TOXICITY OF SOUTH LOUISIANA CRUDE OIL, ALASKAN NORTH SLOPE CRUDE OIL AND DISPERSANT COREXIT 9500 TO GULF KILLIFISH, WHITE SHRIMP, AND EASTERN OYSTER

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Abstract

To address public concern over potential ecological effects on commercially and ecologically important species following use of dispersants during oil spill response efforts, toxicity data was generated for three estuarine species indigenous to the Gulf of Mexico including juvenile Gulf killifish Fundulus grandis, white shrimp Litopenaeus setiferus, and Eastern oyster Crassostrea virginica. The acute toxicity of the dispersant Exxon Corexit 9500, South Louisiana crude oil (SLC), Alaskan north slope crude oil (ANSC) and dispersed oils (SLC+9500 and ANSC+9500) to the species was determined for both nominal concentrations (NC) and hydrocarbon concentrations (HC). Two 24-h field toxicity trials were conducted with the same species in a Louisiana coastal marsh, using ANSC and ANSC+9500, dosed at a NC of 30 ppm.

White shrimp were more sensitive to dispersant, crude oils, and dispersed oils than killifish and oysters. The 96-h NC LC50 for crude oil and dispersed oil ranged from 370 to 4,500 ppm for killifish (HC 7.6 to 18.7 ppm) and 60 to 180 ppm for shrimp (HC 5 to 7.5 ppm). Mortality in oysters was not positively correlated with increasing levels of crude oils, or dispersed oils. Dispersed oils were more toxic than crude oils based on nominal concentrations, but no difference in toxicity of crude oils and dispersed oils was observed based on HC concentrations. No synergistic toxicity action was found between SLC or ANSC and dispersant Corexit 9500 based on HC concentrations.

Survival was relatively high for all three species during the two 24-h field trials, generally exceeding 83% in crude oil and dispersed oil enclosures. Mortality of white shrimp was slightly higher than observed in killifish and oysters. The HC concentration in ANSC+9500 and ANSC enclosures ranged from 14 to 24 ppm and 10 to 11 ppm, respectively, at 0 h and declined to near 0 ppm in 3 hours. The rapid decrease was

attributed to dilution from vertical mixing and tidal action. Both laboratory finding and field studies indicate that short-term exposure to nominal concentrations of ANSC or ANSC+9500 of 30 ppm or less are not likely to have an acute toxic effect on these species.

Chapter 1

Introduction

Louisiana has a significant portion of the USA's riverine, estuarine, and coastal ecosystems. A significant amount of petroleum is refined, stored, or transported through these areas with an annual value of $6.3 billion. Nearly 15% of USA crude oil production is located in Louisiana, and 20% of the nation's oil flows through Louisiana's coastal marshes (Davis and Guidry, 1996). Louisiana is also a national leader in commercial and recreational fisheries production and its coastal wetlands contribute 28 percent to the total volume of domestic fisheries harvest (LAcoast, in "The Cost of Doing Nothing..."; http://www.lacoast.gov/watermarks/1999c-summer/1cost). These wetlands, that provide essential habitat for fisheries species, are vulnerable to oil spills. About 35% to 50% of spillage in USA waters is crude oil. Even though natural seeps of crude oil occur in the ocean floor and stable biotic communities are associated with them, the sudden introduction of high concentrations of hydrocarbons could potentially kill or impose sub-lethal effects in some marine and estuarine species. Contamination by spilled oil can close a commercial shellfish bed for years, resulting in a considerable financial loss to shellfish producers and local economies (National Research Council [NRC], 1989).

To reduce the impact of spilled oil, chemical dispersants and mechanical containment are widely used. Oil can be physically removed from the water surface using barriers to hold or divert it, and skimmers or sorbents are used for recovery. Wetlands are vulnerable to damage during removal operations (OAT, 1990). Chemical dispersants contain surfactants that reduce interfacial tension between oil and water, so they promote the formation of numerous tiny oil droplets and also prevent the coalescence of oil

droplets (NRC, 1989). The first use of chemical dispersants was in 1967 when the tanker Torrey Canyon spill occurred and over 100,000 metric tons of crude oil were released (Smith, 1970). A main concern over the use of dispersant after the Torrey Canyon spill was the toxicity of the dispersants themselves, and the chemical action of the dispersants that increase the solubility of oil components in water, thereby increasing exposure of aquatic organisms to toxic fractions. Public concern over the adverse effects of oil spills stimulated the development of a variety of response techniques to contain or remove spilled oil before it could harm property or the environment (NRC, 1989). Following the Exxon Valdez accident, the federal government passed the Oil Pollution Act of 1990 that established lines of responsibility and a cleanup consortium for future oil spills. Louisiana passed the Oil Spill Prevention and Response Act of 1991 to address statewide oil spill prevention and response (Davis and Guidry, 1996). The dispersants formulated in recent years use non-aromatic hydrocarbons and reduced amounts of toxic surfactants and are considerably less toxic. Chemical dispersants have potential to play an important role in oil spill response and clean-up where mechanical removal may not be possible because of cost or high waves, or to prevent the formation of tar balls.

Despite the effectiveness dispersants in visually removing oil, decision-makers are concerned with the environmental and toxicological effects associated with dispersants and dispersed oil. Regulatory agencies that have a pre-approval process for use of an oil dispersant must be able to assess the potential impact on the aquatic environment (Pace and Clark, 1993). Dispersants have not been widely used in the USA because of complex authorization procedures, logistical difficulties, and a lack of demonstrated effectiveness during actual spills. Currently, insufficient information is

available on the effects and behavior of dispersed oil in the near shore Gulf Coast environment to justify widespread use and application.

Four dispersants - COREXIT 9500, COREXIT 9527, Mare Clean 200 and NEOS AB 3000 – are authorized for use on oil discharge by United States Environmental Protection Agency (USEPA). COREXIT 9500 and COREXIT 9527 are products produced in the USA. COREXIT 9500 is a more recent product and has not been sufficiently tested, especially in conditions common to coastal Louisiana. Toxicological studies are necessary to evaluate the potential impact of dispersants and dispersant by-products on indigenous aquatic organisms and the environment.

To determine the relative toxicity of a chemical on an aquatic organism, an acute toxicity test is first conducted to estimate the lethal concentration of the compound or chemical. Usually, a 48 h or 96 h lethal concentration 50 ("LC50"), which is the concentration estimated to produce mortality in 50% of a test population over a specified time period is reported (Rand, 1995). Many studies have reported the toxicity of crude oil and dispersants from nominal (dose) concentrations rather than measured (analytical) concentrations of hydrocarbons in the water. Toxicity associated with nominal concentrations tends to be larger for crude oil when compared to hydrocarbon concentrations. The more toxic components of crude oil to aquatic organisms include BTEX (i.e., benzene, toluene, ethylbenzene, xylene) and PAH (polycyclic aromatic hydrocarbons), all which have higher water solubility than heavier oil fractions. More recent studies of oil and dispersed oil toxicity to aquatic organisms have reported the water-accommodated fraction (WAF) or chemically-enhanced water-accommodated fraction (CE-WAF) provide more realistic assessments.

The exposure regime to crude oil or dispersed oil will influence their toxicity to aquatic species. Rhoton et al. (2001) reported that the LC50 to marine species exposed to Alaskan north slope crude oil with a spiked exposure (renewable oil exposure) was lower (more toxic) than with flow-through (continuous oil exposure) exposure. The spiked exposure to crude oil is more representative of actual field conditions, and the flow-through tests tend to overestimate the toxic effects of a true oil spill response (Singer et al., 2001; Pace et al., 1995). The standard static non-renewal exposure toxicity test has been used to evaluate oil toxicity to aquatic organisms and compared to other exposure regimes. The Chemical Response to Oil Spills: Ecological Research Forum (CROSERF), a group of individuals from State and Federal government, academia and industry, have worked to develop standard laboratory toxicity test procedures for petroleum dispersants so as to make cross comparison of data possible (Coelho and Aurand, 1997; NRC, 1989).

Most oil toxicity studies have focused on early life stages (egg, embryo, larvae) of test species because these stages are generally more sensitive than adults, but there are exceptions. For example, postlarval white shrimp Litopenaeus setiferus and brown shrimp Penaeus aztecus apparently are more tolerant than juveniles on exposure to No. 2 fuel oil (NAS, 1985).

The objective of this study was to evaluate the acute toxicity of one oil dispersant, Exxon COREXIT 9500 (9500), two crude oils, South Louisiana crude oil (SLC) and Alaskan north slope crude oil (ANSC), and two dispersed oils, dispersed South Louisiana crude oil (SLC+9500) and dispersed Alaskan north slope crude oil (ANSC+9500) to three commercially and ecologically important species indigenous to the Gulf of Mexico: Gulf killifish Fundulus grandis, Eastern oyster Crassostrea virginica and white shrimp

Litopenaeus setiferus. Joint toxicity was examined to describe the effects of crude oil (SLC or ANSC) and 9500 as either additive, synergistic or antagonistic. Joint toxicity, "also referred to as joint action or mixture toxicity, occurs where two or more chemicals are exerting their effects simultaneously" (NRC, 1989).

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Chapter 2

Acute Lethal Toxicity of South Louisiana Crude Oil, Alaskan North Slope Crude Oil and Dispersant Exxon COREXIT 9500 to Gulf Killifish, White Shrimp and Eastern Oyster

Objectives

The objectives of this study were: (1) to determine the acute toxicity of South Louisiana crude oil (SLC), Alaskan north slope crude oil (ANSC), dispersant Exxon COREXIT 9500 (9500), South Louisiana crude oil plus Exxon COREXIT 9500 (SLC+9500), and Alaskan north slope crude oil plus Exxon COREXIT 9500 (ANSC+9500) to Gulf killifish, white shrimp, and Eastern oyster; (2) compare the acute lethal effects of the crude oils, dispersant, and dispersed oils; (3) contrast the comparative tolerance of the three species to the crude oils, dispersant, and dispersed oils; and (4) determine the joint lethal toxicity of crude oil and dispersant for each species.

