the number of mitoses per embryo and an unexplained decrease in anaphase damage. The increase in mitotic activity is referred to as hormesis and is frequently associated with exposure to toxic chemicals. However, unless it is accompanied by a parallel increase in some other visible response (i.e., deformity or death), it is not considered to be indicative of a toxic effect. The decrease in anaphase aberrations may have resulted from a change in water quality or in exposure conditions; however, since there was not an increase in the chromosome damage there was also no indication of increased genotoxicity. Due to lower than expected embryo densities, T = 48 h post-burn sample and its respective control could not be analyzed for genotoxicity. As a result, test endpoints for the 50% post-burn concentration were

compared to the control sent with the 48 h background sample. This post-burn concentration showed no significant difference in the number of mitoses per embryo. However, a significant increase ($P<0.01$) was found in the number of anaphase aberrations.

Bivalve Larvae Test

Mean percent abnormalities in the laboratory-generated samples were relatively low, ranging from 4.9 to 34.7%. Mean abnormality was lowest in the two background samples. However, a two-sample t-test indicated that the background samples were still significantly ($P<0.05$) different from the negative controls. Data sets for both the pre-ignition and post-burn samples passed Shapiro-Wilk's test for normality and Bartlett's test for homogeneity of variance. Results of an analysis of variance indicated that there were significant differences ($P<0.05$) in larval abnormality between these treatments and the negative controls. The NOEC values for the T = 0 h pre-ignition and both post-burn samples were 6.25% (v/v). The NOEC value for the T = 48 h pre-ignition sample was <6.25%. The EC50 values were >100% (v/v) for all samples.

The NOEC value was 6.25% for the T = 48 h pre-ignition sample when statistical comparisons were made against the background. The NOEC and EC50 values remained the same for the other samples.

Mean percent abnormalities were much lower in all the eleven samples collected in the field, ranging from 6.0% to 18.0%. The mean percent abnormality in the background sample, 6.3%, was relatively similar to that obtained for the negative control, 1.8%. A two-sample t-test confirmed that the background sample was not significantly ($P<0.05$) different from the negative control.

Data sets for all the samples passed Shapiro-Wilk's test for normality and Bartlett's test for homogeneity of variance. Results of the analysis of variance indicated that there were significant differences ($P<0.05$) in larval abnormality between treatments. In Burn I, Boat 2, the NOEC values for the pre-ignition, early-burn, late-burn and post-burn samples were 25, <6.25, 6.25 and <6.25% (v/v) respectively. In Burn I, Boat 4, NOEC values were 6.25% (v/v) for the pre-ignition sample and 12.5% (v/v) for the early-burn and late-burn samples. In Burn II, Boat 1, the NOEC values for the pre-ignition, early-burn, late-burn and post-burn samples were <6.25, <6.25, 6.25 and <6.25% (v/v) respectively. Although the NOEC values were low, the data showed that mean abnormalities were

approximately 18% or less which is typically not considered to be biologically significant. The EC50 values were > 100% (v/v) for all samples in both burns.

The NOEC values were higher in the test samples when they were compared to the background sample instead of the negative controls and ranged from 50 to 100% (v/v). The EC50 values were all > 100% (v/v).

There was no adverse effect on bivalve larvae survival as larvae mortality in the laboratory-generated samples and field samples were relatively low.

Inland Silverside Tests

After 48 h, mean survival was 100% in all the laboratory-generated samples except for the T = 0 h post-burn sample. However, mean survival was still high in this sample at 93.3%. The EC50 values was > 100% (v/v) for all the samples.

Mean survival was also high in the four field-burn samples tested. Mean survival was 95% in the background sample and were 95, 100 and 100% in the pre-ignition, early-burn and late-burn test samples, respectively.

DISCUSSION

The absolute concentrations of petroleum hydrocarbons found in both lab and field-burn samples were very low. PAHs, where detectable, were in the μg/L (parts per billion) range. Concentrations found in the full-scale field-burn were much lower than the laboratory-generated samples. This may be accounted for by the natural mixing and dilution that would have occurred in the open sea. Conversely, the oil-to-water ratio in the lab burn units was small, the water confined and there was no opportunity for any dilution. Laboratory-generated samples could be compared to a worst-case scenario for burning in shallow confined waters.

