
Toxicity Bioassays on Dispersed Oil in the North Sea: June 1996 Field Trials

Regulatory Analysis and Scientific Affairs

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Toxicity Bioassays on Dispersed Oil in the North Sea: June 1996 Field Trials

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LIST OF ABBREVIATIONS, SYMBOLS, AND ACRONYMS

<u>Term</u>	<u>Abbreviation, Symbol or Acronym</u>
American Petroleum Institute	API
American Society for Testing and Materials	ASTM
chemically dispersed oil	CDO
Chemical Response to Oil Spills: Ecological Effects Research Forum	CROSERF
Ecosystem Management & Associates, Inc.	EM&A, Inc.
Environmental Protection Agency	EPA
gas chromatography - flame ionization detection	GC-FID
gas chromatography - mass spectrometry	GC-MS
internal standard	IS
liter	L
mass charge (used in GC/MS mass spectra description of fragmentation)	m/z
median effective concentration (concentration at which 50% of population responds)	EC50
median lethal concentration (concentration at which 50% of population dies)	LC50
meters per second	m/s
microliter	μL
microgram	μg
milliliter	mL
miligram	mg
minute	min
nanogram	ng
Newcastle Research Group	NRG
no observable effect level	NOEL
Norwegian Clean Seas Association for Operating Companies	NOFO
part per million (mg/L)	ppm
polycyclic aromatic hydrocarbon	PAH
response factor	RF
rigid hull inflatable boat	RHIB
standard operating procedure	SOP
surrogate standard	SS
total extractable organic content	TEOC
total ion currents	TIC
total petroleum hydrocarbons	TPH
ultraviolet	UV
volatile organic analyte	VOA

EXECUTIVE SUMMARY

This study was part of a larger effort planned and coordinated by IKU Petroleum Institute (Norway) as part of its annual Norwegian Clean Seas Association for Operating Companies (NOFO) on-water exercise held June 11 to 13, 1996. Dispersant testing was an integral part of those studies and focused on the evaluation of a helicopter delivery system (the RESPONSE 3000). At the same time, an extensive scientific program examined the weathering process of both treated and untreated oil and monitored the physical and chemical behavior of an underwater release of a gas-oil mixture (a simulated sub-sea blowout). The toxicity experiments reported here were integrated into the overall IKU Petroleum Institute work plan, and had four main objectives:

- to evaluate the feasibility of selected toxicity tests in near real-time situations under non-optimal conditions at sea;
- to determine the degree of toxicity in the regions of the slick that had the highest oil or dispersed oil concentrations, and to determine how this toxicity varied over depth;
- to collect at-sea toxicity data for future comparisons to laboratory test results; and
- to develop recommendations concerning protocols appropriate for defining ecological impacts in the water column through a combination of field and laboratory data.

Four different toxicity tests were chosen for use during the sea trials. Two of the tests, a bioluminescent bacterial assay (referred to as the “microbial test”) and a rotifer acute toxicity test (referred to as the “rotifer test”) are commercially available, rapid toxicity test kits. They were selected because of their publicized “ease of use” in the field (i.e., at sea). The other tests, oyster embryo development and copepod lethality tests, utilize a traditional 48-hour acute exposure.

The microbial test showed statistically significant effects for dispersed-oil water samples at both 1 meter (m) and 5 m depths. The corresponding total petroleum hydrocarbon values determined by gas chromatography (TPH-GC) beneath the chemically dispersed oil (CDO) slick were

approximately 22 ppm and 2 ppm, respectively. No significant effects were observed for samples taken beneath the untreated oil slick, in which TPH-GC values were reported as 1.4 ppm and 0.5 ppm for 1 m and 5 m depths, respectively. The test results were consistent with the results of the copepod tests and showed a rapid decrease in toxicity over increasing depth beneath the slick. The test is rapid, easy to learn, inexpensive (except for the initial purchase of the equipment), easily transported, and requires little work space. There was no difficulty conducting the tests at sea under the conditions encountered during this study. Preliminary results were available within one hour during this study, and it is the only test evaluated during this study which offers the potential of near real-time information. Before it can be used in any real-time capacity for oil spill response, however, the test must be effectively calibrated against the existing laboratory data to ensure that the results can be properly interpreted.

Survival was low in the reference, control, and test copepod bioassays. This is attributed to the poor shipboard conditions that caused undue stress on the animals. When ANOVA techniques were utilized, the only significant difference noted from the copepod 48-hour lethality test was for the sample water taken at 1 m depth beneath the CDO slick. The TPH-GC value for this sample was 22 mg/L. Although there was a trend that indicated lower survival in the 5 m depth CDO sample (2 mg/L) versus the seawater control, the difference was not statistically significant with the low number of replicates and organism numbers that were used during the bioassay.

Neither the rotifer acute toxicity test nor oyster embryo tests were successful, but the microbial test and copepod toxicity test results allowed many of the project objectives to be met. Even if successful, neither the rotifer test nor the oyster bioassay would have had application as a real-time monitoring assay for spill countermeasure assessment because of their time requirements.

In summary, the study was successful in collecting data to use in comparison with laboratory results, and supports the conventional belief that the zone of biological effects within a dispersed oil plume is limited both spatially and temporally.

Section 1

INTRODUCTION

BACKGROUND

It is generally acknowledged that the use of dispersants in oil spill response can provide protection for shoreline resources, but at the expense of increasing exposure to oil for organisms in the water column (or on the bottom, in shallow water). If this is the case, then it becomes very important to define the level of effect anticipated in the potentially affected habitats, in order to identify the best course of action in terms of planning for response. For the purposes of this discussion, areas of concern for both dispersed and undispersed oil are divided into two categories: 1) shoreline (intertidal) and 2) nearshore subtidal and water column.

Shoreline (Intertidal) and Nearshore Subtidal Effects

Shoreline effects of oil spills have been well studied and are generally predictable if the climate, hydrographic conditions, and habitat type are known. The literature on this habitat is extensive for untreated oil, and is based on experimental work and on observations of effects at actual oil spills (see National Research Council (NRC), 1985 or GESAMP, 1993 for summaries). For dispersed oil, there is little information from actual spills, although Lunel (1997) attempted to estimate the shoreline benefits accrued by oil dispersed at sea during the Sea Empress spill in Milford Haven (United Kingdom). Summaries of real response operations in which oil was successfully dispersed have not made note of adverse shoreline impacts (see Table in Appendix in Lewis and Aurand, 1997). Although this lack of evidence on shoreline effects does not prove that there have been no effects, it does provide some indication that such impacts have not been severe.

