Aquatic Toxicity from *In-situ* Oil Burning

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Abstract

The Newfoundland Oil Burn Experiment (NOBE) was conducted in August 1993 to address issues related to in-situ burning of spilled oil on water. One of the component studies of NOBE was developed to determine the potential toxic effects to aquatic organisms that could result from in-situ burning and how they compare to effects of unburned oil. To assess the effects of in-situ burning, both chemical analysis and aquatic toxicity testing were performed on laboratory-generated burn samples and on the full-scale, field-burn samples.

The primary objective of this study was to compare chemistry and toxicity of background samples (seawater only), pre-burn samples (seawater and unburned oil), and post-burn samples (seawater and burned oil). Testing of the field-burn samples also included analysis of two additional samples collected during the early and late stages of the burn. An additional objective was to evaluate the burn residue remaining after combustion. These samples have been collected and archived pending information on analytical procedures. . . .

The chemical analyses performed on the water samples included identifying 24 target polycyclic aromatic hydrocarbons (PAHs) and determining total petroleum hydrocarbons (TPHs). Results from the laboratory-generated samples indicated a weak trend of increasing detection of PAH compounds, as well as an increasing total PAH concentration from background to pre-ignition and post-burn samples. However, all levels were low, less than 4 μg/L. Levels of total petroleum hydrocarbons were similar for all the laboratory samples and were also relatively low, approximately 40 μg/L. Chemistry results for the field-burn samples found both PAH and TPH to be low. Analysis for PAH compounds found less than 1 μg/L in all the samples, whereas TPH levels were all less than 13 μg/L.

Five toxicity tests were conducted on the laboratory-generated 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. Except for the echinoderm sperm cell test results, little or no toxicity was generally found in the laboratory-generated samples and no discernable difference was apparent between the pre-ignition and post-burn samples. Consequently, when test endpoints indicated a toxic effect, they were most often elevated in both the pre-ignition and post-burn samples. The same suite of toxicity tests, excluding the cytogenetics test, was conducted on the field-burn samples. Toxic effects were found to be even lower in the field-burn samples. As for the laboratory-generated samples, no differences were apparent between the pre-ignition and post-burn samples.

The chemical analyses and toxicity 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. The results from the water analysis performed in this study suggest that the potential for toxicity should not be a factor preventing the use of in-situ burning as a response technique for oil spills.

Résumé

Le projet du "Newfoundland Offshore Burn Experiment" (NOBE) a eu lieu en Août 1993 et avait pour but de répondre aux questions qui sont soulevées lorsque l'on brûle de façon in-situ du pétrole déversé en mer. Une composante, de ces études de mesures d'intervention en cas de déversement du projet NOBE, était de déterminer les effets potentiels de toxicité aquatique sur les tranches d'eau de même que les effets ultérieurs sur les organismes marins. Pour évaluer les effets du brûlage in-situ, des analyses chimiques et des tests de toxicité ont été fait sur des échantillons générés en laboratoire de même que sur des échantillons recueillis sur une échelle plus grande, soit des échantillons dit sur le terrain, c'est à dire lors d'essais directs en mer.

Un objectif premier de cette étude était de comparer des échantillons d'arrière-plan (l'eau de mer seule), des échantillons d'un mélanger d'eau de mer et de pétrole avant le brûlage, et des échantillons d'eau de mer et de pétrole après l'étape du brûlage. Les essais sur les échantillons brûlés incluaient également l'analyse de deux échantillons additionnels dont la collecte fut faite à deux temps différents, soit au début du brûlage et à la fin. Un objectif additionnel était d'évaluer les résidus provenant de la combustion totale du pétrole. Ces échantillons ont été ramassés et rangés aux archives dans l'attente d'informations nécessaires sur les procédures à utiliser pour procéder aux analyses.

Les analyses chimiques faite sur les échantillons d'eau incluaient l'identification de 24 composés cibles d'hydrocarbures polyaromatiques (HPA) et la détermination des hydrocarbures totaux (HT). Les résultats provenant des échantillons générés en laboratoire indiquent une tendance faible mais accrue de la détection de composés HPA, de même qu'une augmentation totale dans la concentration de HPA de l'arrière-fond des échantillons pré- et post-brûlés. Cependant tous les niveaux détectés étaient faibles; l'ordre de concentration étant de moins de 4 µg/L. Les niveaux totaux d'hydrocarbures étaient similaires entre eux pour tous les échantillons de laboratoire et possédaient des concentrations relativement basses, soit de l'ordre d'environ 40 µg/L. Les résultats chimiques pour les échantillons dit de terrain ont également révélés des niveaux de HPA et HT relativement faibles. Les analyses pour les HPA ont donné des valeurs de moins de 1 µg/L dans tous les échantillons, tandis que les niveaux de HT étaient tous de moins de 13 µg/L.

Cinq différents essais de toxicités ont été fait sur des échantillons générés en laboratoire: l'essai sur spermatozoïde des échinodermes, l'essai sur larve d'échinodermes, l'essai cytogénétique des échinodermes, l'essai de larve bivalve et l'essai sur le poisson Menidia beryllina. Exception faite des résultats de l'essai sur spermatozoïde des échinodermes, très peu ou aucun signe de toxicité fut trouvé de façon générale dans les échantillons générés en laboratoire et aucune différence discernable n'est apparue entre les échantillons de pré-ignition et ceux post-brûlés. Par conséquent lorsque les essais de point de fin ont démontré un effet toxique, ils étaient le plus souvent élevés dans les échantillons de pré-ignition et post-brûlés. Cette série d'essais de toxicité, exclusion faite de l'essai de cytogénétique, fut également menée sur les échantillons dit de terrain. Les résultats ont démontrés que les effets toxiques ici, étaient encore plus bas pour ces échantillons. Quant aux échantillons générés en laboratoire, aucune différence marquée n'a été observée entre les échantillons de pré-ignition et ceux post-brûlés.

Les analyses chimiques et les essais de toxicité fait lors de cette étude ont démontré que le brûlage in-situ n'affectait pas de façon négative la tranche d'eau autre que par les effets déjà associés avec le pétrole non-brûlé. Les résultats d'analyse d'eau fait lors de cette étude suggèrent que le potentiel de toxicité ne devrait pas être un facteur empêchant l'utilisation du brûlage in-situ en tant que mesure d'intervention lors de déversements d'hydrocarbures en mer.

Foreword

This document reports on one of the component studies of the Newfoundland Offshore Burn Experiment (NOBE) held in August of 1993. The objectives of NOBE were:

- to obtain measurements of critical burn parameters and to collect and analyze chemical emissions needed for comparison with datasets and models that are currently based on laboratory and medium-scale tests;
- to obtain samples for analysis of the smoke plume, water, and gaseous emissions needed to determine whether the environmental impact of burning is acceptable;
- to conduct a large-scale oil burning experiment in realistic, open-ocean conditions to demonstrate contained burning as a spill response technique;
- to develop a response protocol that will establish operational strategies for burning and safety procedures under a variety of environmental and operational conditions.

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1.0 Introduction

1.1 Background

In-situ burning of marine oil spills has been a controversial issue since it was first tried over 25 years ago. This technique involves the intentional ignition and combustion of spilled oil on the water surface very shortly after the accidental release of the oil. Burning has some distinct advantages over other countermeasures. These include the rapid reduction in the quantity of oil before it spreads out of control and/or contaminates shorelines, and the fact that burning requires little supporting infrastructure/ equipment and can therefore 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 also remain on health and safety risks, environmental effects, and operational constraints.

While substantial information was available about burning from both laboratory and meso-scale experimentation, comparable experiments conducted under realistic, full-scale conditions were lacking. Consequently, in August 1993, a group of 25 agencies from Canada and the United States conducted a major offshore burn near Newfoundland, Canada, involving over 20 vessels, 7 aircraft, and 230 field personnel. Two oil spills of about 50 tons each were released into a fire-proof boom. Each burn was monitored for emissions and physical parameters, yielding data on over 2000 parameters or substances.

It was not known to what extent *in-situ* burning would change the aquatic toxicity of the oil or the toxicity within the water column below the oil slick. Consequently, one of the component studies of the overall burn project was designed to address this issue. This particular study included the chemical analytical and biological effects (toxicity) testing of both laboratory-generated burn samples and full-scale, field-generated burn samples.

Tests were conducted on water samples generated from laboratory burn experiments from June 15 to 19, 1993 and on water samples collected from two full-scale *in situ* oil burn trials conducted off the coast of Newfoundland on August 12, 1993. The toxicity tests conducted in this study included: the echinoderm sperm cell fertilization, the echinoderm larvae, the echinoderm cytogenetic, the bivalve larvae, and the inland silverside juvenile fish tests. Chemical analyses were performed by Environment Canada's Environmental Technology Centre (ETC) and included the determination of 24 target polycyclic aromatic hydrocarbons and total petroleum hydrocarbons.

1.2 Project Objectives

The objectives of the aquatic toxicity studies were to:

- compare the aquatic toxicity (lethal and sublethal) of water beneath unburned and burned crude oil collected in both laboratory and field experiments;
- compare the concentrations of polycyclic aromatic hydrocarbons and total petroleum hydrocarbons in water beneath unburned and burned crude oil collected in both laboratory and field experiments; and
- evaluate the toxicity of solid and semi-solid burn residues remaining after crude oil combustion in laboratory and field experiments. Note: Results from this testing will be provided in a future report.

2.0 Laboratory *In-situ* Burn Testing

2.1 Methods

2.1.1 Burn Apparatus Setup and Testing

The first stage of this project was to generate *in-situ* burn water samples in the laboratory for both toxicity testing and chemical analyses. To safely burn small volumes of crude oil in the laboratory, six identical stainless steel burn units (Figure 1) were designed by the Environmental Technology Centre (Lambert *et al.*, 1993). Each unit consisted of a burn chamber surrounded by a water bath. The units supported a burn of a 1-cm layer of crude oil (100 mL) and generated 1 L of sample water for testing. ASMB Standard Light Crude oil used for testing was supplied by the Environmental Technology Centre (ETC). Two 2-L containers of oil were received on May 24, 1993 and stored in the dark at 4°C.

It was important that each test sample be generated in a consistently reproducible manner and in the shortest possible time. Therefore, trial burns were conducted on May 28, 1993 to refine sample collection skills and to ensure that the burn chambers functioned properly and yielded similar burning conditions (e.g., burn height, burn time, temperature of test water before and after the burns, and sample volume). All burns were conducted at B.C. Research in Vancouver, British Columbia. The capacity of the walk-in fumehood in this facility enabled four burns to be conducted safely at one time. The number of burns conducted simultaneously ranged from one to four.

All burns and sample collections were performed by personnel from EVS Consultants. Burn chambers were cleaned in a fumehood before each use in the following manner, as instructed by the ETC laboratory:

- excess oil was drained;
- remaining oil was removed using 3M polypropylene sorbent pads;
- charring was removed from the edges of the burn chambers using steel wool;
- crucibles were rinsed with methylene chloride and allowed to air dry; and
- crucibles were then rinsed three times with the unfiltered, unsterilized seawater used in the experiments.

The units were transported to the walk-in fumehood and 1 L of unfiltered, unsterilized seawater was added to each burn chamber. Unfiltered, unsterilized seawater was used to represent natural samples that would be collected in the field. Unfiltered, unsterilized seawater at 28 ppt was also added to the surrounding water bath. The seawater used for the burn experiments was obtained from Burrard Inlet, Vancouver, British Columbia, at a depth of 12 m, approximately 200 m from shore. Water used for sample generation was collected the day before use and allowed to equilibrate to room temperature overnight. A 100-mL aliquot of the light crude oil was added to the top of the seawater in the burn chamber using a graduated cylinder to prevent agitation and mixing with the test water. To reduce variability in the replicate burns, the contents of one 2-L sample container were used for the practice burns while the oil from the second 2-L container was used exclusively for generating test samples. The oil was ignited using a propane torch and various parameters of each burn (e.g., burn time, flame height, sample volume generated, and temperature) were recorded.