Materials and Methods

Test Species , Maintenance, and Acclimation

The test species selected for the toxicity evaluations were Gulf killifish Fundulus grandis, white shrimp Litopenaeus setiferus , and Eastern oyster Crassostrea virginica. Fundulus grandis were obtained in May and October 2001 from Gulf Coast Minnows Company, Thibodaux, Louisiana, USA. The fish ranged from 4 to 6 cm total length with a mean weight (mean ± SD) of 2.01 ± 0.83 g. Eastern oysters with mean whole weight (WW ± SD) of 98.4 ± 27.7 g were collected from March to July 2002 from Grand Isle, Louisiana, and transported to the Aquaculture Research Station. White shrimp with a total length ranging from 2 to 3.5 cm (distal end of the rostrum to the tip of the telson) were collected in July and August 2002 from a tidal ditch near Gulf Coast Research Lab,

Ocean Spring, Mississippi. Because of their sensitivity to handling and to avoid stress the shrimp were not weighed, but were estimated to range in weight from 4 to 6 grams based on the length-weight regression equations for Litopenaeus setiferus (Fontaine and Neal, 1971).

All test species were maintained in 2,000 L fiberglass tanks at 8-9 ppt salinity supplied with constant aeration at the Aquaculture Research Station, LSU AgCenter, Baton Rouge, Louisiana, until used in the study. Baton Rouge municipal supply water was aerated for 2 days to remove chlorine, and the mineral concentration of the holding and acclimation water adjusted to a salinity of 8-9 ppt with artificial crystal sea salt (Crystal Sea Marinemix, Marine Enterprises International). Gulf killifish were fed a 36% crude protein catfish fingerling feed (Land O'Lakes Farmland Feed, Fort Dodge, IA) once daily. Oysters were fed with a concentrated algae paste (Green Water Formula, Innovative Aquaculture Products, Skerry Bay, Lasqueti Island British Columbia, Canada) once daily prior to use in the tests. The shrimp were not fed because they were used 2 days after collection.

Test organisms were acclimated to ambient laboratory conditions prior to use in the exposure tests as follows: Gulf killifish, 7 days; Eastern oyster, 2 days; and white shrimp, 2 days (USEPA, 1975). Salinity and water temperature during acclimation were maintained within ±3 ppt and ±3 ^{o}C of the exposure conditions (ASTM 8010 E, 1992). Test organisms were subjected to a 12-h light and 12-h dark cycle provided by cool white fluorescent bulbs (Osram Sylvania, Danvers, MA). Feeding ceased 24 hours before exposure for Gulf killifish and Eastern oysters (American Public Health Association, 1965) and animals were not fed during the toxicity tests.

Exposure Chemicals and Test Solutions

Toxicity tests were conducted using chemical solutions made from dispersant, crude oil or dispersed oil (mixture of crude oil and dispersant) from the following compounds: (1) the dispersant COREXIT 9500 (9500) (Nalco/Exxon Energy Chemicals, L.P. Sugarland, Texas); (2) South Louisiana crude oil (SLC), and (3) Alaskan north slope crude oil (ANSC). The crude oils were collected directly from production lines on oil platforms in Louisiana, and Alaska. The oils were stored in tightly covered containers at 4^{o}C to minimize evaporation of volatile components.

COREXIT 9500 contains both anionic and nonionic properties, and an oleophilic solvent carrier designed to work on a wider range of oils including the higher viscosity oils and emulsions (Singer et al., 1996; George-Ares, 2000; Rhoton et al., 2001). SLC is high in light aromatic hydrocarbons and low molecular weight n-alkanes. The oil also contains low levels of sulfur and polycyclic aromatic hydrocarbons (Devai et al., 1998; Lindau and Delaune, 2000). ANSC contains nearly one-third (w/w) volatile, compounds with a boiling point of 204 to 274 ^{o}C (Rhoton et al., 2001). The ANSC appeared more viscous than SLC. The density (mean ± SD) of SLC and ANSC were 889.3 ± 1.16 mg/mL and 939.3 ± 10.07 mg/mL, respectively. The oils were disturbed (stirred with glass rod in their storage container) prior to each test to dispense the oil components evenly. The dispersed SLC (SLC+9500) and dispersed ANSC (ANSC+9500) were made by pre-mixing them at an oil:dispersant ratio of 20:1 (v:v) in a 250 mL glass beaker for 2 minutes on a magnetic stir plate (610 T model, Fisher Scientific) with a 25-mm stir bar (Becker, 1993). The dispersed oils were mixed immediately prior to dosing.

Acute Toxicity Test

A static non-renewal exposure regime was used to evaluate the lethal effect of five exposure chemicals: SLC, ANSC, SLC+9500, ANSC+9500 and 9500 alone to each of three test species over an exposure period of 96 hours (APHA 1965). Organisms were exposed to non-toxicant control (dilution water only) and either five or six toxicant concentrations. The exposure solutions were made by adding the oil, dispersed oil, or dispersant directly in the exposure water (Figure 2.1). Eight-liter volume capacity glass aquaria (30 cm × 15 cm × 20 cm) with glass tops were used as exposure containers. The aquaria were randomly assigned to a test concentration for each of the five toxicants and the non-toxic control for each species. A notch (0.75-cm^2) was placed at two corners of each glass top to accommodate air tubing. A preliminary range-finding test was conducted to define the range of oil, dispersed oil or dispersant dilutions to be used in the definitive tests. Two definitive tests were conducted using Fundulus grandis and Litopenaeus setiferus and three definitive tests for Crassostrea virginica. Ambient water temperatures during the tests ranged from 22 to 23 ^{o}C. One additional acute lethal toxicity test on Crassostrea virginica was conducted under a higher temperature at 28 ^{o}C. A HOBO temperature data logger (Onset Computer Corporation, Bourne, MA) was placed in the control aquarium to continuously monitor water temperature during each 96-h exposure trial.

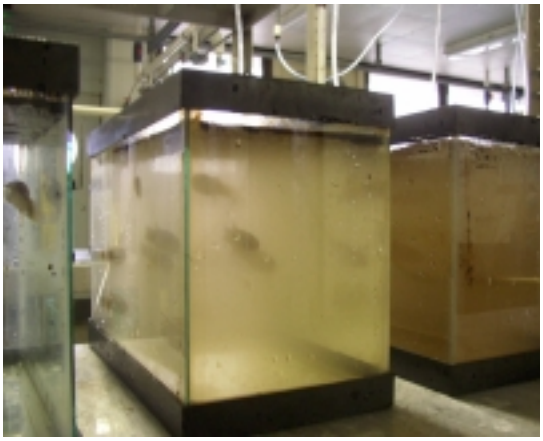

Figure 2.1. SLC exposure setting for Fundulus grandis.

The oil, dispersed oil or dispersant was injected 1-cm below the water surface with either a 5-mL pipettor (< 20 mL of toxicant) or 10 mL syringe (> 20 mL of toxicant) (Becton Dickison & Company, Franklin Lakes, NJ), and the solutions mixed twice with a plastic disposable pipette for 1 minute. This mixing procedure of crude oil and dispersed oil was used in an attempt to simulate field conditions in which an oil spill might occur in a Louisiana coastal marsh with minimal tidal exchange and wave action. Oil-free air was supplied to each exposure container via a glass tube (5-mm dia) with a 1.5-mm outlet at a rate of about 150 bubbles per minute (5.03 mL/min) to maintain sufficient dissolved oxygen concentrations for the test (Webber, 1993; Rhoton et al., 2001). Aeration was minimized to reduce loss of volatile oil components to the atmosphere from water turbulence. Prior to placement of the test organisms in the aquaria, air volume was increased briefly to clear oil from an area about twice the size of the test organisms, through which organisms were introduced into the exposure solution. Either 8 or 10 test organisms per aquarium were added. The tests were terminated after 96 hours.

Dead organisms were removed from each aquarium at 24-hour intervals, and behavior of live animals was noted. Organisms that showed no response to gentle prodding were considered dead (Fuller et al., 2001).

Water Analysis

Water samples were collected for determination of salinity, pH, un-ionized ammonia, dissolved oxygen, and total hydrocarbons. To minimize contamination by feces and oil, a 10-mL water sample was collected from the middle of the water column by siphoning through a plastic tube (5-mm dia) with one end connected to a plastic pipe and syringe.

Water was analyzed daily for salinity (YSI Model 33, Yellow Springs Instrument Co., Inc., Yellow Spring, Ohio), pH (glass electrode) and un-ionized ammonia (HACH Company, Loveland, Colorado). Dissolved oxygen (Winkler method) (Tucker and Boyd, 1992) was measured at 0, 48, and 96 hours. Total hydrocarbons were measured for crude oils and dispersed oil solutions at dosing and at 24-hour intervals by fluorometery (Turner Quantech Digital Filter Fluorometer Model FM109535) using a method modified from (Booksh, 1996). A standard curve for total hydrocarbons for both SLC and ANSC were made by dissolving the crude oils at concentrations of 0, 10, 25, 50 and 100 ppm in hexane and reading the fluorescence. The fluorometer has calibrated at 0 and 50 ppm of standard oil solutions, prior to taking reading of the water samples. Water samples for hydrocarbons analysis were stored at 4 ^{0}C for no more than 96 hours prior to fluorometric analysis.

Statistical Analysis

All statistical analyses were conducted using Statistical Analysis System (SAS) software (Statistical Analysis System software version 8.2 for Windows, SAS Institute, Inc., Gary, NC). Where possible, the probit analysis was used to estimate the 96-h LC50 with 95% confidence limits for each of the five chemical toxicants for each species using both nominal (dosed, NC) concentrations of the oils, dispersed oils, and dispersant, and analytical (measured) concentration of total hydrocarbons (HC) for oils and dispersed oils. Because concentrations of hydrocarbons changed during the 96-hour tests, the weighted mean of the measured total hydrocarbons concentration (HC) at 0, 24, 48, 72, and 96 hours was used in the statistical analyses according to the following equation:

$$HC \text{ (weighted mean)} = [HC_{0hr} + 2 \times (HC_{24hr} + HC_{48hr} + HC_{72hr}) + HC_{96hr}] / 8$$

The PROC PROBIT analysis, which uses a maximum-likelihood estimation procedure, was used for probit models (Finny, 1971; Ellersieck and La Point, 1995). The Spearman-Karber technique (distribution-free estimator) was used when the statistical criteria for proper use of the probit analysis were not met (Hamilton et al., 1977, 1978). The binomial method was used to estimate the 96-h LC50 where only 0% and 100% mortality occurred (Bliss, 1967). The LC50 value was determined from data using the highest toxicant concentration at which 0% mortality occurred and the lowest concentration at which 100% mortality occurred (Gelber et al., 1985).