The most meaningful field experiments defining the consequences of dispersant use in intertidal and nearshore areas were a series of three experiments implemented in the early 1980s. The first was conducted at a site on Baffin Island in the Canadian Arctic (BIOS Study; Sergy, 1985), the second, at a small embayment near Searsport, Maine (Gilfillan *et al.*, 1983, 1985), and the third, in a tropical embayment containing mangroves, sea grass beds, and corals in Panama (TROPICS

Study; Ballou *et al.*, 1989; Dodge *et al.*, 1995). In all three studies, both pre-dispersed and untreated crude oil were released slightly offshore or subtidally, and allowed to strand on the intertidal zone. In all studies, significant shoreline contamination was seen in the high intertidal zone with the untreated oil, but not with the pre-dispersed oil. The ecological effects of this untreated oil contamination varied widely, depending upon the intertidal species, but were severe in the mangrove community in Panama. Initial impacts in the water column and in benthic organisms were higher in the presence of the pre-dispersed oil, but the effect was temporary and conditions returned to normal within one year (in the arctic) to several years (in Panama). The Searsport study was only short-term. Monitoring at the BIOS site continued for several years and documented long-term contamination of the sediments from erosion of beach sediments at the untreated oil site. In Panama, the TROPICS sites were monitored several times during the two years immediately following the experimental spill, and then revisited at the ten year point (Dodge *et al.*, 1995). At the end of two years, the authors concluded that the undispersed oil had severely damaged or killed the mangrove trees in the test area, while there was little or no damage to trees from the pre-dispersed oil. Sea grasses were not affected by either treatment, but invertebrates present in the sea grass beds were severely affected initially by the pre-dispersed oil. Recovery appeared complete after two years. Corals were also significantly affected by the pre-dispersed oil in the first two years, but not by the untreated oil. The evaluation at ten years confirmed the significant effect of the untreated oil on mangroves, since the area now contained even fewer living trees (approximately half of the original population). In contrast, no explicit mortality of trees exposed to the pre-dispersed crude oil was observed. The corals, which had shown an effect from the pre-dispersed oil at the two-year point, had now recovered and no significant difference existed between sites.

Benthic habitats immediately adjacent to oiled shoreline may be affected by erosion of contaminated sediment from the intertidal zone. Contamination of intertidal sediment is reduced or eliminated by the use of dispersants. Effects on birds and marine mammals are well understood, but are more difficult to quantify except when connected to specific geographic locations (e.g., haul-out areas for marine mammals or rookery sites for nesting birds). It is generally accepted that reducing oil on the water surface will protect marine mammals and birds

at sea. Preventing oil from stranding in nesting or haul-out areas is an obvious benefit of dispersant use.

Water Column Effects

Based on our knowledge of the fate and behavior of oil, water column effects are much less significant than intertidal effects, except under unique conditions; therefore, they have been much less studied (NRC, 1989). There have been a number of field studies to examine the physical fate of dispersed versus untreated oil at sea, and this process is well-understood. In the late 1970s, the U.S. Environmental Protection Agency (EPA) and the American Petroleum Institute (API) conducted at-sea studies on both the East and West Coasts of the United States to evaluate dispersant performance, the effects of various application methods, and the fate of both treated and untreated oil (McAuliffe *et al.*, 1980; McAuliffe *et al.*, 1981). Throughout the 1980s and 1990s, sea trials have continued in the North Sea by researchers from both Norway and the United Kingdom (UK) (for examples of the most recent work see Lunel, 1994, 1995; Lewis and Davies, 1996; Lewis *et al.* 1995; and Brandvik *et al.* 1996a,b). None of these studies focused on long-term biological effects in the water column, although they provided data on water column concentrations of hydrocarbons, which can be used to make estimates of potential effects.

Soule and Oguri (1985) did conduct an evaluation of short-term water column biological effects in conjunction with the 1979 EPA/API experiment off the U.S. East Coast. The authors collected water samples from beneath dispersed and undispersed oil slicks (Murban and La Rosa crude oils) at depths of one and three meters. Using net-collected zooplankton samples from uncontaminated control areas, they conducted shipboard toxicity tests in ambient temperature water baths for either three or twelve hours, and then used a vital stain to identify living zooplankton in test and control samples. The distribution of zooplankters was highly variable, and the survival patterns were inconsistent, leading to inconclusive results. However, the authors reported that there was a tendency for higher mortality beneath the dispersed oil slicks, based on survival of adults of the three most common species, all calanoid copepods (*Centropages typicus*, *Paracalanus parvus*, and *Acartia tonsa*). Of the three, *A. tonsa* was the most resistant, not showing any statistically significant mortality. Some significant results were obtained for the other two species. Chemical data were available from water samples taken at approximately the

same time (approximately one-half hour after release and dispersion), with no “oil” concentrations reported in excess of 0.5 mg/L (ppm). This value is lower than any of the LC50 (96 or 72-hour) values reported for copepods in NRC (1985, Table 5-5). Given that this exposure concentration was maintained for only a brief time in comparison to the laboratory tests, the basis for the observed effects is unclear. Baker (1993) used the ppm-hour concept (Anderson *et al.*, 1984; 1987), in which the units are the product of the measured mean concentration and time. She estimated that the lowest reported LC50 value was about 8 ppm-hour, a value much higher than the apparent exposure in the Soule and Oguri (1985) study.

It is important to note that these early studies that examined water column effects did not utilize highly sophisticated means of chemical analysis (such as gas chromatography). Many of the early studies relied on infrared or fluorescence methods to quantify exposure. Although these methods of analysis are still used for at-sea monitoring work, it is not appropriate to directly compare these toxicity results with those which utilize fully quantitative analytical methods.

There have been some attempts to estimate water column effects at actual oil spill events. In general, these studies have indicated a very transient impact of little or no significance when oil was not dispersed (for examples see Johansson *et al.*, 1980; Celewycz and Wertheimer, 1996). There are no reported water column studies to date at spills where dispersants were used. In the case of the MV *Braer* spill off the Shetland coast, the severe weather and chemical and physical properties of the spilled oil (Gullfaks crude) essentially led to a total, natural dispersion of the approximately 85,000 tonnes of cargo, along with a small volume of fuel oil. In the three months after the incident, over 800 water samples were taken for hydrocarbon analysis. Concentrations of total hydrocarbons in the water column within 100 to 200 yards of the tanker were as high as 10,000 ppm (a 1% oil-in-water dispersion). However, by the time the plume was 2 km away from the wreck, concentrations were approximately 100 ppm, a level that was observed as far as 24 km north of the wreck (Thomas and Lunel, 1993). These levels were apparently maintained over much of the six days it took for the wreck to break up, and then declined rapidly. Within another three days, concentrations in Garth Ness (the small embayment where the wreck grounded) were only 25 ppm (Thomas and Lunel, 1993). Ritchie and O’Sullivan (1994)

characterized concentrations in the water column “near” the tanker as “in the range of several hundred ppm soon after the grounding.” They also summarized concentrations over the next few days as around 50 ppm in areas affected by the spill. Obviously, concentrations were highly variable in both time and space but it is clear that dilution was quite rapid after the release ended and as the plume moved away from the site.

Laboratory toxicity tests are the most frequently encountered data when planners attempt to evaluate potential risks and benefits of dispersant use. One must keep in mind that crude oil is a complex mixture of thousands of compounds of varying volatility and a wide range of solubilities in water, and that the addition of dispersants can create a multi-phase system (e.g., water, oil, dispersant, dispersed oil droplets), all of which have a different affinity for the compounds present in the oil. Because of these characteristics of oil and dispersant interaction, it should not be surprising that test results have been greatly influenced by factors such as the nature and degree of agitation, the type of test container, whether the test containers are open or closed, the oil:water ratio, the source of the crude oil, or its weathering state, to name only a few (Aurand, 1995).