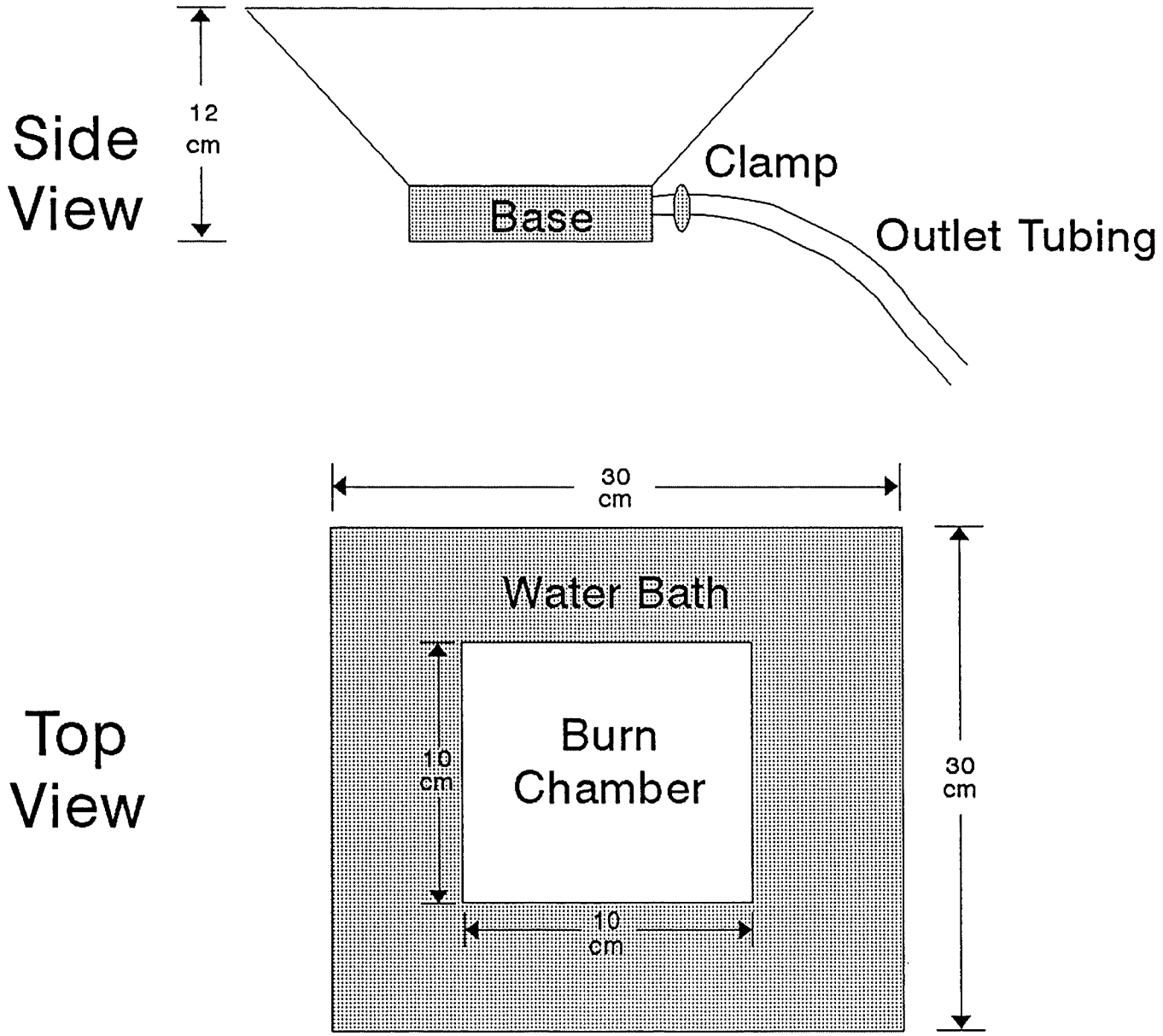


Figure 1 Oil Burn Chamber

Water samples were removed from the burn chamber through the outlet tubing attached to the bottom of the unit. A system of hose clamps and valves were arranged to prevent contamination of the water samples with residue from the burn. Underlying water was drawn out slowly so as to minimize disturbance of the residual surface slick.

2.1.2 Generation of Laboratory Test Samples

Replicate Chemistry Samples - Using the pre-tested burn chambers, water samples were generated for chemical analyses on June 1, 1993. All samples were generated with the methods used during the trial burns except that 1050 mL of unsterilized, unfiltered seawater were added to each crucible. During the trial burns, it was found that this extra volume was required in order to generate the 1-L samples since it was not possible to recover all the test water, as some invariably mixed with the remaining unburned oil and residue. All samples were collected in clean 946-mL glass containers prepared according to the following procedures provided by the ETC laboratory:

- washed with detergent and rinsed with tap water;
- rinsed three times with deionized water;
- rinsed once each with acetone, hexane, and dichloromethane; and
- air-dried.

Five 1-L replicates of the following different water samples were collected:

- **Background** - 1050 mL of unsterilized, unfiltered seawater was placed in the burn chamber and held for 30 minutes before withdrawing the sample.

- **Pre-ignition** - 1050 mL of unsterilized, unfiltered seawater and a 1 cm thick layer of ASMB Light Crude oil (100 mL) was placed in a burn chamber and held for 30 minutes before withdrawing the sample. Samples were collected so that crude oil was not drawn into the sample.

- **Post-burn** - 1050 mL of unsterilized, unfiltered seawater, with a 1 cm thick surface layer of ASMB Light Crude oil (100 mL), was placed in a burn chamber and held for 30 minutes. The oil was then ignited and allowed to burn. After the burn was completed, the water sample was removed.

Remaining burn residue, a thick liquid material found in the interface between the seawater and the remaining unburned oil, was also collected from the post-burn treatments in pre-cleaned glass jars using the same method as for the water samples. The residue samples have been archived at -20°C until an analytical procedure has been finalized by the Scientific Authority. These results will be provided in a future report.

All water samples were kept cool and sent for analysis to ETC by overnight courier. The samples were shipped on ice in two different coolers on the day of generation. One of the coolers containing three samples of each water type (i.e., background, pre-ignition, and post-burn) was received by the chemistry laboratory on June 2, 1993. The other cooler containing the remaining two samples of each water type was misplaced by the courier service and not received until the following day (June 3, 1993). Three additional background samples (Background 6 to 8) were later requested by the analytical laboratory to

provide confirmation since initial test results were highly variable. These samples were generated on June 17, 1993 according to procedures outlined previously and were received by the chemistry laboratory the following day.

Biological Effects Samples - Samples of background, pre-ignition, and post-burn water for toxicity testing were generated in the same manner as the replicate chemistry samples. In order to initiate toxicity testing immediately, sample generation was conducted on June 15, 1993 beginning at 12:00 midnight. Enough sample of each water type was prepared to perform toxicity tests immediately (T = 0 h) and again 48 h later (T = 48 h) to determine possible effects of sample-holding time. Sample-holding time was of concern because field-generated samples could not be tested immediately after generation due to the unavoidable transit time involved in shipping the field samples to the testing laboratory. Differences between samples tested immediately (T = 0 h) and those held for 48 hours (T = 48 h) could indicate possible changes in sample toxicity during the holding period. Samples for holding time (T = 48 h) testing were stored at 4°C in the dark.

Background and pre-ignition samples were prepared at the EVS Consultants laboratory. Post-burn samples were prepared in the walk-in fumehood at B.C. Research. A sufficient volume of each water type was generated to meet the toxicity testing requirements to conduct five separate tests: the echinoderm sperm cell test, the echinoderm larvae test, the echinoderm cytogenetics test, the bivalve larvae test, and the inland silverside juvenile fish test. A total of 7 L was prepared for the background sample, which was tested at 100% concentration only. A total of 11 L was generated for both the pre-ignition and post-burn samples as these samples were tested at various concentrations. The 1-L samples for background, pre-ignition, and post-burn water were generated separately and then composited to yield the three final test samples. All water samples were kept refrigerated until tested. Burn residue samples were generated as described previously and stored at -20°C.

Before initiating toxicity testing at T = 0 and T = 48 h, a 1-L sample was removed from each composite and sent to the ETC for chemical analysis. Additional 1-L samples from the undiluted test solutions used in the inland silverside test were sent for chemical analysis after toxicity tests were completed.

2.1.3 Chemistry

Chemical analyses for the laboratory-generated water samples were carried out at the Environmental Technology Centre (ETC) in Gloucester, Ontario. The samples were analyzed for 24 target polycyclic aromatic hydrocarbons (PAHs) as well as for total petroleum hydrocarbons (TPHs). The report submitted by the ETC is provided in Appendix A.

2.1.4 Toxicity Testing

Toxicity of the water samples generated for biological analysis was determined using three standard marine test organisms: echinoderms (*Dendraster excentricus*), bivalves (*Crassostrea gigas*), and inland silversides (*Menidia beryllina*). The toxicity tests conducted are provided in Table 1.

The echinoderm sperm cell fertilization, echinoderm larvae, and bivalve larvae development tests consisted of duplicate tests (primary and secondary) conducted with two different sets of gametes. The secondary test was counted only if the primary test did not meet specified control criteria or if results needed to be confirmed.

Table 1 Toxicity Tests Conducted

Toxicity Test	Test Organism	Test Endpoints
Echinoderm Sperm Cell Fertilization	*Dendraster excentricus* (Sand Dollar)	Percent Fertilization
Echinoderm Larvae Development	*Dendraster excentricus* (Sand Dollar)	Percent Abnormality Percent Mortality
Echinoderm Cytogenetics	*Dendraster excentricus* (Sand Dollar)	Number of Mitoses per Cell Percent Anaphase Aberrations
Bivalve Larvae Development	*Crassostrea gigas* (Pacific Oyster)	Percent Abnormality Percent Mortality
Inland Silverside Juvenile Fish	*Menidia beryllina* (Inland Silverside)	Percent Mortality

Toxicity testing was initiated immediately following completion of sample generation on June 15, 1993 (T = 0 h) and again 48 h later on June 17, 1993 (T = 48 h) to determine if the toxicity of the original sample changed after a 48-h holding period.

Additional water samples were generated and tested on June 21, 1993 to determine whether filtering and sterilizing seawater and/or holding seawater in the burn chamber had any effects on the toxicity test results. Samples tested were unfiltered unsterilized seawater, unfiltered unsterilized seawater held in the burn chamber for 30 minutes (background sample), and filtered sterilized seawater held in the burn chamber for 30 minutes. These samples were tested using the echinoderm sperm cell fertilization test.

Natural seawater, used for conducting all the toxicity tests and holding the test organisms, was obtained from Burrard Inlet, Vancouver, B.C., at a depth of 12 m, approximately 200 m offshore. Unlike the water used for sample generation, however, this seawater was also filtered through a 5-μm Cuno filter and passed through an ultraviolet-sterilizing apparatus as recommended for toxicity testing. The filtered UV-sterilized seawater was warmed to test temperature, aerated vigorously, and used within 24 h of collection.

Echinoderm Sperm Cell Fertilization Test - The echinoderm sperm cell fertilization toxicity tests using the sand dollar, *Dendraster excentricus*, were conducted according to procedures described in Environment Canada (1992). Tests were conducted at 15°C ± 1°C in a constant environment room and were completed within 3 to 5 h.

Sand dollars used for both tests (T = 0 h and T = 48 h) were collected from Semiahmoo Spit, Washington, on June 3, 1993. The animals were transported in approximately 20 cm of sediment with 10 L of overlying seawater. Following arrival in the laboratory, the sand dollars were held in a flow-through filtered seawater system and fed a daily diet of eel grass. An organism receipt log was completed following receipt and kept on file. A logbook was maintained documenting daily monitoring at the holding facility.

Tests were conducted in 15-mL glass test tubes with 10-mL test volumes. Pre-ignition and post-burn samples were tested at five different concentrations [100, 50, 25, 12.5, and 6.25% (volume/volume)]. These test treatments were prepared simultaneously for both the echinoderm sperm cell fertilization test and the echinoderm larvae test. Background samples were tested only at 100% (v/v). A negative (clean) control consisting of filtered, sterilized seawater was tested concurrently with each sample. Three replicates were prepared for each test concentration and control. 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 0.5 M 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 to 30 minutes. Sperm were collected with a glass pipette, transferred to small beakers, and stored on ice until used. Before beginning the test, the sperm-to-egg ratio yielding closest to 90% fertilization in control seawater was determined. The gametes were examined microscopically to assess their quality, and their densities were adjusted to appropriate levels.

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. The percentage of 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:

$$Mean\ \%\ Unfertilized = \frac{sum\ of\ all\ unfertilized\ eggs\ in\ 3\ replicates}{sum\ of\ all\ eggs\ in\ 3\ replicates} \times 100$$

All data were also corrected for the control response using Abbott's formula:

$$Mean\ Net\ \%\ Unfertilized = \frac{\%\ test\ response\ -\ \%\ control\ response}{100\ -\ \%\ control\ response} \times 100$$

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°C ± 1°C in a constant environment room. A 14 h:10 h, light:dark photoperiod was maintained during the 48-h exposure.

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. Pre-ignition and post-burn samples were tested at the same concentrations used for the echinoderm sperm cell tests [i.e., 100, 50, 25, 12.5, and 6.25% (v/v)]. Background samples were tested only at 100%. Two sets of negative controls were tested concurrently with samples for each test. These negative controls

included seawater alone and 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. 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 to 60 min. Fertilization was accomplished by combining the eggs from several females and 20 to 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 2000 to 3000 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 one set of subsamples 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 10-mL subsample was also collected and archived as a backup 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 test was based on abnormal development and mortality. Larvae that 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 percentage of abnormality was calculated using the following equation:

$$\textit{Mean Abnormality (\%)} = \frac{\textit{sum of all abnormal larvae in 3 replicates}}{\textit{sum of all larvae in 3 replicates}} \times 100$$

Mortality was calculated using the following equation:

$$\textit{Mean Mortality (\%)} = 100 - \frac{(\textit{mean no. of larvae recovered from 3 replicates}) \left(\frac{100\ \textit{mL test volume}}{10\ \textit{mL subsample}} \right)}{\textit{no. of embryos introduced}} \times 100$$

Echinoderm Cytogenetics Test - The echinoderm cytogenetics test was conducted by EVS Consultants and Dr. R. Kocan of the University of Washington. EVS Consultants performed the embryo exposure

along with the sampling and preservation, while Dr. Kocan provided the chromosome analysis and technical interpretation.