With the exception of the echinoderm sperm cell test, little or no toxicity was associated with the laboratory-generated samples of water from beneath oil and burned oil. Although the NOEC values were generally low, the high EC50 values typically indicated that oil combustion caused minimal effects to the aquatic toxicity. Because NOEC's are derived from statistical analyses, there may be statistical significance without biological significance. In most cases, test results were below or near levels expected to be associated with biological significance. The only exception was the T = 0 h echinoderm sperm cell

test results for the post-burn sample where the percentage of unfertilized eggs was found to be 73.3%. In addition, there was no apparent effect of the 48 h holding period.

An unexpected finding from the toxicity tests with the laboratory-generated samples was the level of toxicity associated with the background sample. It was anticipated that the results obtained for this sample would be comparable to those obtained for the negative control. Although this was the case for some of the tests, results from the echinoderm sperm cell and echinoderm larvae tests found the test endpoints to be higher in the background sample than those in the negative control and, in general, more comparable to those found in the pre-ignition and post-burn samples.

The low levels of total petroleum hydrocarbons and polycyclic aromatic hydrocarbons from the lab samples support the toxicity test results. Although there was a weak trend of increasing PAH composition and concentration from the background through to the pre-ignition and post-burn samples, PAH levels in all the samples were very low. Levels of TPH were also low. However, unlike the PAH results, the TPH results did not discern any clear differences between the different water types. Because of the detectable TPH levels in the background sample, PAH levels may be a more suitable measure for interpreting oil related effects.

As for the laboratory-generated samples, the echinoderm sperm cell test was the most sensitive test in identifying possible oil combustion effects in the field study. Responses to the burn-period and post-burn samples generally did not exceed those associated with the unburned oil. Results from the other toxicity tests found very low levels of toxicity, even lower than those obtained from testing the laboratory-generated samples. No clear differences in toxicity were apparent between the boats, the burns or between the pre-ignition and post-ignition (i.e., during-burn and post-burn) samples. Unlike the laboratory-scale study, the background field-burn sample results were comparable to the negative control for all the tests conducted.

Chemical analyses from the field-burn samples again supported toxicity findings since the levels of PAH and TPH were low and not indicative of any adverse effect. In addition, no significant difference existed between any of the samples.

The echinoderm sperm cell test and the determination of polycyclic aromatic hydrocarbons composition were found to be the most sensitive toxicity and chemical tests conducted in this study to identify oil-related effects. However, results from all the tests conducted generally found minimal differences between pre-ignition and post-ignition samples indicating that oil combustion did not adversely affect the water column beneath the oil

beyond those effects already associated with the unburned oil. These results are encouraging in light of the toxicity found to aquatic organisms with the use of some alternative oil spill clean-up methods such as dispersants (Fisher and Foss, 1993, Vigers, 1979). However, while the burning of the surface oil slick does not appear to negatively affect the aquatic organisms tested, it does not alleviate the effects of the oil slick itself. Results from analysis of the burn residue samples will generate a more complete data set from which to identify the effects of oil combustion on aquatic toxicity and thereby, provide a more comprehensive evaluation of the acceptability of *in situ* burning as a response technique for oil spills.

CONCLUSIONS

- Lethal and sublethal aquatic toxicity and concentrations of petroleum hydrocarbons from water in the vicinity of unburned and burned crude oil slicks in the open sea were extremely low. There were no significant differences in the measurements of either toxicity of petroleum hydrocarbons between water samples associated with unburned oil, burning oil or post-burn stage of the *in situ* field trial.

- Toxicity and petroleum hydrocarbon concentrations in water samples from a closed-system small-scale laboratory burn unit were higher than those found in the field. The data indicated a weak trend of increasing toxicity and PAH concentrations from samples beneath unburned oil to those beneath burned oil. Nevertheless, values are still considered to be very low and the conditions of sample generation to be skewed to worst-case.

- The echinoderm sperm cell test was the most sensitive of the five toxicity tests in detecting oil-related effects.

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