Laboratory toxicity tests do not relate directly to field conditions unless the exposure regimes are comparable. Standard acute toxicity tests with a static exposure to a toxicant for a period of time (usually 96 hours) cannot be used to evaluate episodic, short duration events followed by rapid dilution, which is the case even for relatively large oil spills. It seems clear, at least in some instances, that using results from standard 96-hour constant exposure tests has led regulators to over-estimate potential water column effects from dispersed oil. On the other hand, recent efforts in the United States and elsewhere to expand the use of dispersants to areas of shallow water near shore (or in estuaries) in an attempt to protect sensitive intertidal resources, does increase the possibility of more extended exposures in the water column and to benthic communities. This has led to renewed interest in developing laboratory approaches more directly related to field conditions.

Singer *et al.* (1990) developed a flow-through test chamber to simulate short-term “spiked” exposures for small marine species, with the express intent of developing data that more closely resemble potential field exposures. In this system, a maximum initial concentration (to simulate application) declines at a fixed rate, as if by natural dilution. This apparatus has now been installed at several laboratories in an attempt by interested state and Federal agencies and industry to develop a better toxicity data base. This work is coordinated through the Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF), which is working to 1) develop improved research protocols, and 2) integrate laboratory, mesocosm, and field research into a body of knowledge appropriate for decision-makers (the most recent report from this group is Coelho and Aurand, 1997). As part of this effort, the group has been trying to integrate the results of toxicity tests conducted under the new exposure regimes with those from traditional, continuous-exposure, 96-hour tests, and then relate both approaches to conditions observed in field trials.

One key issue is to confirm through studies at sea that dispersed oil found in the environment under natural conditions is effectively modeled by dispersed oil created in the laboratory in terms of toxicity. Intuitively, the mixing regime during laboratory preparations of dispersed oil droplets in sea water is much different from the natural process in the ocean, but the operating assumption is that, with sufficient energy and mixing, the resultant droplets are not likely to be significantly different than those produced in nature.

Increased interest in dispersant use in nearshore situations has led some regulatory agencies in the United States to request consideration of ecological monitoring programs in the event of dispersant use. Traditional toxicity tests, which require anywhere from two days to a week or more to complete, would be of no value except in terms of retrospective analyses. There are some tests, most notably the microbial test, that offer the possibility of more timely information if they can be properly interpreted. When the microbial test analyzer is hooked up to a portable computer with its proprietary software, a user can analyze a sample, download it directly from the analyzer to the computer, and have statistically analyzed toxicity data available in 30 to 45 minutes.

In 1994 and 1995, in conjunction with at-sea trials off the southeast coast of the UK, the authors made attempts to transport water samples to shore laboratories for use in standard toxicity tests and the microbial test (Wright *et al.*, 1994; Coelho *et al.*, 1995). Severe logistical problems occurred in both studies, although data confirming the lack of toxicity at low concentrations of hydrocarbons in the water samples were obtained. A major conclusion of those efforts was that, except in unusual circumstances, transporting water samples back to a laboratory for further biological testing is impractical. Unfortunately, this leaves a less than ideal alternative - toxicity testing at sea. On that basis, the work described below was undertaken.

OBJECTIVES OF THIS STUDY

This study was part of a larger effort planned and coordinated by IKU Petroleum Institute (Norway) as part of their annual NOFO on-water exercise held June 11 to 13, 1996. NOFO is the Norwegian abbreviation for the "Norwegian Clean Seas Association for Operating Companies." Dispersant testing was an integral part of those studies and focused on the evaluation of a helicopter delivery system (the RESPONSE 3000). At the same time, an extensive scientific program examined the weathering process for both treated and untreated oil and monitored the physical and chemical behavior of an underwater release of a gas-oil mixture (a simulated sub-sea blowout). Extensive hydrocarbon chemistry was done throughout the experiment. These studies are described in Strøm-Kristiansen *et al.* (1996) and Brandvik *et al.* (1996a).

The purpose of the study described in this report was to gain more information on water column impacts by taking advantage of the ongoing efficacy and monitoring studies done by NOFO in order to conduct field toxicity tests. As shown in the background discussion, there have been extensive field studies of shoreline/nearshore communities. These show lasting and sometimes severe impacts for massive exposures. More limited data are available on water column impacts, and available data from ambient community studies are highly variable and inconclusive. The goal of this study was to obtain field effects data using shipboard, real-time toxicity tests with field water. This data can then be used in the future to link field effects to laboratory toxicity data. This toxicity study had four main objectives:

- to evaluate the feasibility of selected toxicity tests in near real-time situations under non-optimal conditions at sea;
- to determine the degree of toxicity in the regions of the slick that had the highest oil or dispersed oil concentrations, and to determine how this toxicity varied over depth;
- to collect at-sea toxicity data for future comparisons to laboratory test results; and
- to develop recommendations concerning protocols appropriate for defining ecological impacts in the water column through a combination of field and laboratory data.

Section 2

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Field Trial Design

The experimental field site was located near the Frigg field in the North Sea in an area centered around N59°59' - E002°27' (Figure 2-1). Troll crude oil, produced by Norsk Hydro, was released on two consecutive days. Trial 1 (11 June, 1996) involved two surface releases of Troll crude oil by means of a Transrec hose and skimmer head. Each slick was 15 m³ in size and was released over 200 meters. The first release (Charlie slick) served as a control slick that was not treated.

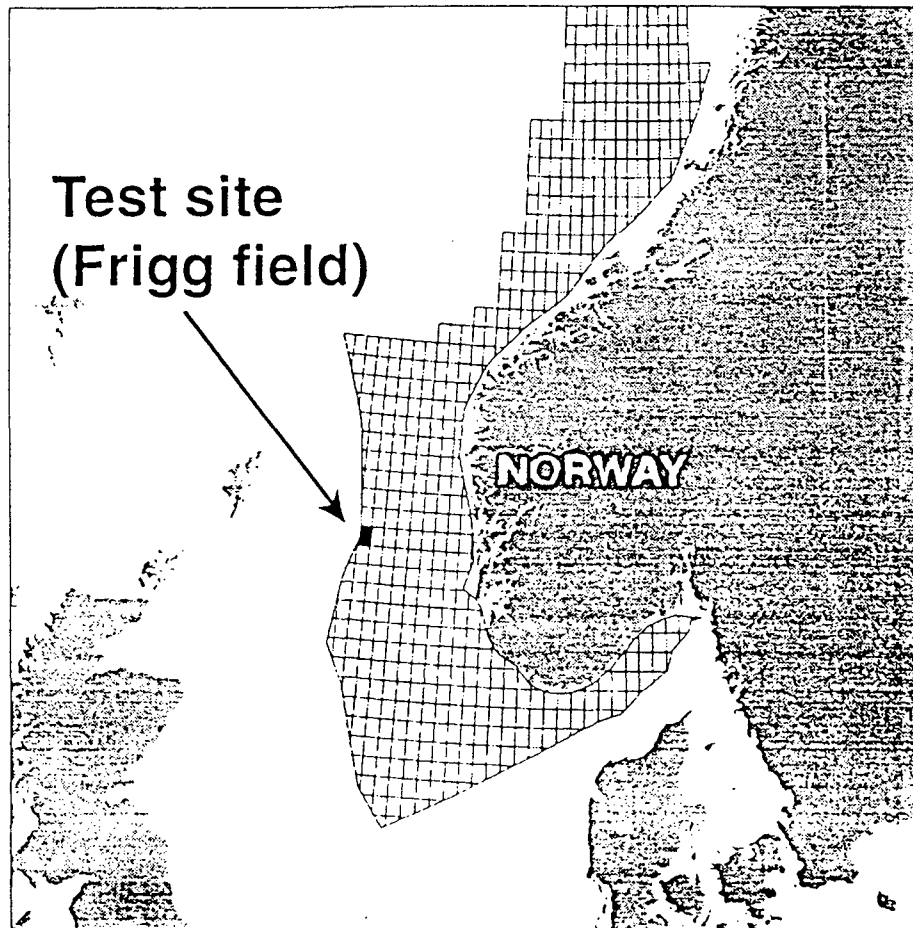


Figure 2-1. Location of Experimental Field Site (Used with permission from Brandvik *et al.*, 1996a).