Echinoderm cytogenetics tests using the sand dollar, *Dendraster excentricus*, were conducted based on procedures described in ASTM (1989), PSEP (1991), Liguori and Landolt (1984), Kocan *et al.* (1982), and Nichols *et al.* (1977). Embryo exposure was conducted at 15°C ± 1°C in a constant environment room and a 14 h: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 setup and spawning of the sand dollars were conducted according to methods described for the echinoderm larvae test. Within 2 h of fertilization, test containers were inoculated with approximately 3000 embryos. Embryo inoculation was conducted in the same manner as 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. The first set of preserved samples was sent to Dr. Kocan. The second set of 10-mL aliquots was collected in the event that results needed to be confirmed. 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.

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 to 3 mL) was added to 100 mL of clean seawater and treated in the same manner as the test containers.

Following receipt at the University of Washington laboratory, 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 to 1000 magnification. Due to the lower than expected embryo densities in the samples, it was necessary to send the backup vials and pool the two sample sets.

The two endpoints measured in the echinoderm cytogenetics test were the mean number of mitoses and the percentage of abnormal anaphase nuclei. Toxic effects were indicated by a decrease in mitotic activity or 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). Tests were conducted at 20°C ± 1°C in a constant environment room. A 14 h:10 h, light:dark photoperiod was maintained during the 48-h exposure.

Oysters were obtained from Kim Siewers Inc. in California. The test organisms were transported by overnight courier in styrofoam coolers and received on June 3, 1993. Following arrival in the laboratory, the oysters were held in a flowthrough seawater system and fed a daily diet of a mixture of re-suspended

algal paste and microencapsulated feed. The oysters were maintained in spawning condition by thermal conditioning, increased photoperiod, and increased feeding. An organism receipt log was completed upon arrival and kept on file. A logbook was maintained documenting daily monitoring at the holding facility.

The tests were conducted in 15-mL glass test tubes with 10-mL test volumes. Pre-ignition and post-burn samples were tested at five concentrations [100, 50, 25, 12.5, and 6.25% (v/v)]. Background samples were tested only at 100%. Two sets of negative controls were tested concurrently with samples for each test and included seawater alone and additional "zero-time" seawater controls to confirm embryo inoculation density and monitor larval development.

Three replicates were prepared for each control and test concentration. Measurements of water quality parameters (pH, dissolved oxygen, salinity, and temperature) were recorded in the controls as well as in the 100, 25, and 6.25% (v/v) sample concentrations 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 to 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 counting the number of embryos in three 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 that 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:

$$\textit{Mean Abnormality (\%)} = \frac{\textit{sum of all abnormal larvae in 3 replicates}}{\textit{sum of all larvae in 3 replicates}} \times 100$$

Mortality was calculated using the following equation:

$$Mean\ Mortality\ (\%) = 100 - \left(\frac{mean\ no.\ of\ larvae\ recovered\ from\ 3\ replicates}{no.\ of\ embryos\ introduced} \right) x\ 100$$

Inland Silverside Test - Acute lethality tests with juvenile inland silverside (*Menidia beryllina*) were conducted as described in U.S. EPA (1991). Tests were conducted in a constant environment room at 25°C ± 2°C. A 16 h:8 h, light:dark photoperiod was maintained for the 96-h exposure.

For the T = 0 h testing, 10-day-old inland silverside juveniles were obtained from Aquatic Resources in California on June 11, 1993. These fish were 14 days old when the tests were initiated on June 15, 1993. Nine-day-old inland silversides were obtained from the same supplier on June 15, 1993. These fish were 11 days old at test initiation on June 17, 1993 for the T = 48 h tests. On both occasions, the test animals were transported by overnight courier in styrofoam coolers. The juvenile fish were held in a 38-L aquaria containing natural seawater and fed < 24-h-old *Artemia* nauplii before testing. Seawater in the holding containers was replaced every second day. Injured, unhealthy, or dead animals were discarded before testing. An organism receipt log was completed following receipt of each supply of fish and kept on file.

The test containers were 1-L glass beakers. Pre-ignition and post-burn samples were tested at five concentrations [100, 50, 25, 12.5, and 6.25% (v/v)]. Background samples were tested only at 100%. A negative control consisting of natural seawater was tested concurrently with each sample. Three replicates were prepared for each control and test concentration.

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. 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 (≥ 90%) after the 96-h exposure for both the T = 0 h and T = 48 h tests. Control survival did meet necessary criteria after 48-h exposure. The 48-h exposure results were reported as additional data.

2.1.5 Statistical Methods

Two-sample t-tests were performed using the STATISTIX computer program (NH Analytical Software, 1986) to compare the background samples to the negative controls. Statistical analyses of the test samples were performed using the TOXSTAT computer program (Gulley *et al.*, 1990) to calculate the NOEC (No-Observed-Effect-Concentration) values. The data were transformed before 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 before detailed analysis. Significant differences (P <0.05) in percentage of unfertilized eggs or percentage of abnormal larvae development were determined for each sample 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. Two sample t-tests were also performed to compare the 100% (v/v) pre-ignition to the 100% (v/v) post-burn samples. Data from the cytogenetics test were evaluated using an ANOVA and Fisher's Exact Test. Significant differences were determined at $P < 0.05$ or $P < 0.01$. No statistical analyses were performed on the inland silverside data.

2.1.6 Reference Toxicant Tests

Positive (toxic) controls were also conducted using the reference toxicant cadmium chloride ($CdCl_2$) for each of the tests. A series of test concentrations, with two replicates each, were prepared from a 1000 mg/L of Cd stock solution. The reference toxicant tests were set up at the time of sample testing and treated in the same manner. The EC50 values were calculated using the EFFL program (version 1.0) as detailed by Stephan (1977). The results of the reference toxicant test were used to assess the relative sensitivity of test organisms by comparing the EC50 with the range of acceptable values (mean ± 2SD) obtained by the laboratory in previous testing.

2.2 Results

The average length of each of the lab burns was approximately 14 min. Flame heights ranged from 30 to 60 cm. Initial temperatures recorded in the water baths were approximately 20°C. Temperatures recorded in the water baths after the flames had extinguished themselves ranged as follows: 24 to 30°C on the surface, 23 to 24°C halfway down in the water bath, and 20 to 22°C at the bottom of the water bath.

2.2.1 Chemistry

Replicate Chemistry Samples - Results for the replicate chemistry samples (generated on June 1, 1993) are fully described in Appendix A and summarized in Tables 2 and 3. Unfortunately, results for seven of the fifteen samples (Background 1 to 3, Pre-ignition 1 to 2, and Post-burn 1 to 2) suggest that they may have been contaminated since they showed very high concentrations of polycyclic aromatic hydrocarbons (PAHs) and total petroleum hydrocarbons (TPHs). Consequently, these samples have been omitted in the data analysis. Results from the chemical analysis of the remaining samples did indicate a possible trend of PAH composition as well as total PAH concentration from the background samples (Table 2). 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 3.79 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 compounds, and the three post-burn samples contained between five and ten of the target compounds.

Naphthalene and alkyl homologues of naphthalene were the main components in the pre-ignition and post-burn samples. This was likely due to their relatively higher solubilities in water compared to the other PAH target compounds. Burning the oil resulted in a greater number of naphthalene compounds detected as well as other PAHs. However, no PAH compound with higher than three rings was found in any of the samples. The highest ring number PAH was phenanthrene which was found in the post-burn sample.

Table 2 Mean PAH Results for Replicate Chemistry Samples

Compound	Background[1] (μg/L H_2O)	Pre-ignition[1] (μg/L H_2O)	Post-burn[1] (μg/L H_2O)
Sampling Date	June 17, 1993	June 1, 1993	June 1, 1993
naphthalene	ND	0.96	1.70
2-methyl-naphthalene	ND	0.53	0.89
1-methyl-naphthalene	ND	0.43	0.64
biphenyl	ND	ND	0.03
2,6-dimethyl-naphthalene	ND	0.11	0.22
acenaphthylene	ND	ND	0.14
acenaphthene	ND	ND	ND
2,3,5-trimethyl-naphthalene	ND	ND	0.03
fluorene	ND	ND	0.03
dibenzothiophene	ND	ND	0.03
phenanthrene	ND	ND	0.07
1-methyl-phenanthrene	ND	ND	ND
fluoranthene	ND	ND	ND
pyrene	ND	ND	ND
benz(a)anthracene / chrysene	ND	ND	ND
benz(b)fluoranthene / b(k)fluor.	ND	ND	ND
benzo(e)pyrene / benzo(a)pyrene	ND	ND	ND
perylene	ND	ND	ND
indeno(1,2,3-cd) pyrene	ND	ND	ND
dibenz (a,h) anthracene	ND	ND	ND
benzo (g,h,i) perylene	ND	ND	ND
Total PAH	ND	2.03	3.78

[1] The mean values listed for the background, pre-ignition, and post-burn samples exclude those samples suspected of contamination and are averages from five (Background 4 to 8), two (Pre-ignition 4 to 5), and three (Post-burn 3 to 5) measurements, respectively.

ND Not detected

TPH analysis of the water samples showed a more consistent relationship between the background, pre-ignition, and post-burn samples (Table 3). 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. Because TPHs were detected in the background seawater, PAH levels may be a more suitable measure for interpreting oil-related effects. However, the questionable results in the first few individual samples restricted the number of data points, making comparisons between replicates and between different water types less conclusive.

Table 3 Results of PAH and TPH Analyses of Laboratory Chemistry Samples

Sample Number	Total PAH (μg/L H_20)			TPH (μg/L H_20)		
	Background	Pre-ignition	Post-burn	Background	Pre-ignition	Post-burn
3	ND	-[1]	2.12	-[1]	-[1]	37
4	ND	1.94	3.78	26	44	43
5	ND	2.09	5.47	20	38	51
6	ND	-[2]	-[2]	32	-[2]	-[2]
7	ND	-[2]	-[2]	62	-[2]	-[2]
8	ND	-[2]	-[2]	62	-[2]	-[2]
Average	ND	2.03	3.79	40	41	44

[1] Samples were omitted due to possible contamination
[2] No samples were collected
ND Not detected

Biological Effects Samples - 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 (Table 4). As for the replicate chemistry samples, none of the target PAHs was detected in the background water. Water samples sent to the ETC laboratory after the 48-h holding period showed similar total PAH levels, 0.67 μg/L in pre-ignition and 5.05 μg/L in post-burn samples. Samples collected from the undiluted treatments at the termination of each set of 96-h inland silverside tests (i.e., T = 0 h and T = 48 h) showed no detectable target PAH compounds in any of the samples. This decrease in PAH levels may have resulted from uptake by the juvenile fish, adsorption to the fish, or adsorption to the test containers.

Concentrations of TPH 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.

Table 4 Results of PAH and TPH Analyses in Biological Effects Laboratory Samples

Compound	Background Initial[1] (μg/L H_2O)	Pre-ignition Initial[1] (μg/L H_2O)	Post-burn Initial[1] (μg/L H_2O)	Background Final[2] (μg/L H_2O)	Pre-ignition Final[2] (μg/L H_2O)	Post-burn Final[2] (μg/L H_2O)
T = 0 h						
Total PAH	ND	1.83	4.27	ND	ND	ND
Total TPH	30	31	47	129	146	112
T = 48 h						
Total PAH	ND	0.67	5.05	ND	ND	ND
Total TPH	100	77	109	62	80	86

[1] Initial samples were collected before initiating toxicity testing
[2] Final samples were collected at the termination of the 96-h inland silverside test
ND Not detected

2.2.2 Toxicity Testing

Echinoderm Sperm Cell Fertilization Test - Results of the echinoderm sperm cell fertilization tests conducted at T = 0 h and T = 48 h are summarized in Tables 5 and 6. Complete data are provided in Appendix B.

T = 0 h Results - Mean percent fertilization in the seawater controls for each sample exceeded the minimum 50% criterion set by Environment Canada (1992). The sperm-to-egg ratio yielding 70 to 90% fertilization was 200:1.

The differences in mean percent unfertilized eggs between the pre-ignition and post-burn samples are shown in Figure 2. The mean percent unfertilized eggs in the 100% (v/v) post-burn sample, 73.3%, was much higher than that observed in the pre-ignition sample, 36.7%. Mean percent unfertilized eggs was similar at lower concentrations. The background percent unfertilized eggs was similar to that found in the pre-ignition sample and was 38.0%.

A two-sample t-test indicated that the background sample was significantly different ($P<0.05$) than the negative control. 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 ($P<0.05$) in fertilization between treatments. The NOEC value for the pre-ignition was <6.25% (v/v). Dunnett's t-test showed that the post-burn sample was significantly different than the control at 6.25, 50, and 100% (v/v), but not at 12.5 and 25% (v/v). Therefore, a conservative estimate of the NOEC value for the post-burn sample would be <6.25% (v/v). The EC50 values were >100% (v/v) and 76.4% (v/v) for the pre-ignition and post-burn samples, respectively. A two-sample t-test indicated that the 100% (v/v) pre-ignition sample was significantly lower than the 100% (v/v) post-burn sample.