The 96-h LC50 values for each toxicant for each species were calculated for each definitive test separately (non-pooled data) and then combining the raw data from the two test into a single data set and re-calculating the LC50 (pooled data set). The two-way analysis of variance (ANOVA) was used to determine if mortality, as determined from the 96-h LC50, differed among the species, and among different chemicals within a species. Differences in means were declared to be statistically significant at $\alpha < 0.05$.

Joint (Mixture) Toxicity

The joint action (mixture) toxicity of dispersant when combined with crude oil was determined from an isobole (Marking , 1985; Zheng and Weng , 1993) by plotting the 96-h LC50 (± 95% confidence limits) of the crude oil (x-axis) and dispersant (y-axis) for each species (Figure 2.2). Chemical mixture toxicity was of crude oil and the dispersant mixture was interpreted as being additive (AD), synergistic (SY), or antagonistic (AN) to the aquatic animals tested. The 96-h LC50 values of dispersed oils were partitioned into a 96-h LC50 fraction attributed to crude oil alone and a 96-h LC50

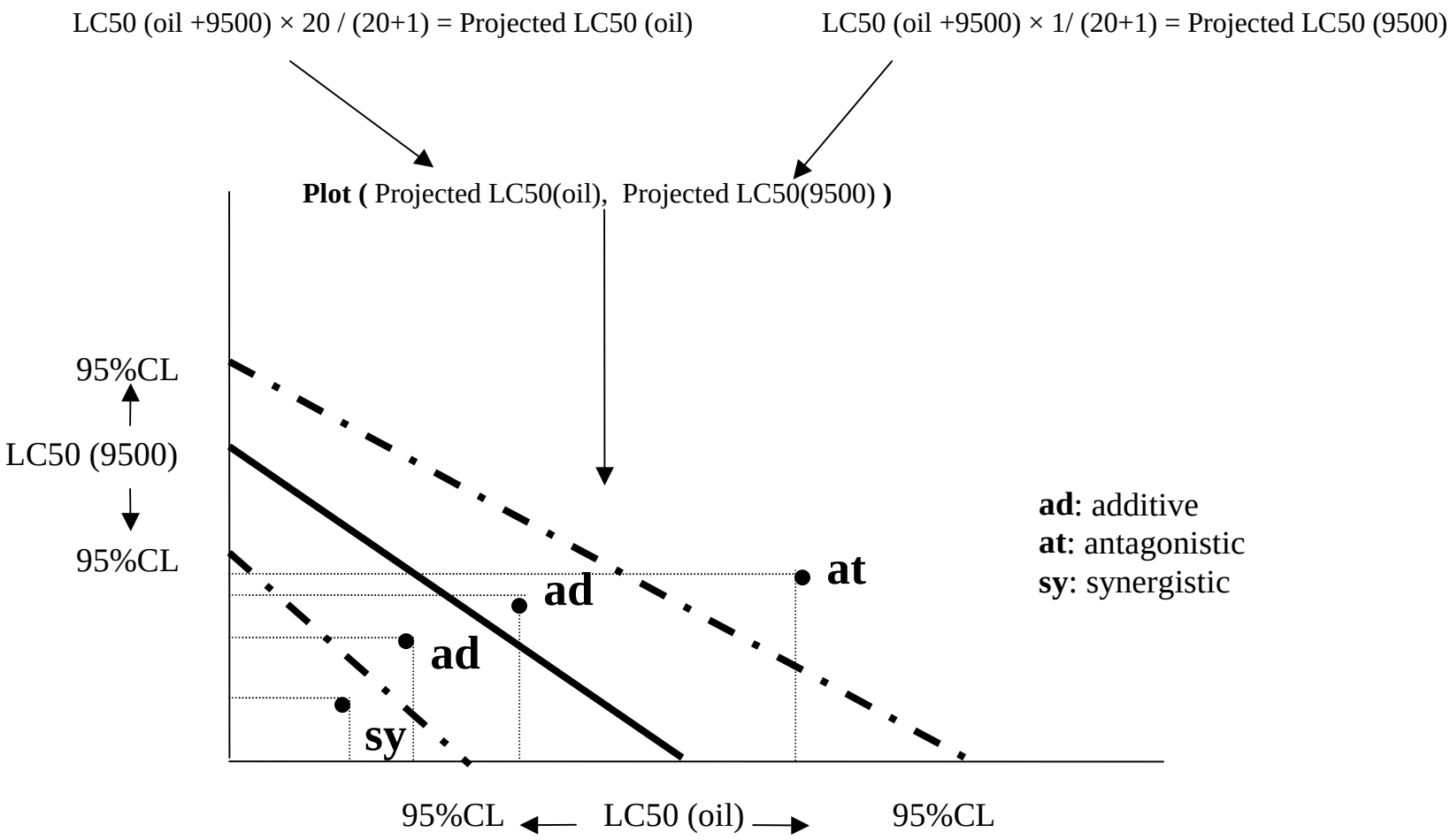

Figure 2.2. Isobole of the crude oil portion and dispersant portion of the 96-h LC50 of dispersed oil and the 96-LC50 values of crude oil and dispersant used to estimate the joint action toxicity of the dispersant and crude oil.

fraction attributed to dispersant alone, based on the oil:dispersant ratio of 20:1 (v:v) that was used in preparing the dispersed oil solutions used in dosing according to the following equations:

$$\text{Projected LC50 of crude oil} = (\text{LC50 dispersed oil}) \times 20 / (20+1) \quad (1)$$

$$\text{Projected LC50 of dispersant} = (\text{LC50 dispersed oil}) \times 1 / (20+1) \quad (2)$$

The projected LC50 values (both crude oil portion and dispersant portion) of dispersed oil determined from equations (1) and (2) were plotted on the appropriate isobole for each species. Projected LC50s that fell within the 95% CI boundaries were considered to have an additive joint toxicity effect (AD); projected LC50s below the lower boundary of the 95% CI were considered to have a synergistic joint toxicity (SY); and projected LC50s above the higher boundary of the 95% CI were considered to have an antagonistic joint toxicity (AN) (Figure 2.2). The joint or mixture toxicity was determined for both nominal (NC) and measured hydrocarbon concentrations (HC). The dispersant portion of nominal 96-h LC50 of dispersed oil was same as the dispersant portion of HC 96-h LC50 of dispersed oil because the dispersant was not amenable to analysis of hydrocarbons.

Results

Acute toxicity

Dispersed oils were more toxic than crude oils ($p < 0.0001$) based on nominal concentrations (NC) (Tables 2.1 – 2.2). For Gulf killifish, the mean 96-h NC LC50 of SLC and ANSC were 4,457 ppm and 4,492 ppm, respectively, 11.5 times and 3.5 times higher than the 96-h NC LC50 of SLC+9500 and ANSC+9500 (Table 2.1). The 96-h NC

Table 2.1. Nominal concentration (NC, ppm) and hydrocarbon concentration (HC, ppm) of South Louisiana crude oil (SLC), Alaskan north slope crude oil (ANSC), dispersed South Louisiana crude oil (SLC+9500), dispersed Alaskan north slope crude oil (ANSC+9500), and dispersant COREXIT 9500 (9500) that are lethal to 50% of Fundulus grandis in a 96-h static non-renewal exposure.

Exposure Chemical	96-h LC50 (ppm)					
	Test 1 (non-pooled)		Test 2 (non-pooled)		Mean of test 1 and test 2 (pooled)	
	Nominal	Hydrocarbon	Nominal	Hydrocarbon	Nominal	Hydrocarbon
COREXIT 9500 (95% C.L.)	153.0 (N/A)	--	180.8 (N/A)	--	172.6 (147.1 –220.9)	--
SLC + 9500 (95% C.L.)	317.7 (76.7 – 501.9)	18.42 (10.72 – 22.24)	431.5 (322.7 –521.6)	17.61 (N/A)	386.8 (299.1 –469.2)	17.86 (15.04 – 19.96)
ANSC + 9500 (95% C.L.)	1,500* (N/A)	20.72 (N/A)	1,200 (941.3 - 1419)	18.67 (N/A)	1,270 (1115 - 1473)	18.67 (N/A)
SLC (95% C.L.)	4,250 (N/A)	(N/A)	4,243 (2977 - 6047)	8.30 (7.08 – 9.77)	4,457 (3633 - 5555)	8.30 (7.08 – 9.77)
ANSC (95% C.L.)	4,243 (2,760 – 6,522)	7.71 (6.84 – 8.84)	4,721 (3581 - 6570)	7.72 (6.93 – 8.76)	4,492 (3494 - 5718)	7.67 (7.04 – 8.30)

1500*: Binomial analysis from only 0% mortality and 100% mortality
--: No hydrocarbon analysis for dispersant

Table 2.2. Nominal concentration (NC, ppm) and hydrocarbon concentration (HC, ppm) of South Louisiana crude oil (SLC), Alaskan north slope crude oil (ANSC), dispersed South Louisiana crude oil (SLC+9500), dispersed Alaskan north slope crude oil (ANSC+9500), and dispersant COREXIT 9500 (9500) that are lethal to 50% of Litopenaeus setiferus in a 96-h static non-renewal exposure.