The second slick (Hotel slick) was released 1km away from Charlie slick. It was allowed to weather for 3.5 hours before being dispersed with Corexit® 9500 via a helicopter bucket. Winds were westerly at 5 m/s during the slick release and dispersant application, and increased to 6-7 m/s after dispersant was applied. Wave heights were at 1.0 m, increasing to 1.5 m with the higher wind. A summary of weather conditions during both Trials is presented in Figure 2-2.

Duplicate 1 L samples of control water were collected at the field site (at 1 m depth) prior to the release of the Charlie slick. Sample water was collected via an array of submerged pumps mounted on the side of a Rigid Hull Inflatable Boat (RHIB). The pumps continuously sampled water at various depths and monitored the hydrocarbon content of the water by *in situ* UV-fluorometers operated by Norwegian field technicians. Details of the UV-fluorometer method are described in the NOFO Report (Brandvik *et al.*, 1996a). One hour after release of Hotel slick (prior to dispersant application) duplicate 1 L samples were collected at 1 m and 5 m depths beneath the slick. Water was only collected when UV-fluorometry indicated the presence of high concentrations of oil, based on the assumption that the entire ocean was not at risk, but only that water which came in contact with the slick or was in the plume of dispersed oil. The sampling transect extended across the entire slick (or through the entire dispersed oil plume) during all sampling collection. One hour after treatment of Hotel slick with Corexit® 9500, duplicate samples were again collected at 1 m and 5 m depths beneath the slick, in areas of high oil concentrations.

Trial 2 (12 June, 1996) involved the underwater release an gas-oil mixture. This was designed to simulate a blowout by releasing 60 m³ Troll oil/gas mixture (at a rate of 1 m³/minute) at a depth of 100 m. Winds were westerly at 8-11 m/s during the release and wave heights were on the average 1.7-1.8 m. Due to the inadequate formation of a surface slick on the second day, dispersant was not applied during Trial 2. Control water could not be collected during Trial 2 because the pump lines had been contaminated with oil. Prior to contamination of pump lines, duplicate samples were collected at 1 m and 5 m depths beneath the resulting surface slick (light sheen developed), in areas where UV-fluorometry indicated higher concentrations of oil.

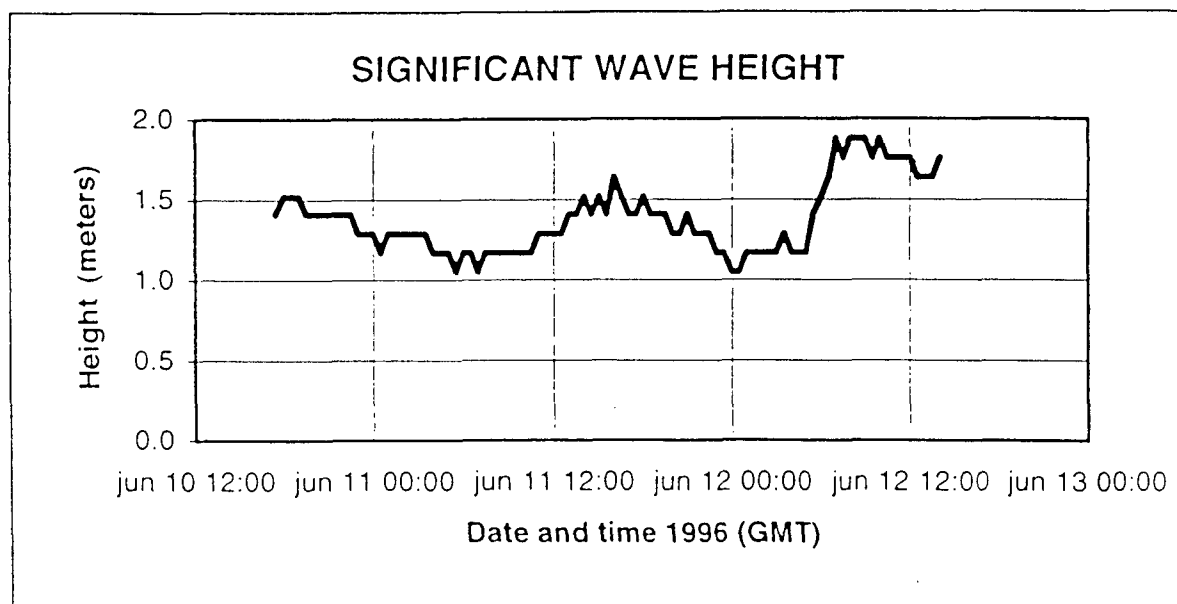
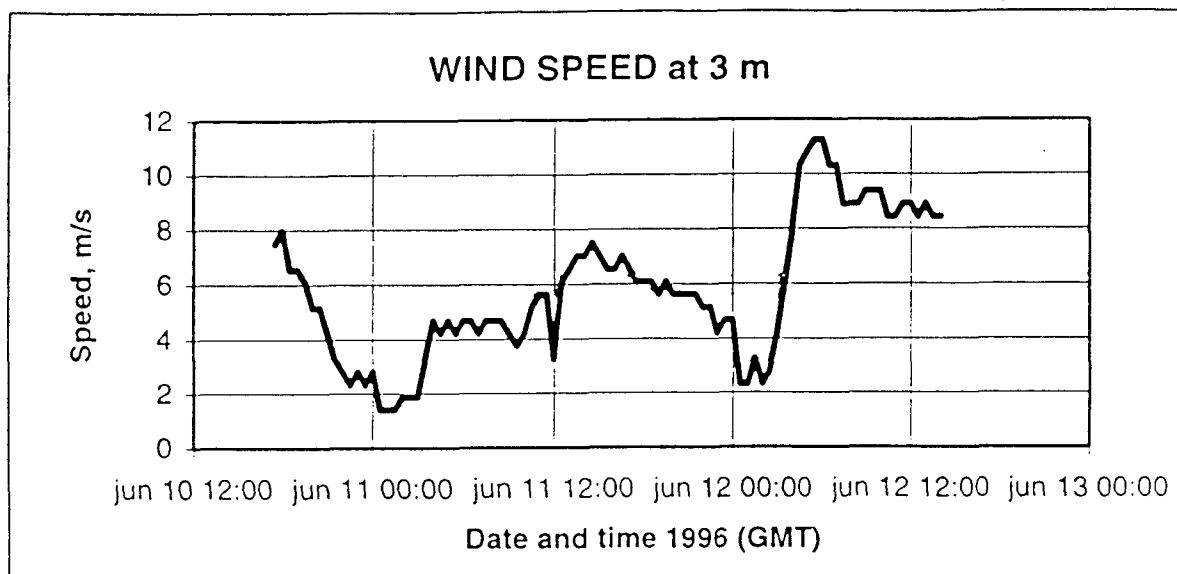


Figure 2-2. Wind Speed (m/s) and Significant Wave Height (m) Recorded During Trials 1-2
 (Used with permission from Brandvik *et al.*, 1996a).