Table 5 Summary of Echinoderm Sperm Cell Fertilization Toxicity Test Results

Test	Sample	Mean % Unfertilized	Mean Net % Unfertilized
T = 0 h	Background		
	100%	38.0*	35.4
	Control	4.0	0.0
	Pre-ignition		
	100%	36.7*	34.0
	50%	26.0*	22.9
	25%	16.7*	13.2
	12.5%	14.7*	11.1
	6.25%	11.0*	7.3
	Control	4.0	0.0
	Post-burn		
	100%	73.3*	69.8
	50%	29.3*	20.0
	25%	16.3	5.3
	12.5%	17.3	6.4
	6.25%	29.0*	19.6
	Control	11.7	0.0
T = 48 h	Background		
	100%	23.7*	15.2
	Control	10.0	0.0
	Pre-ignition		
	100%	24.3	8.8
	50%	28.7	14.1
	25%	32.3	18.5
	12.5%	25.7	10.4
	6.25%	15.7	-1.6[1]
	Control	17.0	0.0
	Post-burn		
	100%	26.0	11.6
	50%	19.3	3.6
	25%	29.7*	15.9
	12.5%	27.7*	13.5
	6.25%	21.3	6.0
	Control	16.3	0.0

* Values significantly different ($P<0.05$) than the control

[1] Negative value indicates that the percentage of unfertilized eggs was lower than the control

Table 6 Summary of Toxicity Endpoints - Laboratory Samples

Sample	Echinoderm Sperm Cell				Echinoderm Larvae				Bivalve Larvae			
	NOEC[1]		EC50[1]		NOEC[1]		EC50[1]		NOEC[1]		EC50[1]	
	vs Control	vs Background	vs Control	vs Background	vs Control	vs Background	vs Control	vs Background	vs Control	vs Background	vs Control	vs Background
T = 0 h												
Pre-ignition	<6.25	100	>100	>100	6.25[3]	100	>100	>100	6.25	6.25	>100	>100
Post-burn	<6.25[2]	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[4]	100	>100	>100	<6.25	6.25	>100	>100	6.25	6.25	>100	>100

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

2 12.5% and 25% (v/v) were not significantly different ($P<0.05$)

3 25% (v/v) was not significantly different ($P<0.05$)

4 50% and 100% (v/v) were not significantly different ($P<0.05$)

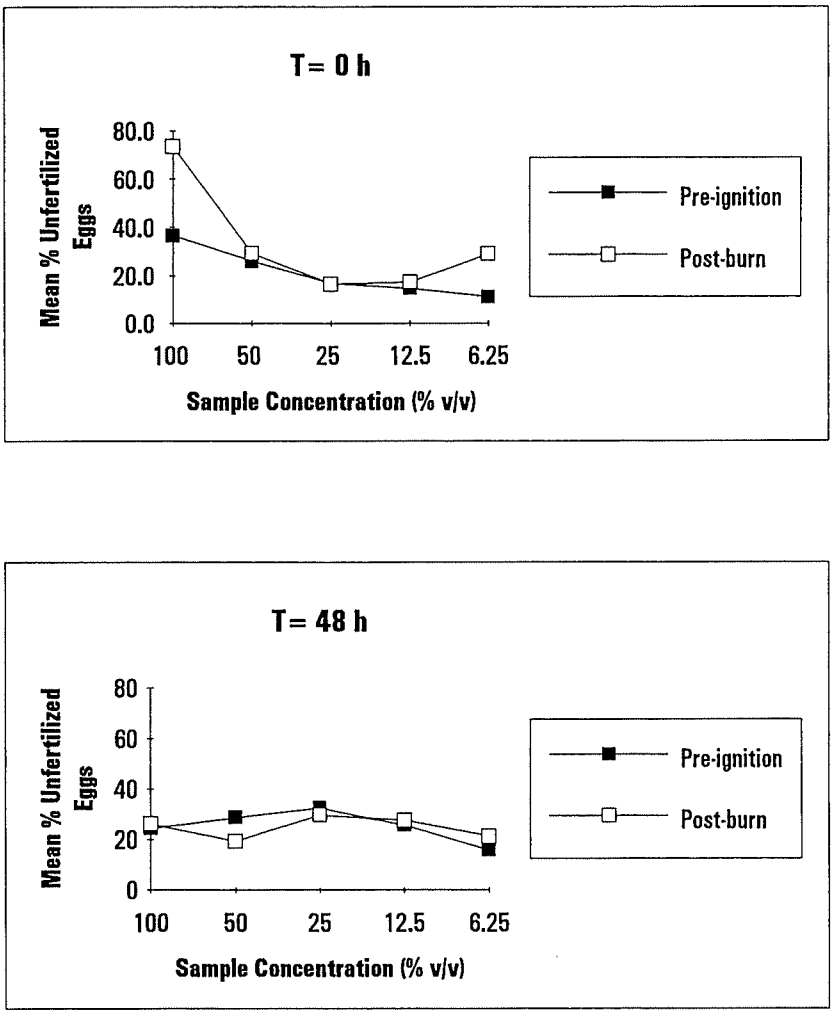


Figure 2 Echinoderm Sperm Cell Fertilization

Statistical comparisons between the pre-ignition and the background found no significant difference between these two samples. However, the post-burn sample was found to be significantly different than the background. Higher NOEC and EC50 values were obtained when compared to the background sample than those reported when the treatments were compared to their control. The NOEC values for the pre-ignition and post-burn samples were 100% and 50% (v/v), respectively. The EC50 value was <100% (v/v) for the pre-ignition sample and 94.2% for the post-burn sample.

Water quality parameter ranges measured in the background, pre-ignition, and post-burn samples before testing were: temperature 15°C; pH 7.6 to 7.7; dissolved oxygen 8.0 to 8.8 mg/L; and salinity 27 to 29 ppt.

The 20-min EC50 value for the reference toxicant, cadmium chloride ($CdCl_2$) was <0.32 mg/L of Cd. A laboratory mean value for cadmium has not yet been generated. The EC50 values for a 60-min sperm exposure obtained by the laboratory ranged from 4.5 to 16.0 mg/L of cadmium.

T = 48 h Results - Mean percent fertilization in the seawater controls for each sample exceeded the minimum acceptable test criteria. For this test, the sperm-to-egg ratio yielding 70 to 90% fertilization was 2000:1.

Mean percent unfertilized eggs were much lower than those obtained from the T = 0 toxicity tests, ranging in the 100% (v/v) sample treatments from 232.7 to 26.0%. The mean percent unfertilized eggs in the post-burn sample, 26.0%, was only slightly higher than that found in the pre-ignition sample, 24.3%. However, the mean percent unfertilized eggs in the background sample was also similar at 23.7%. Figure 2 shows that the mean percent unfertilized eggs in the pre-ignition and post-burn samples were very similar.

A two-sample t-test indicated that the background sample was significantly different ($P<0.05$) than the negative control. 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 ($P<0.05$) in fertilization between treatments. The NOEC value for the pre-ignition sample was 100% (v/v). Dunnett's t-test showed that the post-burn sample was significantly different at 12.5 and 25% (v/v), but not at 6.25, 50, and 100% (v/v). Therefore, a conservative estimate of the NOEC value for the post-burn sample would be 6.25% (v/v). The EC50 values were both >100% (v/v). A two-sample t-test indicated the 100% (v/v) pre-ignition sample was not significantly different than the 100% (v/v) post-burn sample.

Neither the pre-ignition nor the post-burn sample was found to be significantly different from the background sample. The NOEC values were 100% (v/v) for both samples. The EC50 values remained the same at >100% (v/v).

Water quality parameter ranges measured in the background, pre-ignition, and post-burn samples before testing were: temperature 15°C; pH 7.6 to 7.7; dissolved oxygen 8.3 to 8.4 mg/L; and salinity 28 ppt. The 20-min EC50 value for the reference toxicant, cadmium chloride ($CdCl_2$), was 2.9 mg/L of Cd.

Comparison Between Filtered, Sterilized and Unfiltered, Unsterilized Seawater - Results of the echinoderm sperm cell fertilization test comparing unfiltered, unsterilized seawater to filtered, sterilized seawater are summarized in Table 7. Mean percent fertilization in the seawater control exceeded the minimum 50% criterion specified by Environment Canada (1992). The sperm-to-egg ratio yielding 70 to 90% fertilization was 200:1.

The data 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 compared to the seawater control. Dunnett's t-test indicated that the unfiltered, unsterilized seawater, the filtered, sterilized seawater held in the burn chamber, and the background sample were significantly different ($P<0.05$) than the seawater control. Mean percent unfertilized eggs in the unfiltered, unsterilized seawater, the filtered, sterilized seawater in the burn chamber, and the background samples were 56.7, 26.0, and 42.0, respectively.

Echinoderm Larvae Test - Results of the echinoderm larvae toxicity tests conducted at T = 0 h and T = 48 h are summarized in Tables 6 and 8. Complete data are provided in Appendix C.

Table 7 Comparison Between Filtered, Sterilized and Unfiltered, Unsterilized Seawater

Sample	Mean % Unfertilized	Mean Net % Unfertilized
Background	42.0*	32.3
Unfiltered, unsterilized seawater	56.7*	49.4
Filtered, sterilized seawater + 30 min. in burn chamber	26.0*	13.6
Control	14.3	0.0

* Values significantly different ($P<0.05$) than the control

T = 0 h Results - Mean percent abnormality and mortality in the seawater controls were within acceptable criteria (≤10% abnormality and ≤30% mortality) as set by ASTM (1989) and PSEP (1991). The initial density of embryos introduced into each test container was confirmed to be 2300 embryos/100 mL.

Mean percent abnormalities in all three samples were relatively low and ranged in the 100% (v/v) test concentrations from 15.4 to 23.6%. Mean percent abnormalities in the pre-ignition and post-burn samples were very similar to one another, as shown in Figure 3. The percent abnormality in the background was the highest at 23.6%. Due to the high abnormality observed in the background sample, the secondary echinoderm larvae development test was counted to confirm the results. Only the 50 and 100% (v/v) test concentrations were counted for the pre-ignition and post-burn samples. Results indicated that the abnormality was actually lower at 7.0% in the background sample, but were comparable at 23.4 and 27.0% for the 100% (v/v) pre-ignition and 100% (v/v) post-burn samples, respectively. Results from the secondary test are summarized and provided in Appendix C.

A two-sample t-test indicated that the background sample was significantly different ($P<0.05$) than the negative control. 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 ($P<0.05$) in larval abnormality between treatments. Dunnett's t-test showed that the pre-ignition sample was significantly different at the 12.5, 50, and 100% (v/v) concentrations but not at 6.25 and 25% (v/v). A conservative estimate of the NOEC value would therefore be 6.25% (v/v). The NOEC value for the post-burn sample was 12.5% (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 both samples. A two-sample t-test indicated that the 100% (v/v) pre-ignition sample was not significantly different than the 100% (v/v) post-burn sample.

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

Table 8 Summary of Echinoderm Larvae Toxicity Test Results

Test	Sample	Mean % Abnormal	Mean Net % Abnormal	Mean % Mortality[1]
T = 0 h	Background	23.6*	19.5	9.1
	100%			
	Control	5.1	0.0	-12.8
	Pre-ignition			
	100%	16.2*	9.7	-18.1
	50%	10.6*	3.7	-21.7
	25%	9.9	2.9	-12.8
	12.5%	11.5*	4.6	-12.6
	6.25%	9.1	2.1	-4.8
	Control	7.2	0.0	-7.2
	Post-burn			
	100%	15.4*	10.5	-11.2
	50%	13.5*	8.5	-18.4
	25%	12.0*	6.9	-13.6
	12.5%	7.5	2.2	-5.7
	6.25%	6.6	1.2	-7.2
	Control	5.5	0.0	-16.5
T = 48 h	Background			
	100%	8.4*	5.1	-9.3
	Control	3.5	0.0	-12.0
	Pre-ignition			
	100%	12.1*	9.6	0.9
	50%	14.9*	12.6	9.9
	25%	8.5*	5.9	-13.6
	12.5%	9.4*	6.8	0.3
	6.25%	6.5*	3.8	-11.6
	Control	2.7	0.0	-12.4
	Post-burn			
	100%	14.1*	10.9	-8.7
	50%	10.5*	7.1	0.0
	25%	11.0*	7.6	-11.9
	12.5%	13.5*	10.3	-5.5
	6.25%	7.3*	3.8	-11.2
	Control	3.6	0.0	-13.3

* Values significantly different ($P<0.05$) than the control

[1] Negative % mortality values indicate that the surviving larvae concentrations exceeded those found in the "zero-time" controls used to determine the inoculation density. This apparent negative effect is due to variability associated with subsampling as well as in estimating inoculation density.