Exposure Chemicals	96-h LC50 (ppm)					
	Test 1 (non-pooled)		Test 2 (non-pooled)		Mean of test 1 and test 2 (pooled)	
	Nominal	Hydrocarbon	Nominal	Hydrocarbon	Nominal	Hydrocarbon
COREXIT 9500 (95% C.L.)	33.4 (5.6 – 62.9)	--	29.4 (11.4 – 81.1)	--	31.1 (16.1 – 49.4)	--
SLC + 9500 (95% C.L.)	64.6 (38.3 – 96.6)	4.84 (3.95 – 5.65)	37.1 (N/A)	5.55 (N/A)	49.5 (25.1 – 60.7)	5.00 (3.34 – 5.81)
ANSC + 9500 (95% C.L.)	90.6 (36.2 – 241.2)	7.46 (N/A)	42.7 (N/A)	7.60 (N/A)	57.5 (0.3 - 90)	7.50 (7.20 – 7.66)
SLC (95% C.L.)	110.5 (N/A)	6.32 (N/A)	232.6 (N/A)	7.16 (N/A)	132.6 (39.6 – 210.8)	6.49 (5.36 – 6.96)
ANSC (95% C.L.)	110.5 (N/A)	6.05 (N/A)	241.9 (N/A)	7.80 (N/A)	145.4 (39.3 – 241.3)	6.59 (4.31 – 7.64)

--: No hydrocarbon analysis for dispersant

LC50 of SLC and ANSC for white shrimp were 132.6 ppm and 145.4 ppm, 2.7 times and 2.5 times higher than the 96-h NC LC50 of SLC+9500 and ANSC+9500, respectively. No difference was observed in the toxicity of SLC and ANSC, or the dispersed oils, SLC+9500 and ANSC+9500 ($p = 0.2388$ for nominal concentration, $p = 0.7188$ for measured hydrocarbon concentration) for either Gulf killifish or white shrimp. The mean 96-h HC LC50 of SLC and ANSC were 6.49 ppm and 6.59 ppm for white shrimp and 8.30 ppm and 7.67 ppm for Gulf killifish, respectively. The mean 96-h HC LC50 values of dispersed oils (SLC+9500 or ANSC+9500) were about twice that of the crude oils for Gulf killifish and about the same for white shrimp. The dispersant was more toxic to Gulf killifish and white shrimp than the crude oils and dispersed oil ($p < 0.0001$) based on nominal concentration. The mean 96-h NC LC50 of dispersant to Gulf killifish was 172.6 ppm , and 31.1 ppm for white shrimp.

A 96-h LC50 would not be effectively determined for Eastern oysters in crude oils and dispersed oils in any of the definitive tests, because mortality was not positively correlated with increasing levels of crude oils, or dispersed oils (Figure 2.3 - 2.4). The only constant in the oyster studies was low mortality in the controls. Eastern oysters began to die at nominal concentration of crude oils (both SLC and ANSC) exceeding 100 ppm and total hydrocarbon levels exceeding 1.11 ppm for SLC and 1.71 ppm for ANSC, with mortality generally ranging from 10 to 80% near 28 ^{o}C (range: 26 ^{o}C to 31 ^{o}C). Mortality of oysters was substantially lower at 23^{o}C (range: 19 to 25 ^{o}C), ranging from 0 to 30%. Oyster mortality was observed in dispersed oils at nominal concentrations exceeding 500 ppm (both SLC+9500 and ANSC+9500) and total hydrocarbon levels

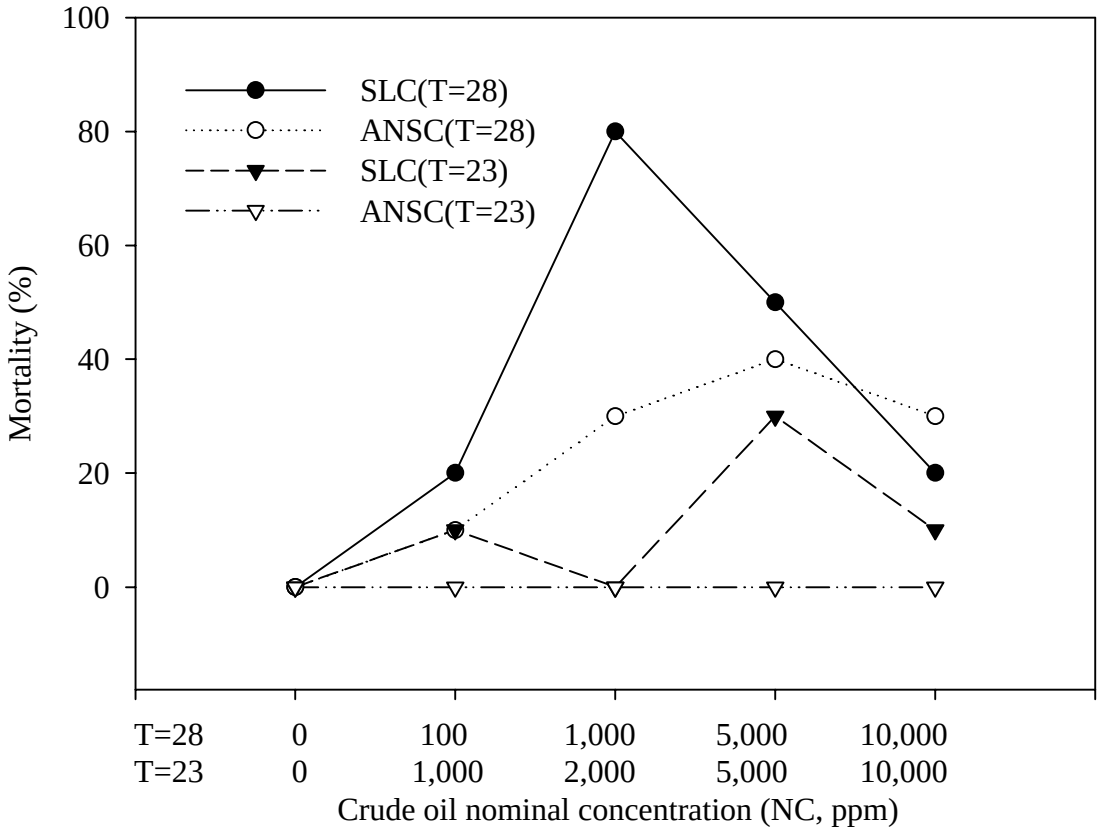

Figure 2.3. 96-h mortality (%) of Eastern oyster with different exposure concentrations (NC, ppm) of South Louisiana crude oil (SLC) and Alaskan north slope crude oil (ANSC) under two different temperatures (23 ^{o}C and 28 ^{o}C).

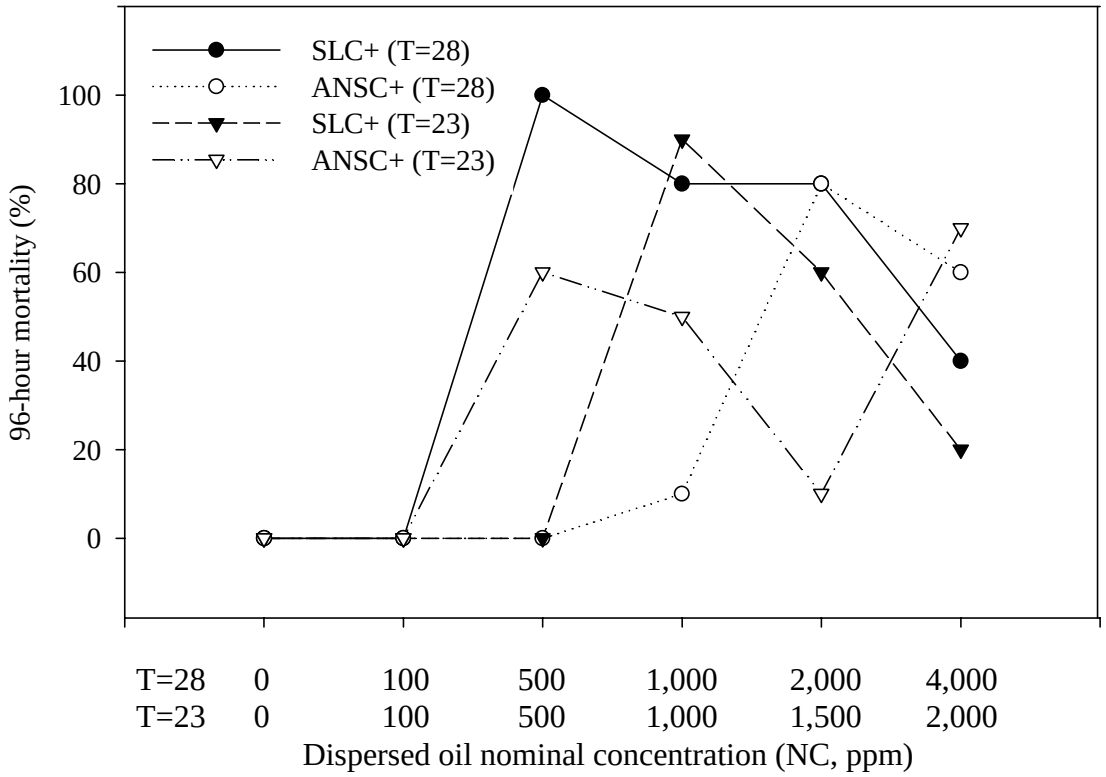

Figure 2.4. 96-h mortality (%) of Eastern oyster with different exposure concentrations (NC, ppm) of dispersed South Louisiana crude oil (SLC+9500) and dispersed Alaskan north slope crude oil (ANSC+9500) under two different temperatures (23 ^{o}C and 28 ^{o}C).

exceeding 26.25 ppm for SLC+9500 and 16.10 ppm for ANSC+9500, with mortality generally ranging from 50 to 100% near 28 ^{o}C (range: 26 ^{o}C to 31 ^{o}C). Mortality of oysters in dispersed oil at 23 ^{o}C appears lower than that observed at 28 ^{o}C. Based on nominal (dosed) concentrations, dispersed oils appeared to be more toxic than crude oils to Eastern oysters. The 96-h LC50 of dispersant COREXIT 9500 (9500) for the Eastern oyster at 23 ^{o}C is 167 ppm. Mortality of oysters in the dispersant at 28 ^{o}C appears higher than observed at 23 ^{o}C (Figure 2.5). This bivalve is an immobile species, and it was not possible to determine visually or by probing whether or not oysters were dead or alive while in the exposure water, so mortality was recorded only after animals were removed and examined after 96 hours of exposure.