Sample and Reference Water Handling

Sample water was collected in 1 L Nalgene® fluorinated DOT bottles (VWR Scientific, Cat. No. 16129-704) which had been pre-cleaned by rinsing with dichloromethane (DCM), then Nanopure deionized water. Two bottles were filled (no headspace) for each water sample, then transferred back to the research vessel via a RHIB. One sample water bottle was immediately stored at 4°C for later TPH and semi-volatile analysis by gas chromatography. Two 40 mL amber VOA vials (VWR Scientific, Trace-Clean, Cat. No. 15900-036,) were immediately filled (no headspace) from the second bottle of sample water, and stored at 4°C for later volatile analysis. Sample water from the second bottle was also used for the toxicity tests. Total time elapsed between water collection at sea and the commencement of the bioassays was about 0.5-1 hour.

In addition to the control water samples taken in the field, sterilized reference seawater was also used. The rationale for using this reference water was to have a background reference in case the control water at the field site was contaminated. In a previous field trial (Wright *et al.*, 1994), toxicity tests were compromised due to low-level hydrocarbon contamination of control water. Reference seawater was prepared one week prior to field testing by collecting water from the mouth of the Patuxent River (from Chesapeake Biological Laboratory, University of Maryland), which was 0.5 µm filtered. Ambient salinity of this water was measured at 8 ppt, and was subsequently taken up to 34 ppt with the addition of Hawaiian Marine sea salt. Previous studies indicate that the test organisms used in this experiment will not survive in reference water made entirely from artificial sea salts, and the only local sea water source was from this estuarine area. After a short mixing period, the water was poured through a 10 µm bag filter to remove excess undissolved salts, sterilized with UV light, then stored (in the dark) in the same type of 1 L Nalgene® bottles described above, and transferred to the field site.

TOXICITY TESTING - DESCRIPTION AND PROTOCOLS

Rotifer Acute Toxicity Test

A commercially available rotifer acute toxicity test kit was obtained from Charles River Aquatic Research Organisms, Wilmington, MA, USA. This kit is a complete off-the-shelf microbioassay

kit for performing 24-hour LC50 aquatic toxicity tests using the marine rotifer, *Brachionus plicatilis*. Marine rotifers are small zooplankters (about 0.25 mm in length) that are ubiquitously distributed in the world's oceans and estuaries. They feed on phytoplankton and are an important food source to larval fish. The kit includes vials of rotifer cysts (dormant eggs), specially designed PVC test plates, salt solutions required for hatching the cysts, micropipettes, reference toxicant, and a Standard Operational Procedure (SOP) guide.

In brief, the SOP involves hatching the rotifer cysts by placing them in 10 mL of 20 ppt seawater and incubating them under light at 25°C for 28-30 hours. Because of incubation time, hatching needs to be initiated 28-30 hours prior to the start of the toxicity test. Once hatched, neonates are rinsed and acclimated to full strength seawater for one hour. They are then transferred to a series of "exposure wells" (five rotifers per well) containing 0.3 mL of reference seawater or sample water. The wells are then covered and incubated under darkness at 25°C for 24 hours. Mortality is assessed at the end of the 24-hour exposure period. A qualitative pre-field trial experiment was conducted following this SOP with use of the reference toxicant potassium dichromate ($K_2Cr_2O_7$) at concentrations ranging from 100-1000 mg/L. The salt solution provided with the kit is intended for estuarine conditions, so full-strength reference seawater (as described in the previous section) was used. This experiment was done to provide familiarization with the SOP and to confirm viable hatching of the cysts. Chemical analysis and endpoint assessment was not completed in this preliminary run.

The Microbial Test

A commercially available bioluminescent bacterial test analyzer was used to screen the test water samples. The test utilizes a suspension of luminescent bacteria that emit light as a natural product of their metabolic processes. When exposed to toxic substances, the light emission is inhibited. The analyzer measures the degree of light reduction associated with toxic sample water, and thus is measuring a change in metabolism of the bacteria when exposed to toxic substances.

The "Basic Test Protocol for Marine Samples" (Microbics, 1992) was followed with the exception that 20 μ L of reagent was transferred to cuvettes B1-B5 during the "Reagent Preparation" step. As recommended by Microbics (1991), reagent was only used for two hours

after reconstitution, then discarded. A new reagent was mixed and used for each two-hour period of testing. Acceptable initial light intensity (I_0) levels on the reagent (taken after the 15-minute reagent stabilization wait time) were set by the researchers at $\pm 20\%$ variation, as recommended by Microbics. If any of the I_0 readings were outside of this range, the reagent batch was discarded and the test was rerun with a new reagent batch. After sample water was transferred to the reagent vials, light emission from the bacteria was recorded at both $T=5$ minute (I_5) and $T=15$ minute (I_{15}). Some toxicants require up to 15 minutes before full luminescent response occurs; although typical response time is 5 minutes. Therefore, the researchers opted to record light output at both time intervals. Due to space limitations aboard ship, the analyzer was used without a computer. All timing was performed with a stopwatch, and data were recorded manually. Both data reduction and statistical analyses were performed using the manufacturer's software (Microbics, 1994).

Oyster Embryo Development Test

Adult oysters (*Crassostrea gigas*), with pre-determined gender, were obtained from Guernsey Sea Farms (Park Lane, Vale, Guernsey, Channel Islands) by airmail shipment on 5 June 1996. They were kept refrigerated in damp paper towels until transferred to the ship, where they were placed in a shipboard incubator kept at 8°C until needed. The oysters were then acclimated to room temperature ($\sim 20^\circ\text{C}$) for 1-2 hours prior to use.

The protocol used for the embryo 48-hour EC50 test was modified from the standard ASTM method (ASTM Designation E 724-89). Approximately six hours prior to desired exposure start time, 2 male oyster gonads were stripped, filtered through $20\ \mu\text{m}$ Nitex™ and flushed with 500 mL diluent (reference seawater) into 1 L glass beakers. Two female oyster gonads were then stripped, filtered through $77\ \mu\text{m}$ Nitex™ and pooled into a single beaker, then diluted to ~ 5000 eggs/mL. An assessment of egg health was not made for two reasons: 1) the microscope on board did not have an adequate lens for inspection, and 2) the motion of the ship caused excessive movement of the eggs, making observation difficult. The eggs were assumed to be healthy. The sperm were examined under a microscope after 15 minutes, and the beaker containing the healthier (more motile) sperm was diluted to 1.5 L. Two milliliters of this sperm

suspension was added to the beaker of eggs to initiate fertilization (in Trial 2, sperm were diluted to 1.0 L to conserve reference seawater, and 1.5 mL of sperm suspension was used to initiate fertilization). The resultant suspension was gently agitated periodically. Since it was not possible to assess the embryo cell stage on board the ship, it was assumed that the embryos reached the 4-8 cell stage after 4 hours. They were then concentrated to ~6000 embryos/mL by removing diluent through a 77 μ m Nitex™ filter. The approximate concentration was determined by means of a Sedwick-Rafter counting cell. Then 0.5 mL of the embryo suspension was added to 30 mL of test solution in a 60 mL glass screw top bottle. (Note in Trial 2, embryos were concentrated to ~5000 embryos/mL and 1.0 mL of the embryo suspension was added to each bottle.) The bottles were sealed with Teflon®-lined caps and maintained at room temperature.