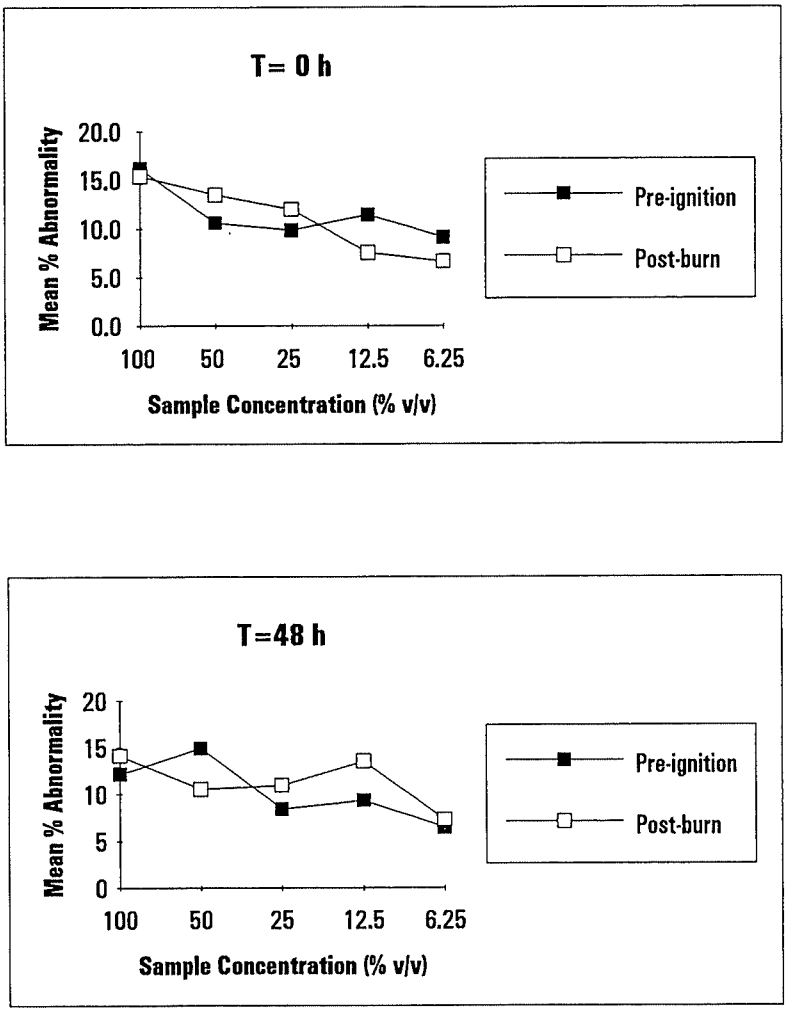


Figure 3 Echinoderm Larvae Abnormality

Water quality parameter ranges measured in the background, pre-ignition, and post-burn samples before testing were: temperature 15°C; pH 7.6 to 7.7; dissolved oxygen 8.0 to 8.8 mg/L; and salinity 27 to 29 ppt. The water quality parameters measured at the end of the test were: temperature 16°C; pH 7.7; dissolved oxygen 7.8 to 8.1 mg/L; and salinity 30 to 31 ppt.

The 48-h EC50 value for the reference toxicant, cadmium chloride ($CdCl_2$), was 13.9 mg/L of Cd. A laboratory mean value for cadmium has not yet been generated. However, this value was consistent with the range of values (8 to 10 mg/L of Cd) obtained by another toxicity testing laboratory that conducts the same test, using the same test organism and reference toxicant.

T = 48 h Results - Mean percent abnormality and mortality in the seawater controls were within acceptable test criteria. The initial density of embryos introduced into each test container was confirmed to be 2500 embryos/100 mL.

As was reported for the T = 0 h toxicity tests, mean percent abnormalities in the background, pre-ignition, and post-burn samples were relatively low, ranging from 8.4 to 14.1% in the 100% (v/v) test treatments. Mean abnormalities in the pre-ignition and post-burn samples remained similar to T = 0 h values as shown in Figure 3 and were 12.1 and 14.1%, respectively. However, the mean percent abnormality in the background of 8.4% was much lower than the T = 0 value of 23.6%. Results from the secondary test showed similar mean abnormalities and ranged from 7.9 to 15.3% in the 100% (v/v) test treatments.

A two-sample t-test indicated that the background sample was still significantly different ($P<0.05$) than the seawater control. 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 ($P<0.05$) in larval abnormality between treatments. The NOEC values for the pre-ignition and post-burn samples were both <6.25% (v/v). Although the NOEC values were low, the data showed that mean abnormality was approximately 15% or less at all concentrations. As for the T = 0 h tests, these relatively low abnormalities are not considered to be biologically significant. The EC50 values were >100% (v/v) for both samples. A two-sample t-test indicated that the 100% (v/v) pre-ignition sample was not significantly different than the 100% (v/v) post-burn sample.

Statistical comparisons between the pre-ignition and post-burn samples and the background yielded lower NOEC values than those obtained when the treatments were compared to their respective negative control. The NOEC values for the pre-ignition and post-burn samples were 25 and 6.25% (v/v), respectively. The EC50 values remained the same at >100% (v/v) for both samples.

Water quality parameter ranges measured in the background, pre-ignition, and post-burn samples before testing were: temperature 15°C; pH 7.6 to 7.7; dissolved oxygen 8.3 to 8.4 mg/L; and salinity 28 ppt. The water quality parameters measured at the end of the test were: temperature 15°C; pH 7.7 to 7.9; dissolved oxygen 7.8 to 8.1 mg/L; and salinity 27 to 28 ppt.

The 48-h EC50 value for the reference toxicant, cadmium chloride ($CdCl_2$), was 8.6 mg/L of Cd which was within the range of values (8 to 10 mg/L Cd) obtained by another toxicity testing laboratory which conducts the same test, using the same test organisms and same reference toxicant.

Echinoderm Cytogenetics Test - Results of the echinoderm cytogenetics test conducted at T = 0 h and T = 48 h are summarized in Table 9. Complete data are included in the report prepared by Dr. Kocan provided in Appendix D.

T = 0 h Results - No toxic response was found in either mitotic activity or in the number of anaphase aberrations in the background sample. A genotoxic effect was found in the 100% (v/v) pre-ignition sample since the mean number of mitoses per embryo was significantly lower ($P<0.05$) than the negative control. The data for the anaphase aberration also showed a genotoxic effect since the pre-ignition sample produced a significant increase in the number of aberrations. In contrast, the 100% (v/v) post-burn sample showed a significant increase in the number of mitoses per embryo and an unexplained decrease in anaphase damage compared to the pre-ignition sample. This 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 either water quality or exposure conditions; however, since there was no increase in

Table 9 Summary of Echinoderm Cytogenetics Toxicity Test Results

Sample ID		Mean # Mitoses/Embryo	Mean % Abnormal Anaphase
T = 0 h (June 15, 1993)			
Background	100%	20.5	2.7
	Control	19.0	1.7
Pre-ignition	100%	20.4+	4.3+
	Control	26.6	1.7
Post-burn	100%	27.1*	5.0
	Control	21.5	7.0
Positive Control[1] (Burn Residue)		31.0+	15.3+
T = 48 h (June 17, 1993)			
Background	100%	26.3	8.1
	Control	30.2	6.5
Pre-ignition	100%	23.4	11.8
	Control	19.9	12.2
Post-burn	50%[2]	31.8	12.5+

* Values significantly different ($P<0.05$) than the control

\+ Values significantly different ($P<0.01$) than the control

1 Statistical comparisons were made between the positive control and the negative control sent with the 0-h background sample.

2 Reliable data could not be obtained for either the 100% post-burn 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.

Note: n = 30 (3 replicates of 10) for all except the following:

T = 0 h	Control (Post-burn)	n = 23 (replicates of 10, 10, and 3)
T = 48 h	Control (Pre-ignition)	n = 28 (replicates of 10, 10, and 8)
T = 48 h	Pre-ignition (100%)	n = 24 (replicates 10, 9, and 5)
T = 48 h	Post-burn (50%)	n = 20 (replicates of 10, and 10)

chromosome damage, there was also no indication of increased genotoxicity. A significant increase was found in both mitotic activity and chromosome damage in the positive control samples spiked with the burn residue when compared to the background negative control.

T = 48 h Results - No significant differences were found in either mitotic activity or in the number of anaphase aberrations in the background or 100% (v/v) pre-ignition sample. Due to lower than expected embryo densities, the negative control sent with the post-burn samples and the 100% (v/v) post-burn test

concentration could not be analyzed for genotoxicity. As a result, test endpoints for the 50% (v/v) 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 - Results of the bivalve larvae toxicity tests conducted at T = 0 h and T = 48 h are summarized in Tables 6 and 10. Complete data are provided in Appendix E.

T = 0 h Results - Mean percent abnormality and mortality in the negative controls were within acceptable criteria (≤10% abnormality and ≤30% mortality) set by ASTM (1989). The initial density of embryos introduced into each test container was confirmed to be 340 embryos/10 mL.

Mean percent abnormalities in the samples were relatively low, ranging from 5.7 to 30.7% in the 100% (v/v) test treatments. The mean percent abnormality in the 100% (v/v) pre-ignition sample, 30.7%, was slightly higher than that observed in the 100% (v/v) post-burn sample, 19.1%, as shown in Figure 4. A two-sample t-test indicated that the 100% (v/v) pre-ignition sample was significantly higher than the 100% (v/v) post-burn sample.

Mean abnormality was lowest in the background sample which reported a value of 5.7%. However, a two-sample t-test indicated that the background sample was still significantly different ($P<0.05$) than the negative control. 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 treatments. The NOEC values for the pre-ignition and post-burn samples were both 6.25% (v/v). Although the NOEC values were low, the data showed relatively low mean abnormalities, approximately 30% or less in all treatments. The EC50 values were >100% (v/v) for both samples. The NOEC and EC50 values remained the same for both the pre-ignition and post-burn samples when statistical comparisons were made against the background sample.

Water quality parameters measured in the background, pre-ignition, and post-burn samples before testing were: temperature 20°C; pH 7.6; dissolved oxygen 8.2 mg/L; and salinity 29 ppt. The water quality parameters measured at the end of the test were: temperature 21°C; pH 7.6 to 7.8; dissolved oxygen 7.6 to 7.8 mg/L; and salinity 27 to 28 ppt.

The 48-h EC50 value for the reference toxicant, cadmium chloride ($CdCl_2$), was 1.0 mg/L of Cd. This value was within the range of values (0.3 to 2.8 mg/L of Cd) obtained by the laboratory in previous testing. A laboratory mean (± 2 SD) for cadmium has not yet been generated.

T = 48 h Results - Mean percent abnormality and mortality in the seawater controls were within acceptable test criteria. The initial density of embryos introduced into each test container was confirmed to be 330 embryos/10 mL.

Table 10 Summary of Bivalve Larvae Toxicity Test Results

Test	Sample	Mean % Abnormal	Mean Net % Abnormal	Mean % Mortality[1]
T = 0 h	Background			
	100%	5.7*	2.7	-4.9
	Control	3.1	0.0	0.9
	Pre-ignition			
	100%	30.7*	27.9	-0.9
	50%	22.1*	18.9	8.1
	25%	13.7*	10.1	-5.4
	12.5%	11.8*	8.2	3.0
	6.25%	6.1	2.2	-11.1
	Control	3.9	0.0	0.6
	Post-burn			
	100%	19.1*	15.9	3.8
	50%	16.2*	13.0	7.0
	25%	12.8*	9.4	16.3
	12.5%	10.4*	6.9	14.9
	6.25%	6.5	2.9	6.2
	Control	3.7	0.0	-3.1
T = 48 h	Background			
	100%	4.9*	3.3	-10.8
	Control	1.6	0.0	-4.6
	Pre-ignition			
	100%	34.0*	31.9	-3.0
	50%	15.2*	12.4	-12.6
	25%	10.4*	7.5	-10.5
	12.5%	9.4*	6.4	-12.2
	6.25%	6.6*	3.6	-6.8
	Control	3.1	0.0	-3.7
	Post-burn			
	100%	32.6*	30.3	-6.6
	50%	17.7*	14.9	-3.0
	25%	14.7*	11.7	-10.5
	12.5%	11.2*	8.1	-1.4
	6.25%	5.4	2.1	-4.8
	Control	3.4	0.0	-2.1

* Values significantly different (P<0.5) than the control

1 Negative % mortality values indicate that the surviving larvae concentrations exceeded those found in the "zero-time" controls used to determine the inoculation density. This apparent negative effect is due to variability associated with subsampling, as well as in estimating inoculation density.

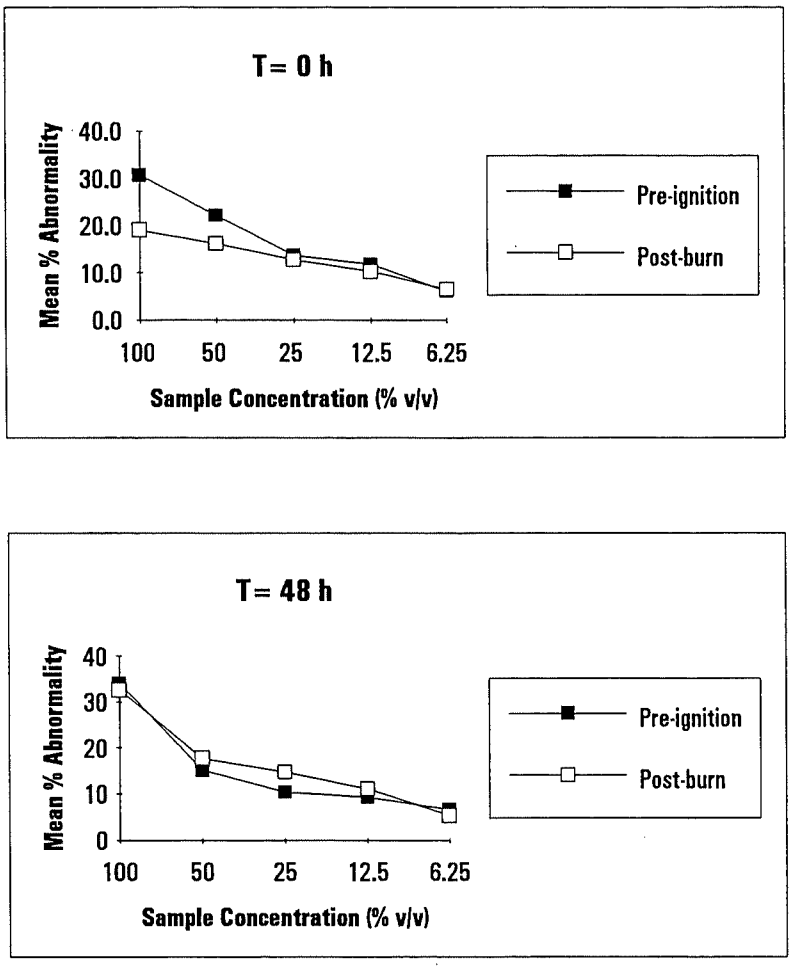


Figure 4 Bivalve Larvae Abnormality

Mean percent abnormalities were relatively low in the 100% (v/v) test treatments, ranging from 4.9 to 34.0%. Figure 4 shows that the mean percent abnormalities in the pre-ignition and post-burn samples were very similar at all test concentrations. A two-sample t-test indicated that the 100% (v/v) pre-ignition sample was not significantly different than the 100% (v/v) post-burn sample.