White shrimp were more sensitive to the five toxicants than Gulf killifish or Eastern oysters. The 96-h HC LC50s of either oils or dispersed oils were less than 8 ppm for white shrimp (Table 2.2). Shuba and Heikamp (1989) reported that 96-h NC LC50 of SABL crude oil to Litopenaeus setiferus (mean length TL 4.1 ± 0.5 cm) was 180 ppm in a static acute toxicity test, which was comparable to the findings of this study (132.6 ppm for SLC and 145.4 ppm for ANSC). Most of the mortality occurred within 48 hours of exposure, and nearly 100% mortality had occurred in 100 ppm dispersed oil (NC) after 48 hours exposure. The tolerance of Gulf killifish to dispersed oils was about 2.8 times higher than white shrimp.

Water Environmental Conditions

Generally, the pH, dissolved oxygen (DO), un-ionized ammonia (NH_3-N) and temperature were maintained in an acceptable range for all three test species (Table 2.3 - 2.5). In one incident during the second definitive test for ANSC on Gulf killifish, an oil

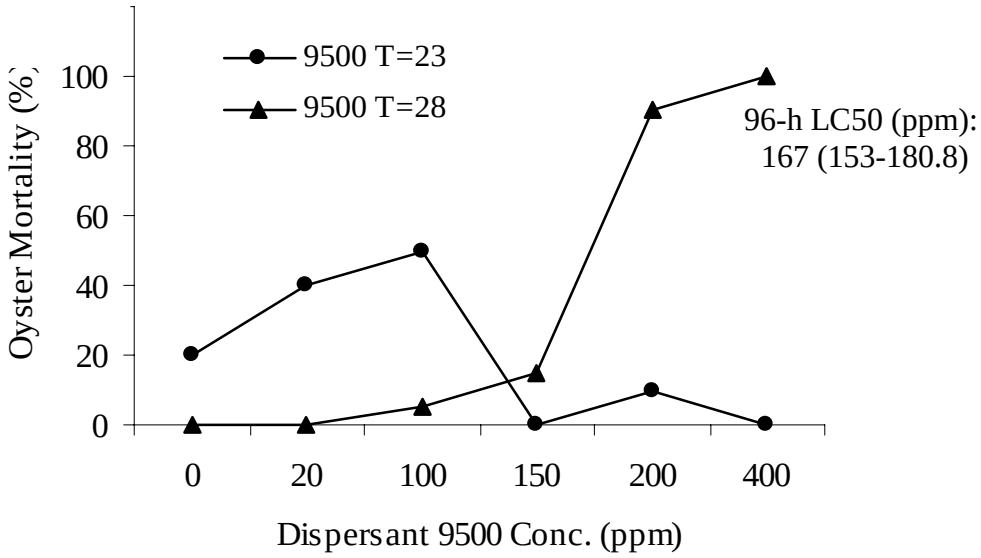

Figure 2.5. 96-h mortality (%) of Eastern oyster with different exposure concentrations (NC, ppm) of dispersant COREXIT 9500 (9500) and under two different temperatures (23 ^{o}C and 28 ^{o}C).

Table 2.3. Water quality parameter range during toxicity tests of South Louisiana crude oil (SLC), Alaskan north slope crude oil (ANSC), dispersed South Louisiana crude oil (SLC+9500), dispersed Alaskan north slope crude oil (ANSC+9500), and dispersant COREXIT 9500 (9500) to Gulf killifish, Fundulus grandis.

Water parameter	Time (hr)	Exposures					
		Control	SLC	ANSC	SLC+9500	ANSC+9500	9500
Dissolved Oxygen (mg/L)	0				8.22		
	24	6.3	2.8 – 4.2	2.1 – 4.2	--	--	4.6 - 5.4
	48	4.1	2.6 – 3.0	2.8 – 5.1	2.4 – 6.8	3.1 – 5.2	2.8 - 6.2
	72	3.8	2.7 - 3.9	0.2 – 5.3	3.1- 7.0	2.1 – 5.0	3.1 - 5.7
	96	4.0	2.0 – 4.1	3.1 – 6.3	3.2 – 4.9	3.0 – 5.5	3.2 - 4.9
pH	0				8.0		
	24	8.0	7.5 - 8.0	7.5 - 8.0	7.5 - 8.0	7.5 - 8.0	7.5 - 8.0
	48	7.5	7.5 - 8.0	7.5	7.5 - 8.0	7.5 - 8.0	7.5 - 8.0
	72	8.0	7.5 - 8.0	7.5	7.5	7.5 - 8.0	7.5 - 8.0
	96	8.0	7.5 - 8.0	8.0	7.5 - 8.0	8.0	8.0
Un-ionized Amnonia (mg/L)	0				--		
	24	0.03	0.06	0.05	0.03	0.05	0.02
	48	0.02	0.04	0.05	0.04	0.03	0.03
	72	0.03	0.06	0.03	0.04	0.03	0.03
	96	--	0.05	0.05	0.03	0.04	0.03
Water Temperature (^{o}C)	0				23		
	24	22	22	22	22	22	22
	48	23	23	23	23	23	23
	72	22	22	22	22	22	22
	96	20	20	20	20	20	20

--: no observation.

Table 2.4. Water quality parameter range during toxicity tests of South Louisiana crude oil (SLC), Alaskan north slope crude oil (ANSC), dispersed South Louisiana crude oil (SLC+9500), dispersed Alaskan north slope crude oil (ANSC+9500), and dispersant COREXIT 9500 (9500) to Eastern oyster, Crassostrea virginica.

Water parameter	Time (hr)	Exposures					
		Control	SLC	ANSC	SLC+9500	ANSC+9500	9500
Dissolved Oxygen (mg/L)	0				7.92		
	24	--	--	--	--	--	--
	48	3.86	2.84 – 3.63	2.54 – 3.79	2.47 – 3.36	2.42 – 3.03	2.37 – 3.12
	72	--	--	--	--	--	--
	96	3.28	2.25 – 3.22	2.25 – 3.68	2.32 – 3.24	2.84 – 3.51	2.32 – 3.48
pH	0				8.0		
	24	8.0	8.0	7.5 - 8.0	8.0	7.5 - 8.0	7.5 - 8.0
	48	8.0	7.5 - 8.0	8.0	7.5 - 8.0	7.5 - 8.0	8.0
	72	7.5	7.5	7.5	7.5 – 8.0	7.5 - 8.0	7.5 - 8.0
	96	8.0	8.0	8.0	7.5 - 8.0	8.0	8.0
Un-ionized Ammonia (mg/L)	0				0.01		
	24	0.03	0.04	0.05	0.05	0.06	0.04
	48	0.05	0.05	0.06	0.04	0.05	0.05
	72	0.05	0.05	0.04	0.05	0.03	0.05
	96	0.05	0.06	0.05	0.03	0.05	0.06
Water Temperature (^{o}C)*	0				20, 26		
	24	22, 27	22, 27	22, 27	22, 27	22, 27	22, 27
	48	23, 28	23, 28	23, 28	23, 28	23, 28	23, 28
	72	23, 28	23, 28	23, 28	23, 28	23, 28	23, 28
	96	23, 27	23, 27	23, 27	23, 27	23, 27	23, 27

* : two different temperatures (average 23 ^{o}C and 28 ^{o}C) used in this test, the low temperature controlled by air-condition.
--: no observation.

Table 2.5. Water quality parameter range during toxicity tests of South Louisiana crude oil (SLC), Alaskan north slope crude oil (ANSC), dispersed South Louisiana crude oil (SLC+9500), dispersed Alaskan north slope crude oil (ANSC+9500), and dispersant COREXIT 9500 (9500) to white shrimp, Litopenaeus setiferus.

Water parameter	Time (hr)	Exposures					
		Control	SLC	ANSC	SLC+9500	ANSC+9500	9500
Dissolved Oxygen (mg/L)	0				7.66		
	24	--	--	--	--	--	--
	48	5.82	3.88 – 5.26	3.96 – 5.64	3.50 – 5.12	3.22 – 3.96	4.46 – 5.06
	72	--	--	--	--	--	--
	96	4.58	3.64 – 4.52	3.84 – 4.34	3.96 – 5.28	3.02 – 3.68	3.76 – 4.88
pH	0				8.0		
	24	8.0	8.0	7.5 - 8.0	8.0	8.0	8.0
	48	8.0	7.5	8.0	8.0	8.0	8.0
	72	8.0	8.0	7.5	8.0	7.5 - 8.0	8.0
	96	8.0	8.0	8.0	7.5	8.0	8.0
Un-ionized Ammonia (mg/L)	0				0		
	24	0.02	0.04	0.03	0.03	0.04	0.03
	48	0.03	0.03	0.04	0.04	0.04	0.03
	72	0.03	0.04	0.04	0.04	0.03	0.04
	96	0.02	0.02	0.03	0.03	0.04	0.04
Water Temperature (^{o}C)*	0				20		
	24	20	20	20	20	20	20
	48	20	20	20	20	20	20
	72	20	20	20	20	20	20
	96	20	20	20	20	20	20

* : the temperature was controlled by air-condition.
--: no observation.

particle clogged the glass pipet supplying supplemental air in three of five dose levels, which reduced oxygen concentration to low levels, but it did not appear to affect the 96-h LC50 when values were compared to the first definitive test. The pH maintained from 7.5 to 8.0. Un-ionized ammonia levels were less than 0.05 mg/L in most tests.