At the end of the exposure period (48 hours), 1 mL of 4% formalin (in seawater) solution was added to each test bottle. Each bottle was then gently swirled to mix, and set aside for subsequent counting. Counting was performed by gently mixing the bottle and removing two separate 1 mL subsamples, that were each placed on Sedgewick-Rafter counting cells and assessed under a microscope for normal and abnormal D-hinge (Prodissoconch I) and undifferentiated trochophore development (as per ASTM E 724-89 guidelines).

Copepod Lethality Test

A 48-hour lethality toxicity test was performed using the copepod, *Acartia tonsa*, which were obtained from a culture maintained by Dr. Dag Altin of ALLFORSK (Trondheim, Norway). The shipment of synchronous batch copepods (10 days old) was received on 10 June and immediately transferred to the ship, where it was aerated, fed algae, and maintained at room temperature (~20°C). All algal food was comprised of a 1:1 mixture (v/v) *Monochrysis sp.* and *Thallasiosira sp.* On each test date, copepods were removed from the culture and concentrated down to ~10 times initial culture density. Sets of 10 copepods were pre-counted under a microscope into 5 mL plastic weigh boats with 1-2 mL of reference seawater and set aside until needed (not more than 2.5 hours). No consideration was given to the sex of the copepod during counting.

A series of 60 mL screw cap bottles were set up with 20 mL of sample water in each. The pre-counted copepods were randomly assigned to the bottles, and were transferred to them by

flushing them in with an additional 10 mL of sample water, to give a final volume of 30 mL sample water with 10 copepods per bottle. The test copepods were then fed by transferring 0.5 mL algae to each bottle. The exact algal concentration was not known, due to the difficulties associated with cell counting on board ship. The bottles were then covered (not sealed) and maintained at room temperature. While all tests were begun at sea, the ship returned to port before the end of the 48-hour incubation period, and the samples were transferred to shore for the duration of the tests.

At the end of the exposure period (48 hours), the copepods were filtered (77 μ m Nitex™) from the sample water and rinsed into a Petri dish with reference seawater. They were assessed under a microscope as dead or live organisms. Dead organisms were qualitatively evaluated for color and level of decomposition.

Reference Tests

Although the standard protocols for each of these bioassays recommends the use of reference toxicants (e.g., CdCl₂ for copepods and oysters, phenol for the microbial test, and K₂Cr₂O₇ for the rotifer test) to assess the quality of the test organisms, it was not practical to conduct reference tests concurrent with sample water exposures aboard ship with the limited time, space, and manpower available. It was hoped that follow-up experiments could be conducted in the laboratory to correlate these field tests to land-based laboratory standard toxicity tests that incorporated reference toxicants. A follow-on study was not within the scope of the current field study.

ANALYTICAL METHODS

Water, oil, and dispersant samples were analyzed by Newcastle Research Group (University of Newcastle upon Tyne, Fossil Fuels and Environmental Geochemistry Department). The total petroleum hydrocarbon (TPH) content, volatile organic fraction, and semi-volatile fraction (polycyclic aromatic hydrocarbons) were quantified for each sample. The methods used for the hydrocarbon analysis were essentially those detailed in the relevant EPA protocols with modification where necessary for compounds of particular interest.

Volatiles

Analysis of the volatile component followed EPA Method #8260A: "Volatile organic compounds by gas chromatography-mass spectrometry: capillary column technique," also known as "Purge & Trap." These analyses were subcontracted to AES Laboratories (Newcastle, England) which is one of the largest government-accredited water industry laboratories in the United Kingdom that routinely do Purge & Trap analysis. Quantification was based on the internal standard specified in this method (4-bromofluorobenzene) and calibrated against a 40-component calibration mixture which included all analyzed compounds with the exception of n-C₅, which was quantified using the same response factor as n-C₆.

Semi-volatiles (PAH)

Extraction of the organic fraction from the water was achieved using EPA Method #3510B: "Separatory funnel liquid-liquid extraction." This method involves extraction with dichloromethane (DCM) at pH>11, then the extract is dried with anhydrous sodium sulfate. The method was followed with the following modifications:

- (1) The volume extracted was 500 mL, and
- (2) The concentration technique used was a rotary evaporation under reduced pressure using a Buchi Rotavapor.

Separation of the organic solvent extract into a TPH fraction was achieved using a modified EPA Method #3630B: "Silica gel clean-up." Briefly, this involves adding the DCM extract to a small portion of alumina (~1 g) and allowing the DCM to evaporate. The alumina containing the absorbed extract is then transferred to the top of the gravity column. This is a silica gel (10 g) and sodium sulfate (1 g) column made up in DCM, pre-eluted with 40mL pentane. The absorbed extract is eluted with 25 mL pentane, followed by 40mL pentane/DCM mixture (3:2 v/v). The final concentration step was to 100 µL rather than the 1 mL stated in the EPA protocol in order to allow determination of lower concentrations of components.

Gas chromatography-mass spectrometry (GC-MS) analyses of the petroleum hydrocarbon fractions were carried out using EPA Method #8270B: "GC-MS for semi-volatiles: capillary

column technique.” This method was followed with the exception of the GC oven temperature profile. A slower heating rate was utilized to help ensure good separation of the various components. The GC oven profile used was: 40°C for 4 minutes, 40-300°C at 4°C/minute, 300°C for 21 minutes. GC-MS was carried out on a Hewlett Packard (HP) 5890 Series II GC connected to an HP 5972 MS detector. All analyses were performed on an HP-5 column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) using helium as the carrier gas. Split/splitless injection via an HP 7673 autosampler was used. Quantification was performed with the HP Environquant software.

A Base-Neutrals Surrogate Spike Mix (Supelco Cat. No. 4-8925) containing nitrobenzene-d5, p-terphenyl-d14, and 2-fluorobiphenyl was used as the surrogate standard mix, and 100 µg of each component was added to every 500 mL sample prior to extraction. A semi-volatile internal standard mix (Supelco Cat. No. 4-890) containing 1,4-dichlorobenzene-d4, naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12 was used as the internal standard for the PAH fractions.

Quantitation/matrix spike mixtures containing the following compounds at five different concentrations were prepared: naphthalene, 1-methylnaphthalene, 2,6-dimethylnaphthalene, 1,1'-biphenyl, fluorene, dibenzothiophene, phenanthrene, fluoranthene, pyrene, chrysene, and perylene. These compounds gave a wide range of molecular weights, together with those of particular interest for degradation and weathering studies of petroleum hydrocarbons. Appendix A shows the quantitation compounds used for calibrating other compounds for which standards were not available.

Total Petroleum Hydrocarbon (TPH)

GC analysis was performed using similar conditions and the same column on an HP 5890 Series II GC fitted with a flame ionization detector. Data was collected on a Fisons Multichrom data system (Micro VAX 3100 based). Quantification of TPH was achieved by summing the total GC area in the TPH fraction, using p-terphenyl as an internal standard, and using a response factor (RF) of one. The surrogate standard used during these analyses is described above.

Section 3

RESULTS

TOXICITY TEST RESULTS

A total of six water samples were used to conduct bioassays during the Trial 1 surface oil release.