Abnormality responses in the background and pre-ignition samples were similar to those found in the T = 0 h testing. In contrast, the abnormality for the post-burn sample was higher at 32.6% than that observed in the T = 0 h testing, which was 9.1%.

Although, the mean percent abnormality was low in the background sample (4.9%), a two-sample t-test indicated that it was still significantly different ($P<0.05$) than the negative control. 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 ($P<0.05$) in larval abnormality between treatments. The NOEC values for the pre-ignition and post-burn samples were <6.25 and 6.25% (v/v), respectively. Although the NOEC values were low, the data showed that mean abnormalities were still relatively low, approximately 34% or less at all concentrations. The EC50 values were >100% (v/v) for both samples.

The NOEC value was 6.25% for the pre-ignition sample when it was compared to the background. All other NOEC and EC50 values for the pre-ignition and post-burn samples remained the same.

Water quality parameters measured in the background, pre-ignition, and post-burn samples before testing were: temperature 21°C; pH 7.6 to 7.7; dissolved oxygen 8.2 mg/L; and salinity 28 ppt. The water quality parameters measured at the end of the test were: temperature 21°C; pH 7.7 to 7.9; dissolved oxygen 7.6 to 7.8 mg/L; and salinity 28 ppt.

The 48-h EC50 value for the reference toxicant, cadmium chloride ($CdCl_2$), was 1.3 mg/L of Cd. This value was within the range of values (0.3 to 2.8 mg/L of Cd) obtained by the laboratory in previous testing.

Inland Silverside Tests - Results of the inland silverside tests conducted at T = 0 h and T = 48 h are summarized in Table 11. Complete data are provided in Appendix F.

T = 0 h Results - Mean survival in the seawater controls at 48 h met the minimum criteria of ≥90% survival set by U.S. EPA (1991). Mean survival in the background sample was 100% at 48 h. Mean survival was also high in the 100% (v/v) pre-ignition and post-burn samples at 100 and 93%, respectively. The EC50 values for the pre-ignition and post-burn samples were both >100% (v/v).

Water quality parameter ranges measured in the background, pre-ignition, and post-burn samples during testing were: temperature 24 to 25°C; pH 7.4 to 7.9; dissolved oxygen 3.0 to 6.8 mg/L; and salinity 27 to 30 ppt. The low end of the dissolved oxygen range was a result of a decrease in levels at 48 h.

According to protocol, aeration was introduced at this time and maintained for the duration of the test. Since results have been reported at 48 h and survival was >90% in all samples, it did not appear that these low dissolved oxygen levels had affected reported test results.

The 48-h EC50 value for the reference toxicant, cadmium chloride ($CdCl_2$), was 2.9 mg/L of Cd. A laboratory mean value for cadmium has not yet been generated.

T = 48 h Results - Mean survival in the seawater controls at 48 h met the minimum test criteria for survival. Mean survival in the background sample was 100% at 48 h. Mean survival was also 100% in the 100% (v/v) pre-ignition and post-burn samples. The EC50 values for the pre-ignition and post-burn samples were both >100% (v/v).

Water quality parameter ranges measured in the background, pre-ignition, and post-burn samples during testing were: temperature 24.0 to 25.5°C; pH 7.4 to 7.9; dissolved oxygen 3.0 to 8.1 mg/L; and salinity 28 to 31 ppt. As dissolved oxygen levels were low at 48 h, test chambers were aerated for the remainder of the test. Apparently, this decrease did not affect reported test results since survival was again high in all test concentrations. The 48-h EC50 value for the reference toxicant, cadmium chloride ($CdCl_2$), was 2.2 mg/L of Cd.

Table 11 Summary of Inland Silverside Toxicity Test Results

Test	Sample	Mean % Survival at 48 h
T = 0 h	Background	
	100%	100
	Control	100
	Pre-ignition	
	100%	100
	50%	100
	25%	100
	12.5%	100
	6.25%	100
	Control	100
	Post-burn	
	100%	93.3
	50%	100
	25%	100
	12.5%	100
	6.25%	100
	Control	100
T = 48 h	Background	
	100%	100
	Control	100
	Pre-ignition	
	100%	100
	50%	100
	25%	100
	12.5%	100
	6.25%	96.7
	Control	100
	Post-burn	
	100%	100
	50%	96.7
	25%	100
	12.5%	100
	6.25%	96.7
	Control	100

3.0 Field *In-situ* Burn Testing

3.1 Methods

3.1.1 Generation of Field Samples

The *in situ* oil burn field experiment consisted of two separate burns conducted sequentially on August 12, 1993 off the coast of Newfoundland. Samples were collected from each burn to perform the same suite of chemical analyses and toxicity tests as were conducted for the laboratory-generated samples. Samples to be collected from each burn included background, pre-ignition, early-burn, late-burn, and post-burn samples. Except for the initial grab background samples, all other samples were collected using remote-controlled boats. Due to problems with the remote water samplers, not all the samples could be collected. Most of the intended samples (i.e., pre-ignition, early-burn, late-burn, and post-burn) were collected by the two boats used in Burn I (Boats 2 and 4) and by one of the two boats used in Burn II (Boat 1). Boats 2 and 1 were the lead boats in their respective burns (i.e., they were the closest boats trailing the burn). A background sample was collected before the first burn but no background sample was collected before the second burn. Chemistry samples were sent directly from Newfoundland to the Environmental Technology Centre (ETC) for analysis. Samples for toxicity testing were held overnight at 4°C and shipped the following morning to the EVS laboratory in Vancouver, where they arrived on August 13, 1993.

3.1.2 Chemistry

Chemical analyses for the target polycyclic aromatic hydrocarbons (PAHs) and total petroleum hydrocarbons (TPHs) were conducted on the field-burn samples by the ETC using the same methods as for the laboratory-generated samples. Two samples were collected for each water type from each of the burns and boats.

Samples sent for toxicity testing were also analyzed for chemistry upon completion of the inland silverside test. Unfortunately, there was insufficient volume to send samples before the toxicity testing was initiated. The report submitted by the chemistry laboratory is provided in Appendix G.

3.1.3 Toxicity Testing

Due to problems with the remote water samplers, several of the anticipated samples either could not be collected or did not contain sufficient volumes to conduct the full suite of biological toxicity tests performed on the laboratory-generated samples. As a result, none of the samples could be tested using the echinoderm cytogenetics test and various combinations of the remaining four tests were used depending on sample volumes. The toxicity tests performed on each sample received are summarized in Table 12. All toxicity tests were initiated on the day the samples were received, August 13, 1993. Unlike the laboratory-generated samples, these field samples were not re-tested 48 h later.

A background sample was collected only before the first burn. This sample was tested using the echinoderm sperm cell, echinoderm larvae, bivalve larvae, and inland silverside tests. All tests were

conducted at 100% (v/v) only. A complete set of four samples (i.e., pre-ignition, early-burn, late-burn, and post-burn) was collected from the lead boat in the first burn (Burn I, Boat 2). However, only the first three samples had sufficient volumes to be tested using all four tests. These samples were tested at 100% (v/v) for the inland silverside test and at a series of concentrations [6.25, 12.5, 25, 50, and 100% (v/v)] for the other three tests. Only the bivalve larvae and echinoderm sperm cell tests could be performed due to the limited volume of post-burn sample available. This sample was tested at the same five concentrations used for the other samples. The post-burn sample was missed by the trail boat in the first burn (Burn I, Boat 4). The other three samples - pre-ignition, early-burn, and late-burn - were tested using only the bivalve larvae and echinoderm sperm cell tests. These samples were also tested at five different concentrations [6.25, 12.5, 25, 50, and 100% (v/v)]. All four samples were collected by the lead boat in the second burn (Burn II, Boat 1). These samples were analyzed using the same two tests and five concentrations as the samples collected by the trail boat (Boat 4) in the first burn. No samples were collected by the trail boat in the second burn.

All toxicity tests were performed according to methods described for the testing of the laboratory-generated samples. The only exception is that a new supply of test organisms was obtained for some of the tests. Eight-day-old inland silversides were obtained from Aquatic Research Organisms, New Hampshire, and received at the laboratory on August 12, 1993. These test organisms were 9 days old at test initiation on August 13, 1993. A new supply of oysters was received on July 27, 1993 from Kim Siewers Inc., California and a new supply of sand dollars was collected on July 22, 1993 from Semiahmoo Spit, Washington.

Table 12 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	✓	-	-	✓	-

3.2 Results

3.2.1 Chemistry

Chemistry Field Samples - Water chemistry results are summarized in Table 13. Both PAH and TPH levels were quite low in all field-burn samples. Analysis for the target PAH compounds found less than 1.00 μg/L in all the samples, with levels ranging from <0.10 to 0.45 μg/L. These values were generally much lower than those obtained from analysis of the laboratory-generated samples. This decrease in concentrations may have resulted from increased mixing and dilution in the water beneath the oil slick during the field trial compared to the confined nature of the laboratory-scale experiments. Naphthalene was the compound detected in the pre-ignition, early-burn, late-burn, and post-burn samples.

No discernable differences were apparent between the different water samples. The TPH levels were also relatively low and ranged from 3 to 11 μg/L in the pre-ignition samples, 2 to 9 μg/L in the during-burn samples, and 4 to 12 μg/L in the post-burn water samples. These values were also substantially lower than those obtained from analysis of the laboratory samples.

Biological Effects Field Samples - Due to volume restrictions, not all the samples received for toxicity testing could be tested using inland silversides. Tests were conducted with the initial background sample, as well as the pre-ignition, early-burn, and late-burn samples collected by Boat 2, Burn I.

In all toxicity test water samples taken at the end of the test, none of the four samples tested contained any of the target PAH compounds (Table 14). These results were similar to those obtained from the analysis of laboratory-generated samples. In general, TPH levels were much lower than those detected in the laboratory-generated samples, ranging from only 4 to 22 μg/L.

3.2.2 Toxicity Testing

Echinoderm Sperm Cell Fertilization Test - Results of the echinoderm sperm cell toxicity tests are summarized in Tables 15 and 16. Complete data are provided in Appendix H. Mean percent fertilization in the negative controls exceeded the minimum 50% criterion. The sperm-to-egg ratio yielding 70 to 90% fertilization was 1200:1.

The mean percent unfertilized eggs 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 different ($P<0.05$) than the negative control. As shown in Figure 5, 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. Mean percent unfertilized eggs in the 100% (v/v) test concentrations ranged from 28.7 to 30.7%. A two-sample t-test indicated that the 100% (v/v) pre-ignition sample was not significantly different than the 100% (v/v) post-burn sample.

Table 13 Results of PAH and TPH Analyses of Chemistry Field Samples

Sample			Total PAH (μg/L H_2O)	Total TPH (μg/L H_2O)
Pre-ignition (PI)				
Burn I	Boat 2	PI1	ND	11
		PI2	0.10	4
	Boat 4	PI1	0.45	5
		PI2	ND	3
Burn II	Boat 1	PI1	ND	8
		PI2	ND	3
Early-burn (EB)				
Burn I	Boat 2	EB1	ND	9
		EB2	ND	5
	Boat 4	EB1	ND	5
		EB2	0.12	3
Burn II	Boat 1	EB1	0.15	4
		EB2	0.15	3
Late-burn (LB)				
Burn I	Boat 2	LB1	0.10	2
		LB2	0.10	4
	Boat 4	LB1	0.10	4
		LB2	0.10	5
Burn II	Boat 1	LB1	0.15	4
		LB2	ND	3
Post-burn (PB)				
Burn I	Boat 2	PB1	0.11	4
		PB2	0.12	12
Burn II	Boat 1	PB1	ND	7
		PB2	0.15	4

ND Not detected

Two samples were collected for each water type, 1 and 2

Table 14 Results of PAH and TPH Analyses of Biological Effects Field Samples

Sample	Total PAH (μg/L H_2O)	Total TPH (μg/L H_2O)
Background	ND	7
Burn I Boat 2 Pre-ignition	ND	18
Early-burn	ND	22
Late-burn	ND	4

ND Not detected

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 in the post-burn sample was lower at 24.7%. A two-sample t-test indicated that the 100% (v/v) pre-ignition sample was significantly higher than the 100% (v/v) post-burn sample. Figure 5 shows the differences in mean percent unfertilized eggs between the samples collected in Burn I, Boats 2 and 4, and Burn II, Boat 1.

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 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. The statistical analysis showed that the post-burn sample was significantly different at 50% (v/v) only, but not at 6.25, 12.5, 25, and 100% (v/v). Therefore, a conservative estimate of the NOEC value for this 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 NOEC values similar to 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%.