Test solutions

The concentration of total hydrocarbons dissolved or dispersed in water increased from the initial dosing until termination of the tests at 96-hour (Figure 2.5), and generally ranged from 5 to 15 ppm HC for both crude oil and dispersed oil. An exception was SLC+9500, which has a high initial level of soluble hydrocarbons (37 ppm HC). The solubility of hydrocarbons in SLC was higher in the first 48-hours than for ANSC, the concentrations near termination of the test were comparable. The addition of the dispersant to the both crude oils increased the concentration of soluble hydrocarbons in the exposure solutions (Figure 2.6), particularly within the first 48 hours post-dosing, but final concentrations of HC in crude oil and dispersed oil generally ranged between 12 and 15 ppm after 96 hours post-dosing.

Joint (Mixture) Toxicity

Assessments of the joint toxicity action of dispersed oil mixtures compared to the parent compounds (crude oil and dispersant) for Gulf killifish and white shrimp are presented in Tables 2.6 - 2.13, and Figures 2.7 - 2.14. The results for oyster mortality did not lend itself to assessment of joint toxicity because of the inability to determine a LC50 for the crude oils, dispersed oils, and dispersant. The oil portion of 96-h HC LC50 for Gulf killifish increased from 8.30 ppm for SLC to 17.86 ppm for SLC+9500, and the

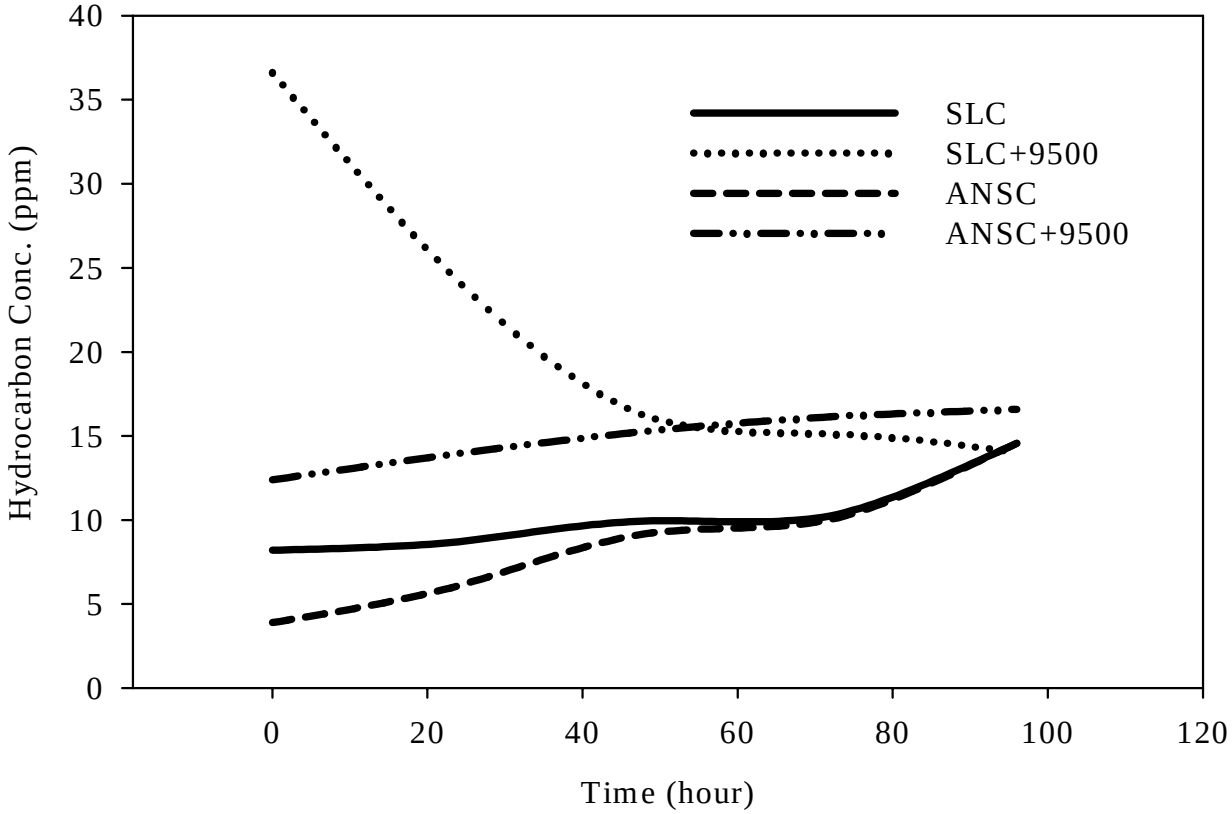

Figure 2.6. The hydrocarbon concentration change with time for South Louisiana crude oil (SLC), Alaskan north slope crude oil (ANSC), dispersed South Louisiana crude oil (SLC+9500) and dispersed Alaskan north slope crude oil (ANSC+9500).

Table 2.6. The portions of 96-h nominal LC50 of South Louisiana crude oil (SLC) and dispersed South Louisiana crude oil (SLC+9500) and the joint toxicity of SLC and COREXIT 9500 to Gulf killifish Fundulus grandis.

Components	SLC only	SLC+9500
	96-h LC50 (oil, ppm)	
	4457	386.8
SLC	4457	368.38
9500	0	18.42

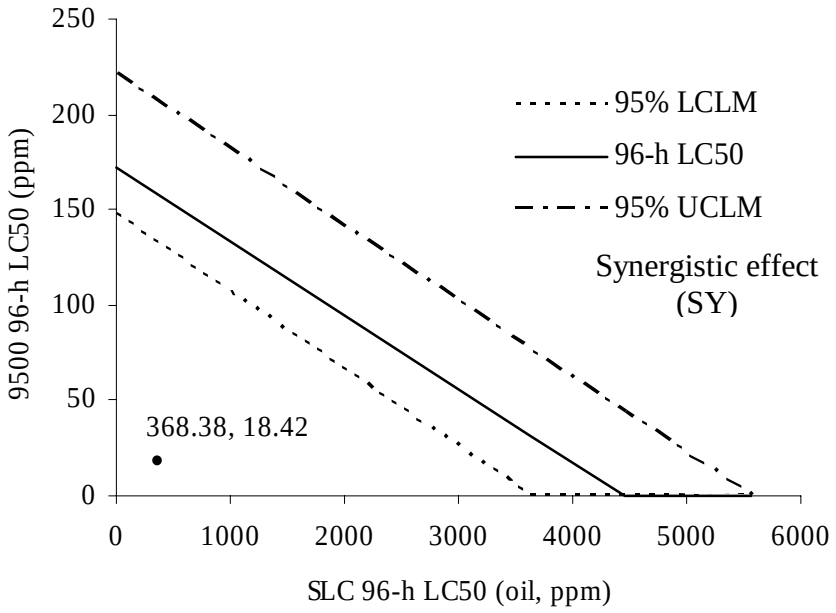

Figure 2.7. Joint Toxicity of South Louisiana crude oil (SLC) and dispersant COREXIT 9500 to Fundulus grandis based on the nominal SLC 96-h LC50 (oil, ppm) with 95% confidence limits, 9500 96-h LC50 (ppm) with 95% confidence limits and the portion of dispersed South Louisiana crude oil (SLC+9500) 96-h LC50 (oil, ppm).

Table 2.7. The portions of 96-h total dissolved hydrocarbon LC50 of South Louisiana Crude oil (SLC) and dispersed South Louisiana crude oil (SLC+9500) and the joint toxicity of SLC and COREXIT 9500 to Fundulus grandis.

Components	SLC only	SLC+9500
	96-h LC50 (HC, ppm)	
	8.30	17.86
SLC	8.30	17.86
9500	0	18.42

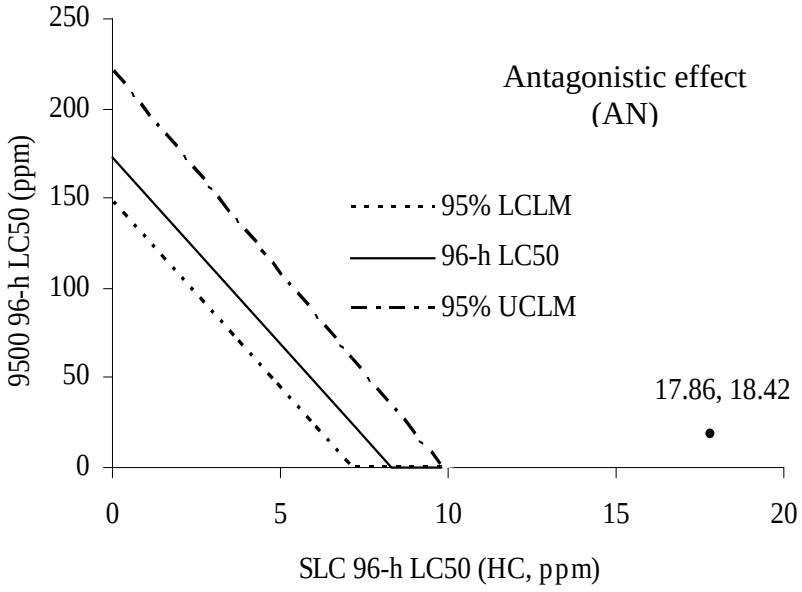

Figure 2.8. Joint Toxicity of South Louisiana crude oil (SLC) and dispersant COREXIT 9500 to Fundulus grandis based on the total dissolved hydrocanbon SLC 96-h LC50 (HC, ppm) with 95% confidence limits, 9500 96-h LC50 (ppm) with 95% confidence limits and the portion of dispersed South Louisiana crude oil (SLC+9500) 96-h LC50 (HC, ppm).

Table 2.8. The portions of 96-h nominal LC50 of Alaska north slope Crude oil (ANSC) and dispersed Alaska north slope crude oil (ANSC + 9500) and the joint toxicity of ANSC and COREXIT 9500 to Fundulus grandis.