- These included the reference water, control seawater (collected prior to oil release), oil only samples (1 m and 5 m depths), and chemically dispersed oil (CDO) samples (1 m and 5 m depths). Four water samples were used for the Trial 2 subsurface oil release tests. These included the reference water, control water (that was collected during Trial 1, since it was not possible to collect clean seawater during Trial 2), and oil-only samples at 1 m and 5 m depths. Dispersant was not used during Trial 2 therefore CDO samples were not collected.

Two of the four bioassays, the rotifer test and the oyster embryo development test, failed prior to completion. Although there is no way to definitively identify the causes for this, some potential sources for test failure are discussed in Section 4.

Results for the microbial tests are summarized in Tables 3-1 and 3-2, and are presented as % bacterial light emission for either T=5-minute exposures (I_5) or T=15-minute exposures (I_{15}). This % light reading is a measure of the metabolic activity of the bacteria. The “Basic Test Protocol for Marine Samples” was utilized during this experiment, based on preliminary experiments indicating that it was the most appropriate method for concentrations of oil and dispersed oil which were likely to be observed at sea. This method requires a 500 μ L volume of sample (treatment) water to be added to a 520 μ L volume of bacterial solution. This causes the treatment water to dilute down to 49% of its original concentration. Serial dilutions of this mixture are then prepared (24.5%, 12.3%, 6.1%) and tested to determine an EC50 value. This EC50 value is reported as percent dilution of the initial sample water, and can only be calculated accurately if at least one of the concentration responses in the dilution series results in greater than 50% light attenuation.

Table 3-1. Summary of Microbial Test Light Readings for Highest Sample Concentration Tested (49%) at T=5 and T=15-Minute Incubation Times.

Trial #	Sample Description	Control			49% Sample Concentration		
		I ₀	I ₅	I ₁₅	I ₀	I ₅	I ₁₅
Trial 1	Reference Water	97	86	84	78	96	90
	Seawater Control	95	95	98	96	103	104
	1m CDO*, analysis 1	91	95	94	89	33	34
	1m CDO, analysis 2	111	110	112	100	37	40
	1m CDO, analysis 3	93	99	100	85	32	35
	5m CDO	97	92	91	94	73	71
	1m Oil Only, analysis 1	94	116	--‡	78	104	-- ‡
	1m Oil Only, analysis 2	97	96	95	93	92	89
	5m Oil Only	95	104	104	85	97	101
Trial 2	Seawater Control (from 6/11/96)	96	98	98	91	99	94
	1m Oil Only	86	83	82	83	83	82
	5m Oil Only	97	100	98	95	100	95

*CDO=Chemically dispersed oil; ‡Run was terminated early due to temperature warning light and concern of heat damage to the bacteria.

Table 3-2. Summary of Microbial Test EC50 Calculations for Light Emission Readings at T=5 Minutes and T=15 Minutes (EC50 estimates are reported as percent of initial sample concentration).

Trial #	Sample Description	I ₅ †EC50 (Conf. Interval)	I ₁₅ ‡EC50 (Conf. Interval)	Notes
Trial 1	Reference Water	> highest conc.	> highest conc.	
	Seawater Control	> highest conc.	> highest conc.	
	1m CDO*, analysis 1	25.3 % (7.5%,85.1%)	26.3 % (6.4%,108%)	I ₀ was not within acceptable limits. Test not completed.
	1m CDO, analysis 2	27.4% (24.4%,30.7%)	30.1% (24.7%,36.7%)	
	1m CDO, analysis 3	23.1% (16.7%,31.8%)	26.5% (18.7%,37.4%)	
	5m CDO	97.7% (3%,3169%)	> highest conc.	
	1m Oil Only, analysis 1	> highest conc.	no data	Analyzer began to overheat. Test not completed.
	1m Oil Only, analysis 2	> highest conc.	> highest conc.	
	5m Oil Only	> highest conc.	> highest conc.	
Trial 2	Seawater Control (from 6/11/96)	> highest conc.	> highest conc.	
	1m Oil Only	> highest conc.	> highest conc.	
	5m Oil Only	> highest conc.	> highest conc.	

*CDO=Chemically dispersed oil; †I₅=5minute light reading; ‡I₁₅=15minute light reading

In cases where an EC50 is calculated, a confidence interval is provided to indicate the power of the test. In cases where there is little toxicity, and little change in light output, the microbial test will not attempt to estimate an EC50, and will report it as “greater than highest concentration” tested. In cases where the highest concentration tested gave a light output value close to 50% light attenuation, the microbial test estimates an EC50 value. However, the confidence interval is very large since no sample concentration was actually greater than the 50% attenuation, and the microbial test cannot make the determination that a linear effect would continue at higher concentrations. This is the case for the 5 m CDO sample in Trial 1 (5-minute incubation period). It is important to keep in mind that the highest concentration tested in any of the microbial tests was 49% of initial sample concentration.

Results for the copepod lethality test are reported in Tables 3-3 and 3-4 for Trials 1 and 2, respectively. The data are presented in terms of survival (% mean live) based on five replicate bottles. A 95% Confidence Interval was calculated for the mean survival based on the following equation:

$$95\% \text{ Confidence Interval } (\alpha = 0.05) = \text{“Mean \% Live”} \pm t_{(1-\alpha/2)} * \frac{s}{\sqrt{n}}$$

where n=5 replicates and t=2.7764 (based on t distribution table). Although each bottle was supposed to receive 10 copepods based on the protocol, there were a few instances where either 9 or 11 copepods were counted at the completion of the test. As a result, the “mean % live” was calculated on actual copepod numbers counted.

Table 3-3. Copepod Test results – Trial 1

Sample Description	Beaker Number	Live Count	Dead Count	% Live	Mean % Live	Standard Deviation	Conf. Interval [#]
Reference Water	1	4	6	40%	37%	8.0%	27-47%
	2	5	5	50%			
	3	3	6*	33%			
	4	3	6*	33%			
	5	3	7	30%			
Seawater Control	1	4	6	40%	44%	8.9%	33-55%
	2	3	7	30%			
	3	5	5	50%			
	4	5	5	50%			
	5	5	5	50%			
CDO - 1 meter	1	0	10	0%	0%	0.0%	0%
	2	0	10	0%			
	3	0	10	0%			
	4	0	10	0%			
	5	0	10	0%			
CDO - 5 meters	1	0	10	0%	24%	18.2%	1-47%
	2	2	8	20%			
	3	5	5	50%			
	4	3	7	30%			
	5	2	8	20%			
Oil Only - 1 meter	1	2	8	20%	35%	13.4%	18-52%
	2	3	7	30%			
	3	4	6	40%			
	4	3	7	30%			
	5	5	4*	56%			
Oil Only - 5 meters	1	4	6	40%	41%	7.3%	32-50%
	2	5	5	50%			
	3	3	7	30%			
	4	4	6	40%			
	5	4	5*	44%			

* Copepod missing; mean based on n=9.