Water quality parameter ranges measured in the samples before testing were: temperature 14 to 16°C; pH 7.6 to 7.9; dissolved oxygen 7.9 to 9.2 mg/L; and salinity 27 to 32 ppt. Due to the small test volumes and short test duration, water quality parameters were not measured at the end of the test.

The 20-min EC50 value for the reference toxicant, cadmium chloride ($CdCl_2$), was 2.8 mg/L of Cd. The EC50 values for a 60-min sperm exposure obtained by the laboratory ranged from 4.5 to 16.0 mg/L of Cd.

Table 15 Summary of Echinoderm Sperm Cell Fertilization Field Toxicity Test Results

Sample (EVS#)		Mean % Unfertilized	Mean Net % Unfertilized
Burn I, Boat 2	Pre-ignition (1)		
	100%	29.3*	20.9
	50%	27.7*	19.0
	25%	23.7*	14.6
	12.5%	27.3*	18.7
	6.25%	38.3*	31.0
	Control	10.7	0
	Early-burn (2)		
	100%	30.0*	22.8
	50%	27.7*	20.2
	25%	25.7*	18.0
	12.5%	24.0*	16.2
	6.25%	16.0	7.4
	Control	9.3	0
	Late-burn (3)		
	100%	28.7*	14.4
	50%	33.7*	20.4
	25%	32.3*	18.8
	12.5%	29.7*	15.6
	6.25%	28.7*	14.4
	Control	16.7	0
	Post-burn (4)		
	100%	30.7*	20.6
	50%	28.7*	18.3
	25%	26.3*	15.6
	12.5%	32.0*	22.1
	6.25%	20.3	8.8
	Control	12.7	0
Background (6)			
100%		15.7	1.2
Control		14.7	0

* Values significantly different ($P<0.05$) than the control

...../cont.

Table 15 (cont.)

Sample (EVS#)		Mean % Unfertilized	Mean Net % Unfertilized
Burn I, Boat 4	Pre-ignition (5)		
	100%	45.3*	33.3
	50%	31.3*	16.3
	25%	25.7*	9.3
	12.5%	35.3*	21.1
	6.25%	35.3*	21.1
	Control	18.0	0
	Early-burn (9)		
	100%	43.3*	27.7
	50%	47.3*	32.8
	25%	57.7*	46.0
	12.5%	37.3*	20.0
	6.25%	37.7*	20.4
	Control	21.7	0
	Late-burn (10)		
	100%	53.3*	36.1
	50%	50.0*	31.5
	25%	45.7	25.6
	12.5%	41.0	19.2
	6.25%	43.0	21.9
	Control	27.0	0
Background (6)			
100%		15.7	1.2
Control		14.7	0

* Values significantly different ($P<0.05$) than the control

...../cont.

Table 15 (cont.)

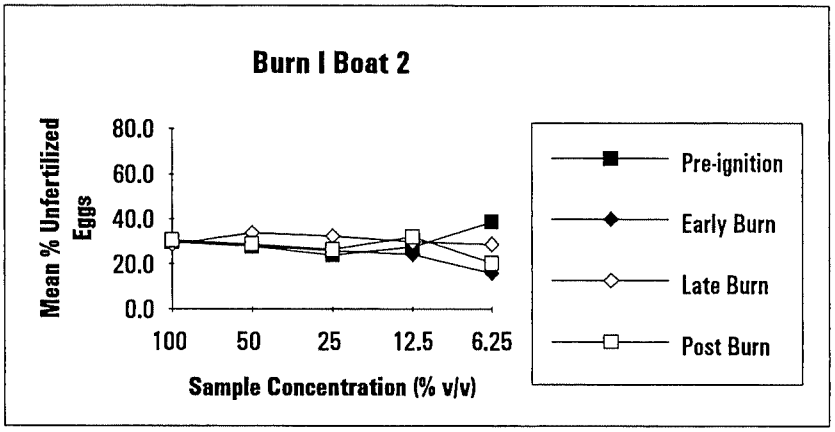
Sample (EVS#)		Mean % Unfertilized	Mean Net % Unfertilized
Burn II, Boat 1	Pre-ignition (1)		
	100%	62.3*	55.0
	50%	42.3*	31.1
	25%	49.3*	39.4
	12.5%	45.3*	34.7
	6.25%	37.7*	25.5
	Control	16.3	0
	Early-burn (2)		
	100%	55.0*	41.3
	50%	51.7*	37.0
	25%	44.0*	27.0
	12.5%	32.0	11.3
	6.25%	27.0	4.8
	Control	23.3	0
	Late-burn (3)		
	100%	55.7*	41.4
	50%	60.3*	47.6
	25%	50.0*	33.9
	12.5%	46.0*	28.6
	6.25%	28.0	4.8
	Control	24.3	0
	Post-burn (4)		
	100%	24.7	9.2
	50%	30.0*	15.7
	25%	23.7	8.0
	12.5%	28.7	14.1
	6.25%	16.0	-1.2
	Control	17.0	0
Background (6)			
100%		15.7	1.2
Control		14.7	0

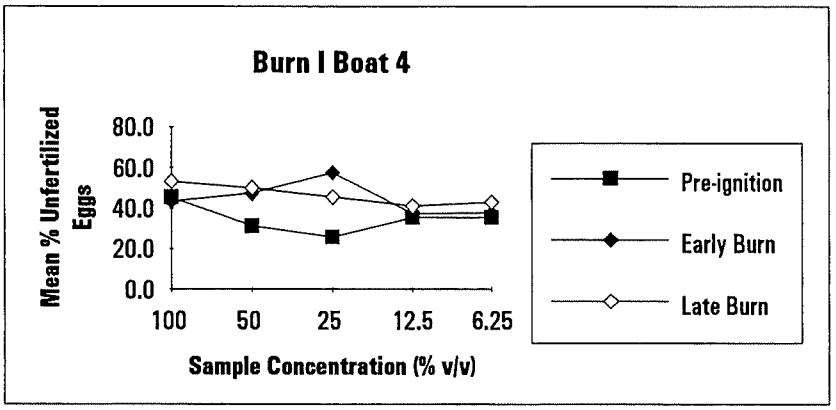
* Values significantly different ($P<0.05$) than the control

Table 16 Summary of Toxicity Endpoints - Field Samples

Sample	Echinoderm Sperm Cell				Echinoderm Larvae				Bivalve Larvae			
	NOEC[1]		EC50[1]		NOEC[1]		EC50[1]		NOEC[1]		EC50[1]	
	vs Control	vs Background	vs Control	vs Background	vs Control	vs Background	vs Control	vs Background	vs Control	vs Background	vs Control	vs Background
Burn I, Boat 2												
Pre-ignition	<6.25	<6.25	>100	>100	12.5[2]	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[3]	6.25	>100	>100	-	-	-	-	<6.25	100	>100	>100

1 All endpoint values are percent volume by volume (%v/v)
2 50% (v/v) was not significantly different ($P<0.05$)
3 100% (v/v) was not significantly different ($P<0.05$)





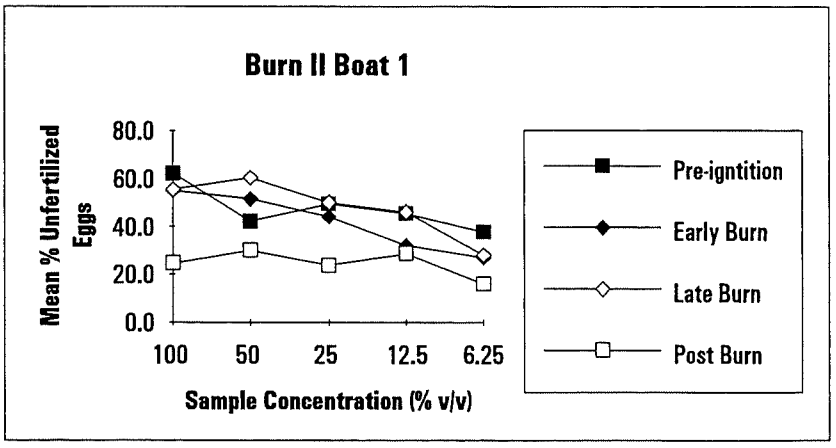


Figure 5 Echinoderm Sperm Cell Fertilization in Burns I and II

Echinoderm Larvae Test - Results of the echinoderm larvae toxicity tests are summarized in Table 16 and Table 17. Complete data are provided in Appendix I. Mean percent abnormality and mortality in the negative controls were within acceptable test criteria (≤10% abnormality and ≤30% mortality).The initial density of embryos introduced into each test container was confirmed to be 3000 embryos/100 mL.

The mean percent abnormality in the background sample, 6.1%, 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 different ($P<0.05$) than the negative control. The mean percent abnormalities in the three samples tested (pre-ignition, early-burn, and late-burn from the lead boat in Burn I) were relatively low, ranging from 10.9 to 18.7% in the 100% test concentrations. As shown in Figure 6, mean percent abnormality was higher in the early-burn and late-burn samples than in the pre-ignition sample at 100% (v/v) concentration.

Data sets for the 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 treatments. Dunnett's t-test showed that the pre-ignition sample was significantly different at 25 and 100% (v/v), but not at 6.25, 12.5, and 50% (v/v). Therefore, a conservative estimate of the NOEC value for this 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. The EC50 values were >100% (v/v) for all three samples. Although the NOEC values were low, the data showed that mean abnormalities were approximately 19% or less at all concentrations which is not considered to be biologically significant.

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).

Water quality parameter ranges measured in the samples before testing were: temperature 15 to 16°C; pH 7.7 to 7.9; dissolved oxygen 8.1 to 8.8 mg/L; and salinity 28 to 32 ppt. The water quality parameters measured at the end of the test were: temperature 15°C; pH 7.8 to 7.9; dissolved oxygen 7.8 to 8.2 mg/L; and salinity 27 to 32 ppt. The 48-h EC50 value for the reference toxicant, cadmium chloride ($CdCl_2$), was 7.0 mg/L of Cd which was within the range of values (8 to 10 mg/L of Cd) obtained by another toxicity testing laboratory.

Bivalve Larvae Test - Results of the bivalve larvae toxicity tests are summarized in Tables 16 and 18. Complete data are provided in Appendix J. Mean percent abnormality and mortality in the negative controls were within test acceptable criteria (≤10% abnormality and ≤30% mortality). The initial density of embryos introduced into each test container was confirmed to be 300 embryos/10 mL.

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 different ($P<0.05$) than the negative control. The mean percent abnormality was generally low, ranging from 6.0 to 18.0% in the 100% (v/v) test concentration for all eleven samples collected in both burns. As shown in Figure 7, no clear trends could be identified within or between each boat since the differences in percent abnormality were relatively small (2 to 10%) and were not considered to be biologically significant.

Table 17 Summary of Echinoderm Larvae Field Toxicity Test Results

Sample (EVS#)		Mean % Abnormality	Mean Net % Abnormality	Mean % Mortality[1]
Burn I Boat 2	Pre-ignition (1)			
	100%	10.9*	6.0	-6.3
	50%	6.4	1.3	-9.0
	25%	8.4*	3.4	-11.0
	12.5%	7.9	2.8	-17.4
	6.25%	6.1	1.0	-15.3
	Control	5.2	0	-14.2
	Early-burn (2)			
	100%	18.7*	14.1	-21.6
	50%	10.3*	5.2	-7.6
	25%	10.5*	5.4	8.7
	12.5%	8.8	3.7	-3.0
	6.25%	8.6	3.4	-3.6
	Control	5.4	0	-13.8
	Late-burn (3)			
	100%	17.0*	13.2	-13.7
	50%	15.0*	11.1	-2.3
	25%	9.9*	5.8	-10.0
	12.5%	7.4*	3.2	-24.2
	6.25%	7.0*	2.8	-18.4
	Control	4.4	0	-8.9
Background (6)				
100%		6.1	-0.8	-11.4
Control		6.9	0	-11.7

* Values significantly different ($P<0.05$) than the control

1 Negative % mortality values indicate that the surviving larvae concentrations exceeded those found in the "zero-time" controls used to determine the inoculation density. This apparent negative effect is due to variability associated with subsampling as well as in estimating inoculation density.

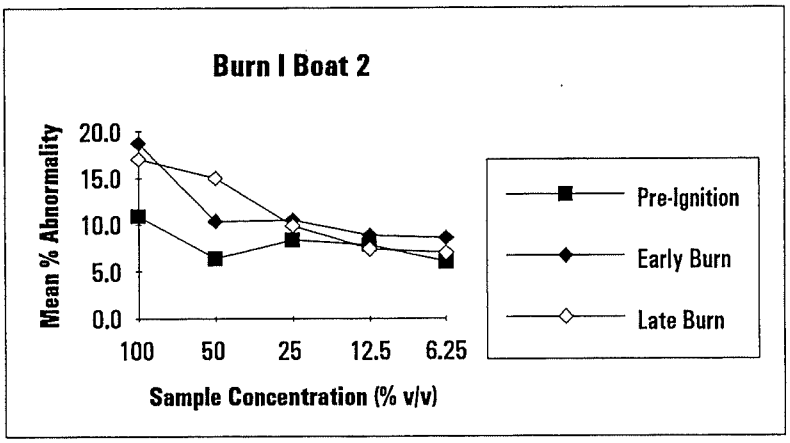


Figure 6 Echinoderm Larvae Abnormality in Burn I

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. A two-sample t-test indicated that the 100% (v/v) pre-ignition sample was not significantly different than the 100% (v/v) post-burn sample.