Components	ANSC only	ANSC+9500
	96-h LC50 (oil, ppm)	
	4492	1270
ANSC	4492	1209.5
9500	0	60.5

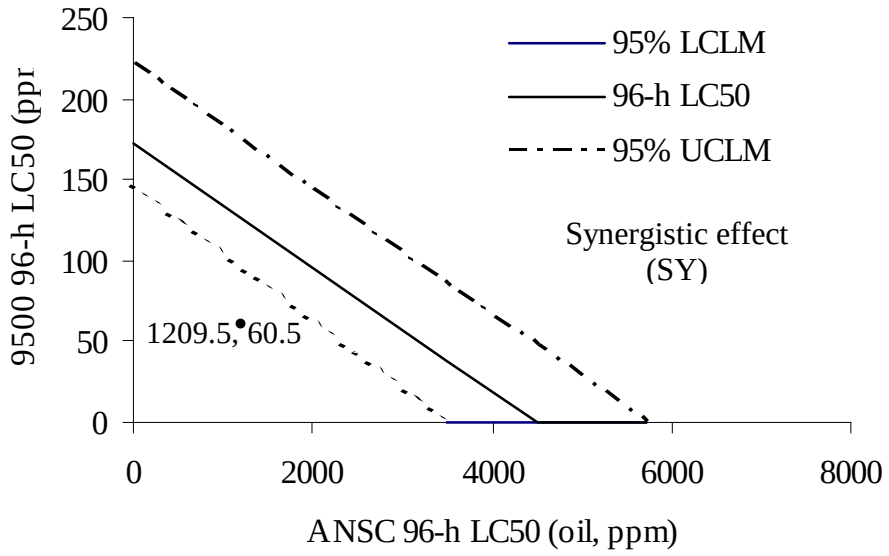

Figure 2.9. Joint Toxicity of Alaska north slope crude oil (ANSC) and dispersant COREXIT 9500 to Fundulus grandis based on the nominal ANSC 96-h LC50 (oil, ppm) with 95% confidence limits, 9500 96-h LC50 (ppm) with 95% confidence limits and the portion of dispersed Alaska north slope crude oil (ANSC+9500) 96-h LC50 (oil, ppm).

Table 2.9. The portions of 96-h total dissolved hydrocarbon LC50 of Alaska north slope crude oil (ANSC) and dispersed Alaska north slope crude oil (ANSC+9500) and the joint toxicity of ANSC and COREXIT 9500 to Fundulus grandis.

Components	ANSC only	ANSC+9500
	96-h LC50 (HC, ppm)	
	7.67	18.67
ANSC	7.67	18.67
9500	0	60.5

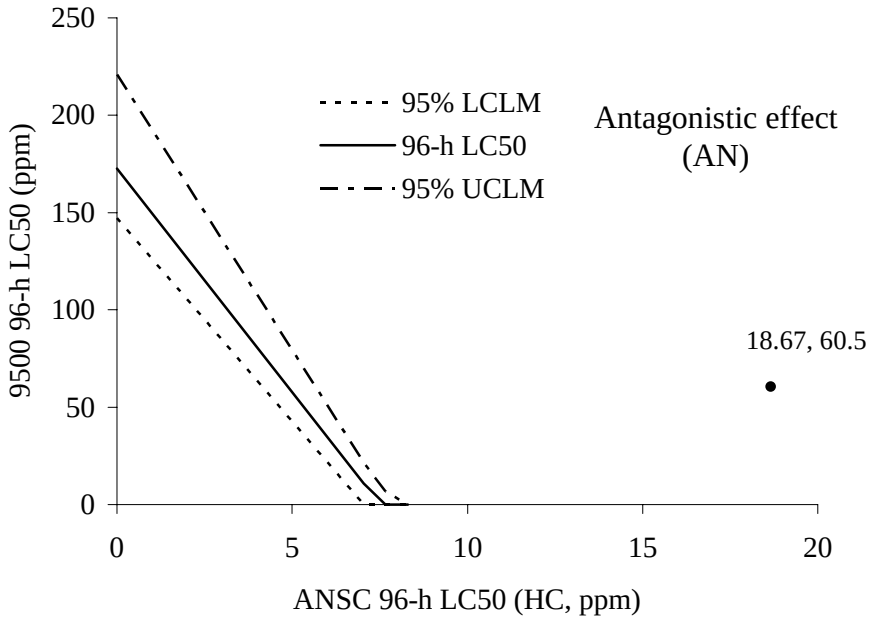

Figure 2.10. Joint Toxicity of Alaskan north slope crude oil (ANSC) and dispersant COREXIT 9500 to Fundulus grandis based on the total dissolved hydrocanbon SLC 96-h LC50 (HC, ppm) with 95% confidence limits, 9500 96-h LC50 (ppm) with 95% confidence limits and the portion of dispersed Alaskan north slope crude oil (ANSC+9500) 96-h LC50 (HC, ppm).

Table 2.10. The portions of 96-h nominal LC50 of South Louisiana crude oil (SLC) and dispersed South Louisiana crude oil (SLC+9500) and the joint toxicity of SLC and COREXIT 9500 to Litopenaeus setiferus.

Components	SLC only	SLC+9500
	96-h LC50 (oil, ppm)	
	132.6	49.5
SLC	132.6	47.14
9500	0	2.36

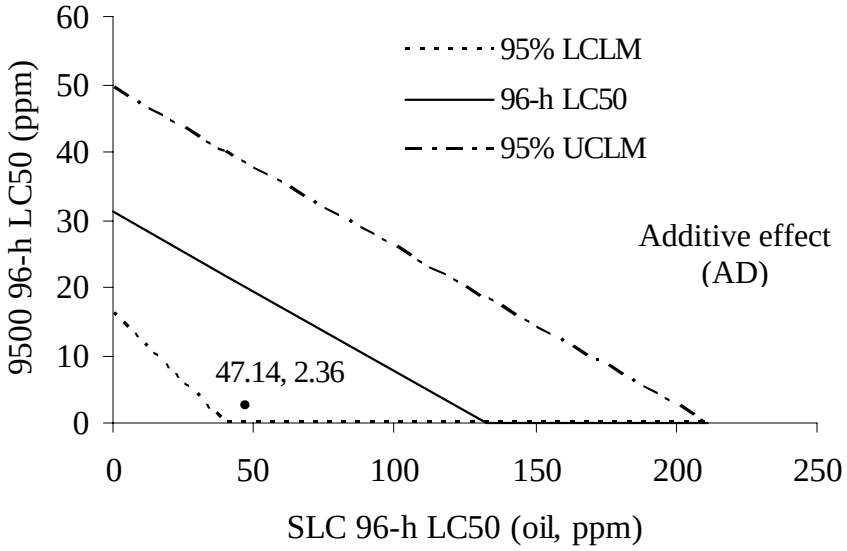

Figure 2.11. Joint Toxicity of South Louisiana crude oil (SLC) and dispersant COREXIT 9500 to Litopenaeus setiferus based on the nominal SLC 96-h LC50 (oil, ppm) with 95% confidence limits, 9500 96-h LC50 (ppm) with 95% confidence limits and the portion of dispersed South Louisiana crude oil (SLC+9500) 96-h LC50 (oil, ppm).

Table 2.11. The portions of 96-h total dissolved hydrocarbon LC50 of South Louisiana crude oil (SLC) and dispersed South Louisiana crude oil (SLC+9500) and the joint toxicity of SLC and COREXIT 9500 to Litopenaeus setiferus.

Components	SLC only	SLC+9500
	96-h LC50 (HC, ppm)	
	6.49	5.00
SLC	6.49	5.00
9500	0	2.36

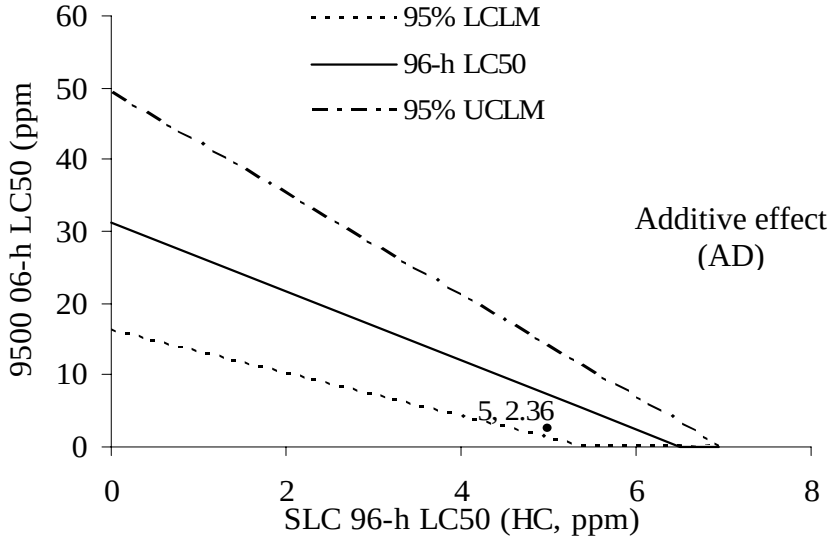

Figure 2.12. Joint Toxicity of South Louisiana crude oil (SLC) and dispersant COREXIT 9500 to Litopenaeus setiferus based on the total dissolved hydrocanbon SLC 96-h LC50 (HC, ppm) with 95% confidence limits, 9500 96-h LC50 (ppm) with 95% confidence limits and the portion of dispersed South Louisiana crude oil (SLC+9500) 96-h LC50 (HC, ppm).

Table 2.12. The portions of 96-h nominal LC50 of Alaska north slope crude oil (ANSC) and dispersed Alaska north slope crude oil (ANSC+9500) and the joint toxicity of ANSC and COREXIT 9500 to Litopenaeus setiferus.