[#] 95% C.I. based on following equation: Mean $\pm t_{(1-\alpha/2)} \cdot s/\sqrt{n}$ where n=5 and t=2.7764 (from t dist. table)

Table 3-4. Copepod Test Results – Trial 2

Sample Description	Beaker Number	Live Count	Dead Count	% Live	Mean % Live	Standard Deviation	Conf. Interval [#]
Reference Water	1	4	6	40%	36%	11.4%	22-50%
	2	5	5	50%			
	3	3	7	30%			
	4	2	8	20%			
	5	4	6	40%			
Seawater Control (from 6/11/96)	1	5	5	50%	38%	8.4%	28-48%
	2	4	6	40%			
	3	3	7	30%			
	4	3	7	30%			
	5	4	6	40%			
Oil Only - 1 meter	1	8	2	80%	41%	22.0%	14-68%
	2	3	7	30%			
	3	3	7	30%			
	4	3	6*	33%			
	5	3	7	30%			
Oil Only - 5 meters	1	5	5	50%	39%	10.1%	26-52%
	2	5	5	50%			
	3	3	7	30%			
	4	4	7**	36%			
	5	3	7	30%			

* Copepod missing; mean based on n=9

** Extra copepod present; mean based on n=11.

[#] 95% C.I. based on following equation: Mean $\pm t_{(1-\alpha/2)} \cdot s/\sqrt{n}$ where n=5 and t=2.7764 (from t dist. table)

SAMPLE WATER HYDROCARBON ANALYSIS RESULTS

During Trial 1, *in situ* fluorometry readings were run continuously across transects through the slicks. This was done to monitor oil concentrations in the dispersed plume, and to assist in tracking slick movement. Figure 3-1 provides a comparison of oil concentrations along a transect through the slick both before and after treatment with dispersant. The UV fluorometer readings were used as a qualitative source of information on oil distribution, and sample water was collected when these fluorometry readings indicated high concentrations of oil. Due to the inhomogeneous nature of oil distribution in the plume, it is not appropriate to compare discreet water sample TPH-GC values (listed in Table 3-7) with fluorometry values (listed in Figure 3-1). The concentration values presented in Figure 3-1 were obtained by calibrating the fluorometer readings to total extractable organic content (TEOC) values determined by GC-FID on samples collected by the Norwegian field team. Detailed methodology is presented in Strøm-Kristiansen *et al.* (1996).

A total of nine samples were collected and shipped to NRG for analysis. These included the seven water samples collected during the field trial, one sample of neat Troll oil and one sample of Corexit® 9500 dispersant. The reference water source (see Section 2) is regularly monitored for hydrocarbon content and is assumed to be free of hydrocarbon contamination. Therefore, due to cost constraints the reference seawater was not sent for detailed hydrocarbon analysis.

Results of the volatile analysis of water samples are presented in Table 3-5. Volatile analyses were not performed on the neat oil and dispersant samples since this information would not assist in interpreting biological effects. Concentrations from semi-volatile analyses are presented in Table 3-6. Most of the values for the volatile and semi-volatile analyses are at least one order of magnitude lower than the minimum detection limits recommended in the EPA analytical protocol (10 µg/L for most semi-volatiles). Although many of the values were below this limit, they were reported since the minimum detection limits of the GC-MS were 0.01 µg/L (i.e., 10 ng/L) for a given analyte. TPH quantities, as determined by GC-FID are presented in Table 3-7. The gas chromatograms of the TPH fractions are presented in Appendix C. The total ion currents (TIC)

from the TPH fraction GC-MS analyses and various partial ion fragmentograms are presented in Appendix D.

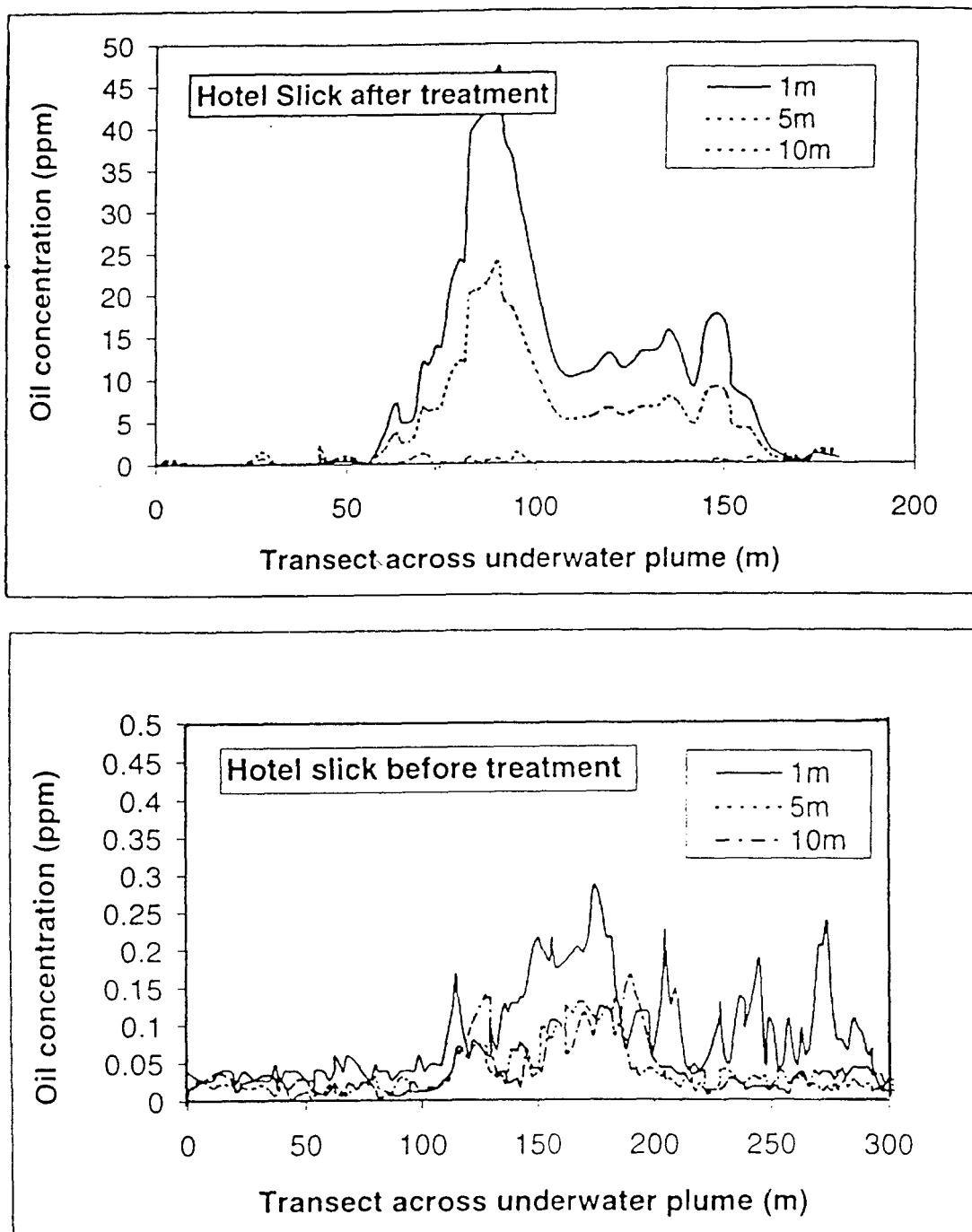


Figure 3-1. Dispersed Oil Concentrations (Measured via *In Situ* Fluorometry) Under the Hotel Slick Before (a) and After (b) Dispersant Application, Approximately 4.5 Hours After Release and 30 Minutes After Dispersant Treatment (Used with permission from Brandvik *et al.*, 1996a).