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. A two-sample t-test indicated that the 100% (v/v) pre-ignition sample was not significantly different than the 100% (v/v) post-burn sample.

Although the NOEC values were low, the data showed that mean abnormality was approximately 18% 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 in both burns.

Statistical comparisons between the test samples and the background indicated that there were no significant differences except for the 100% (v/v) concentrations in the early-burn and late-burn samples collected in Burn II. The NOEC values were higher than those obtained during comparisons with the negative control and ranged from 50 to 100% (v/v). The EC50 values were all >100% (v/v).

Table 18 Summary of Bivalve Larvae Field Toxicity Test Results

Sample (EVS#)		Mean % Abnormality	Mean Net % Abnormality	Mean % Mortality[1]
Burn I, Boat 2	Pre-ignition (1)			
	100%	6.0*	3.6	1.1
	50%	6.3*	4.0	-5.3
	25%	5.0	2.6	1.3
	12.5%	3.8	1.4	-9.2
	6.25%	2.4	0	-2.8
	Control	2.4	0	-11.0
	Early-burn (2)			
	100%	9.8*	8.3	-17.1
	50%	10.4*	8.9	-9.2
	25%	7.1*	5.6	-17.1
	12.5%	5.0*	3.5	-12.7
	6.25%	4.0*	2.4	-92
	Control	1.6	0	-11.6
	Late-burn (3)			
	100%	10.9*	8.8	-8.4
	50%	8.8*	6.7	6.9
	25%	7.4*	5.2	-6.7
	12.5%	5.9*	3.7	2.4
	6.25%	3.6	1.4	-10.1
	Control	2.3	0	-12.1
	Post-burn (4)			
	100%	8.0*	6.9	21.0
	50%	8.2*	7.2	15.1
	25%	9.2*	8.1	19.1
	12.5%	3.8*	2.7	6.7
	6.25%	3.8*	2.7	18.9
	Control	1.2	0	-14.9
Background (6)				
100%		6.3	4.6	-14.8
Control		1.8	0	-5.0

* Values significantly different ($P<0.05$) than the control

1 Negative % mortality values indicate that the surviving larvae concentrations exceeded those found in the "zero-time" controls used to determine the inoculation density. This apparent negative effect is due to variability associated with subsampling as well as in estimating inoculation density.

...../cont.

Table 18 (cont.)

Sample (EVS#)		Mean % Abnormality	Mean Net % Abnormality	Mean % Mortality[1]
Burn I, Boat 4	Pre-ignition (5)			
	100%	8.9*	7.2	21.3
	50%	8.4*	6.7	-4.1
	25%	5.4*	3.6	7.0
	12.5%	5.5*	3.7	-12.0
	6.25%	3.5	1.7	-14.4
	Control	1.8	0	-9.6
	Early-burn (9)			
	100%	12.2*	10.7	3.3
	50%	12.7*	11.3	7.3
	25%	12.7*	11.3	10.0
	12.5%	4.0	2.5	-4.3
	6.25%	2.8	1.2	-7.2
	Control	1.6	0	-10.3
	Late-burn (10)			
	100%	7.4*	5.7	2.4
	50%	6.6*	4.8	4.0
	25%	4.5*	2.7	3.0
	12.5%	3.0	1.1	-1.0
	6.25%	3.4	1.5	-2.2
	Control	1.8	0	-8.1
Background (6)				
100%		6.3	4.6	-14.8
Control		1.8	0	-5.0

* Values significantly different ($P<0.05$) than the control

1 Negative % mortality values indicate that the surviving larvae concentrations exceeded those found in the "zero-time" controls used to determine the inoculation density. This apparent negative effect is due to variability associated with subsampling as well as in estimating inoculation density.

...../cont.

Table 18 (cont.)

Sample (EVS#)		Mean % Abnormality	Mean Net % Abnormality	Mean % Mortality[1]
Burn II, Boat 1	Pre-ignition (11)			
	100%	11.1*	9.9	41.1
	50%	8.0*	6.8	-1.1
	25%	7.5*	6.2	13.8
	12.5%	3.1*	1.8	-3.6
	6.25%	3.7*	2.3	-9.4
	Control	1.3	0	-7.4
	Early-burn (12)			
	100%	18.0*	16.8	0.6
	50%	8.8*	7.5	-2.2
	25%	8.6*	7.3	3.4
	12.5%	6.7*	5.3	0.6
	6.25%	3.7*	2.3	0.6
	Control	1.5	0	-7.0
	Late-burn (13)			
	100%	13.1*	11.2	10.1
	50%	7.2*	5.2	-11.9
	25%	4.7*	2.7	-10.7
	12.5%	5.3*	3.3	-6.0
	6.25%	2.0	-0.1	-9.2
	Control	2.1	0	-9.9
	Post-burn (8)			
	100%	7.9*	6.0	41.2
	50%	5.7*	3.7	22.0
	25%	5.8*	3.8	17.4
	12.5%	6.1*	4.1	-6.8
	6.25%	4.3*	2.3	-7.4
	Control	2.1	0	-11.7
Background (6)				
100%		6.3	4.6	-14.8
Control		1.8	0	-5.0

* Values significantly different ($P<0.05$) than the control

1 Negative % mortality values indicate that the surviving larvae concentrations exceeded those found in the "zero-time" controls used to determine the inoculation density. This apparent negative effect is due to variability associated with subsampling as well as in estimating inoculation density.

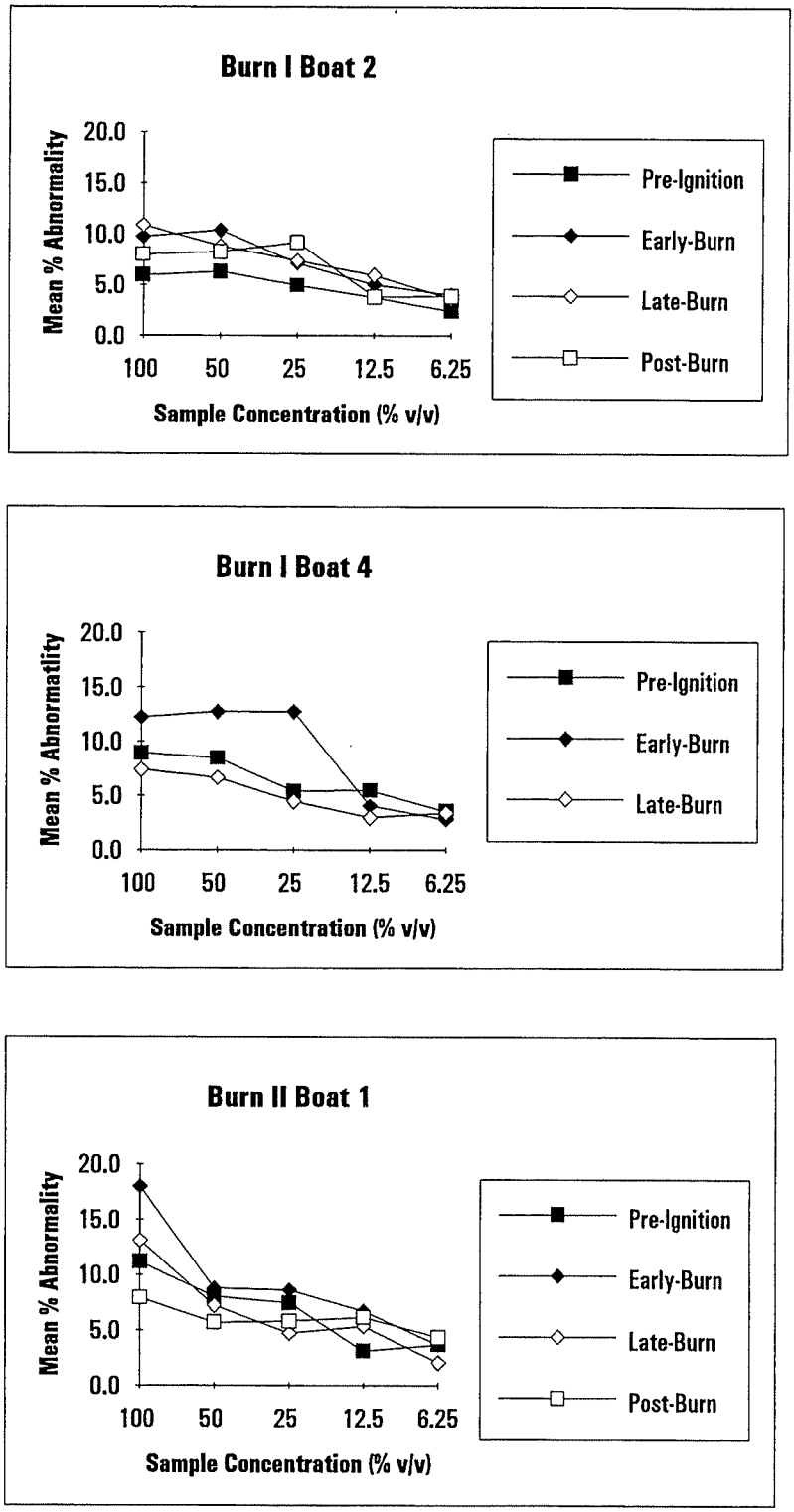


Figure 7 Bivalve Larvae Abnormality in Burns I and II

Water quality parameters measured in the samples before testing were: temperature 19.5 to 21.0°C; pH 7.7 to 7.9; dissolved oxygen 7.2 to 8.2 mg/L; and salinity 27 to 32 ppt. The water quality parameters measured at the end of the test were: temperature 20°C; pH 7.9 to 8.0; dissolved oxygen 7.4 to 7.6 mg/L; and salinity 27 to 36 ppt. The 48-h EC50 value for the reference toxicant, cadmium chloride ($CdCl_2$), was 1.5 mg/L of Cd which was within the range of values (0.3 to 2.8 mg/L of Cd) obtained by the laboratory in previous testing.

Inland Silverside Tests - Results of the inland silverside toxicity test are summarized in Table 19. Complete data are provided in Appendix K. Only four of the eleven samples could be tested using inland silversides. This included the grab background sample as well as the pre-ignition, early-burn, and late-burn samples collected by the lead boat (Boat 2) in Burn I.

Table 19 Summary of Inland Silverside Field Toxicity Test Results

Sample (EVS #)			Mean % Survival at 48 h
Burn I, Boat 2	Pre-ignition (1)	100%	95
	Control		100
	Early-burn (2)	100%	100
	Control		85
	Late-burn (3)	100%	100
	Control		95
Background (6) 100%			95
Control			85

As for the laboratory samples, mean survival in the negative controls failed to meet test acceptability criteria after 96 h (≥90% survival). The mean survival in the controls for the pre-ignition and late-burn samples did meet the minimum criteria for survival after 48 h. Unfortunately, mean survival in the negative controls for both the background and early-burn samples still failed to meet the specified criteria. However, the mean survival in all four test samples was high after 48 h. Mean survival in the background sample was 95% and in the pre-ignition, early-burn, and late-burn test samples was 95, 100, and 100%, respectively.

Water quality parameter ranges measured in the samples during testing were: temperature 23.5 to 25.0°C; pH 7.8 to 7.9; dissolved oxygen 6.0 to 8.4 mg/L; and salinity 28 to 34 ppt. The 48-h EC50 value for the reference toxicant, cadmium chloride, was 1.9 mg/L of Cd.

4.0 Discussion

With the exception of the sperm cell fertilization test, little or no toxicity was associated with the laboratory-generated samples. Although the NOEC values were low, the high EC50 values typically indicated that oil combustion caused minimal effects to the aquatic toxicity. Because NOECs are derived from statistical analyses, there may be statistical significance without biological significance. In most cases, test results were below or close to 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%. Excluding this result, no clear differences were found between the pre-ignition and post-burn samples.

There also appeared to be no effects due to the 48-h holding period as data showed similar test results for samples conducted at T = 0 h and T = 48 h. The only exception was again found in the echinoderm sperm cell fertilization test where the mean percent unfertilized eggs were much lower in the T = 48 h post-burn sample than in the T = 0 h sample.

An unexpected finding from the toxicity tests with the laboratory-generated samples was the level of toxicity associated with the background sample. Results from the echinoderm sperm cell and larvae tests indicated that the test endpoints were higher in the background sample than those in the negative control and were generally more comparable to those found in the pre-ignition and post-burn samples.

Additional testing showed that higher levels of toxicity in the background sample than in the seawater control were due to the use of unfiltered, unsterilized seawater in generating the samples. The results indicated that holding the sample in the burn chamber also contributed to slightly elevated levels of toxicity in the samples.

The low levels of total petroleum hydrocarbons and polycyclic aromatic hydrocarbons 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, in the TPH results, no clear differences were discerned 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. Unfortunately, the suspected contamination in some of the samples restricted the number of data points available for making comparisons and identifying trends.

As was observed in 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 during-burn and post-burn samples generally did not exceed those associated with the unburned oil. 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 PAH composition were found to be the most sensitive toxicity and chemical tests conducted in this study to identify oil-related effects. However, 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 to aquatic organisms found with the use of some alternative oil spill cleanup methods such as dispersants (Fisher and Foss, 1993; Vigers, 1979). While the burning of the surface oil slick does not appear to negatively affect the aquatic organisms tested, however, 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.

5.0 References

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