Components	ANSC only	ANSC+9500
	96-h LC50 (oil, ppm)	
	145.4	57.5
ANSC	145.4	54.76
9500	0	2.74

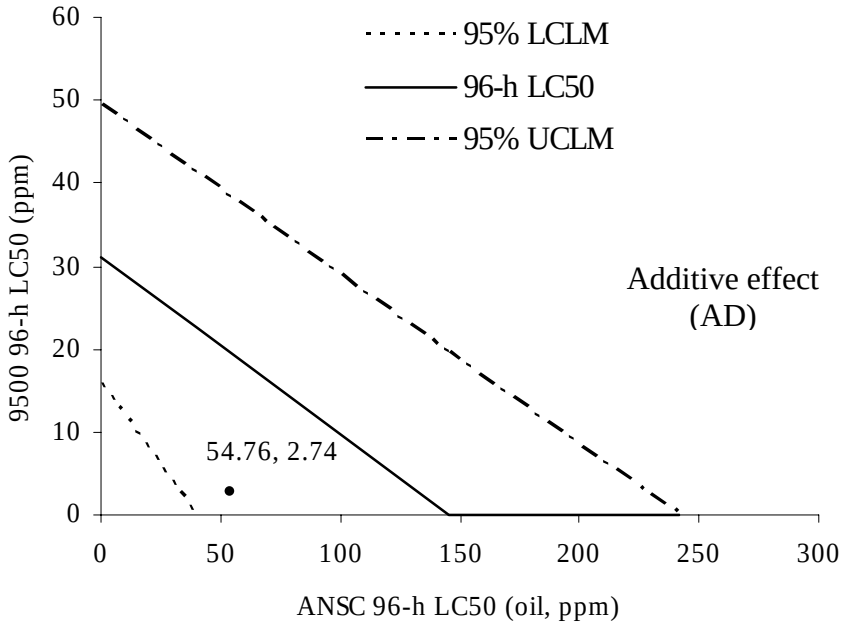

Figure 2.13. Joint Toxicity of Alaska north slope crude oil (ANSC) and dispersant COREXIT 9500 to Litopenaeus setiferus based on the nominal ANSC 96-h LC50 (oil, ppm) with 95% confidence limits, 9500 96-h LC50 (ppm) with 95% confidence limits and the portion of dispersed Alaska north slope crude oil (ANSC+9500) 96-h LC50 (oil, ppm).

Table 2.13. The portions of 96-h total dissolved hydrocarbon LC50 of Alaska north slope crude oil (ANSC) and dispersed Alaska north slope crude oil (ANSC+9500) and the joint toxicity of ANSC and COREXIT 9500 to Litopenaeus setiferus.

Components	ANSC only	ANSC+9500
	96-h LC50 (HC, ppm)	
	6.59	7.50
ANSC	6.59	7.50
9500	0	2.74

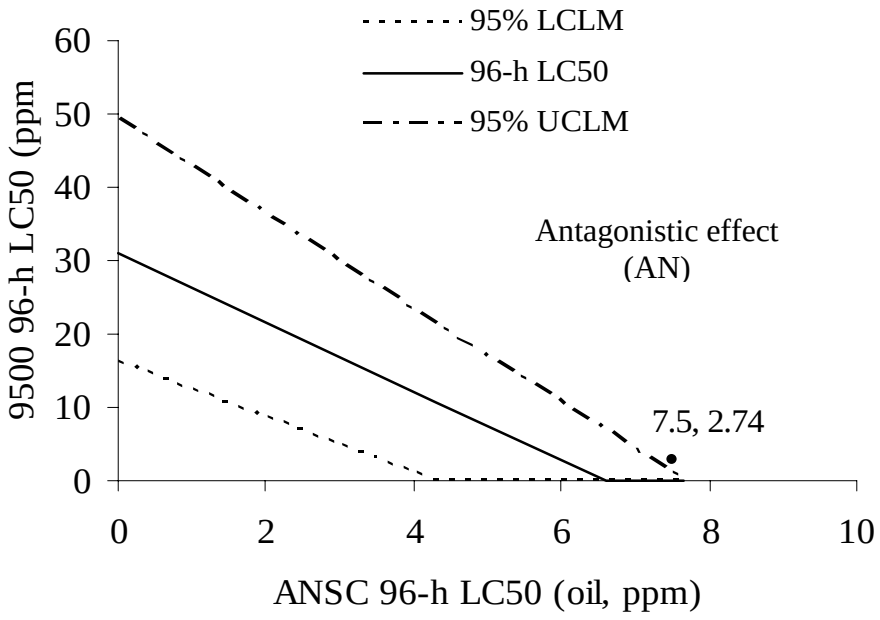

Figure 2.14. Joint Toxicity of Alaska north slope crude oil (ANSC) and dispersant COREXIT 9500 to Litopenaeus setiferus based on the total dissolved hydrocanbon ANSC 96-h LC50 (HC, ppm) with 95% confidence limits, 9500 96-h LC50 (ppm) with 95% confidence limits and the portion of dispersed Alaska north slope crude oil (ANSC+9500) 96-h LC50 (HC, ppm).

dispersant portion increased from 0 ppm to 18.42 ppm (Table 2.8). For Alaska North Slope Crude oil (ANSC), the oil and dispersant portions of HC 96-h LC50s for Gulf killifish also increased from 7.67 ppm and 0 ppm for ANSC to 18.67 ppm and 60.5 ppm for ANSC+9500, respectively (Table 2.10). The joint toxicity of total dissolved hydrocarbons and dispersant COREXIT 9500 was determined to be antagonistic to Gulf killifish (Figure 2.8 and Figure 2.10). In contrast, the oil portions of the nominal 96-h LC50s showed that there was a synergistic effect between SLC and ANSC and the dispersant for Gulf killifish (Figure 2.7 and Figure 2.9).

An additive joint toxicity effect on white shrimp was found between the SLC and dispersant, for both nominal concentrations and hydrocarbon concentrations (Figure 2.11 and Figure 2.12). The joint toxic effect on white shrimp for ANSC and dispersant was also an additive for nominal concentrations (Figure 2.13), but was slightly antagonistic for hydrocarbons (Figure 2.14).

Discussion

Oil and dispersed oil

Although both nominal concentration (NC) and measured total hydrocarbon concentrations (HC) were used in the lethal toxicity analysis, total hydrocarbons are considered more meaningful to explain the toxic effect of oil or dispersed oil because the toxicity is thought to result principally from dissolved hydrocarbons (NRC, 1985). SLC had significantly higher concentrations of soluble hydrocarbons in solution than those of ANSC within the 2 days. Crude oils from different locations can vary in composition and SLC likely had more light-weight, volatile components which usually contributes more than 95% of the water-soluble fraction. The general trend towards

increasing soluble hydrocarbon concentrations with time may have been in part facilitated by the low rates of aeration that facilitated the dissolution of soluble compounds, especially in first 48 h. Conversely, the significant decline in HC levels in SLC after the first two day was likely attributed to the evaporation of volatile components. Payne et al. (1983) reported that the volatile aromatic hydrocarbons in the water-soluble fraction of Prudhoe Bay crude oil were rapidly lost in 40 h. Since evaporation is a surface phenomenon, the layer of non-soluble crude oil on the surface likely reduced the rate of surface volatilization of hydrocarbons from the water column, so it is likely that the rate of dissolution exceeded the rate of loss of hydrocarbons from surface volatilization for SLC and ANSC which would explain their increase during the 96-h tests.

Dispersants reduce the surface tension in oil and the oil forms into tiny droplets in the water in both stable and unstable emulsions (Decola, 1999). Not all crude oils are equally dispersible (Canevari, 1987). The SLC was more easily dispersed than ANSC possibly due to its lower viscosity. The ability of a crude oil to disperse after addition of a chemical dispersant depends on many physical and chemical properties of the oil rather than on the viscosity only. Canevari (1985) diluted the LaRosa crude (73 cSt viscosity) to the same viscosity as Murban crude (6 cSt) and found that the dispersion of LaRosa crude was 50 percent compared to 78 percent Murban crude (NRC, 1989). The high dispersion level of the SLC, compared with ANSC, would also potentially expose aquatic organisms to higher levels of toxic aromatic hydrocarbons in SLC+9500 solutions. The level of soluble hydrocarbons in SLC+9500 mixture declined rapidly within 24 h, and this was a further indication that SLC had much higher levels of volatile and soluble hydrocarbons

than ANSC. Although ANSC contains almost one-third volatile components (Rhoton et al., 2001), its level of solubility in water, was not as high in the first 48 hours as for SLC.

Dispersant

Early developed dispersants (solvents) contained high concentrations of aromatic hydrocarbons that were highly toxic to aquatic organisms. Nagell et al. (1974) and Wells et al. (1985) reported the toxicity of soluble hydrocarbons decreases in the following order: aromatic hydrocarbons > saturated hydrocarbons > glycol ethers > alcohols. Dispersant solvents in use today are ethers and alcohols (NRC, 1989). Although the dispersant COREXIT 9500 is a chemical mixture, the levels of soluble components were not measured in this study. Singer et al. (1996) reported that the solubility of COREXIT 9500 in water was more than 500 ppm at 7 ^{o}C and 1,000 ppm at 15 ^{o}C. George-Ares and Clark (2000) reported on the toxicity of COREXIT 9500 with COREXIT 9527, a more widely tested and used dispersant, to over 60 species of fishes, crustaceans, and mollusk, and they reported that these dispersants had low to moderate toxicity to most aquatic species in laboratory tests. Crustaceans were generally more sensitive to COREXIT 9500 (48-96 h LC50 ranged from 3.5 – 36 ppm) than juvenile and adult fishes (48-h to 96 h LC50 ranged from 50 – 354 ppm). Results from this study on the acute toxicity of COREXIT 9500 to Gulf killfish (172.6 ppm) and white shrimp (31.1 ppm) were comparable to that reported by George-Ares and Clark. In this study, the toxicity of dispersant to Eastern oysters increased with higher water temperature and similar effects have been found in other molluskan species. Ordzie and Garofalo (1981) reported that the LC50 for the dispersant COREXIT 9527 was 200 ppm at 20 ^{o}C, 1,800 ppm at 10 ^{o}C, and

2,500 ppm at 2 ^{o}C. Concentrations of dispersant that were not lethal to scallops during the winter caused greater than 50% mortality at summer temperatures.

Oil and Dispersed Oil toxicity

The 96-h LC50 of crude oil determined from measured hydrocarbon concentrations (HC) may not effectively illustrate the nominal concentration required to obtain the same lethal effect. For Gulf killifish, about 7 to 8 ppm of total hydrocarbons caused 50% mortality in 96 hours, and this was equivalent to a nominal dosing level of about 4,400 ppm of crude oil. In contrast, total hydrocarbons concentration of about 6 to 7 ppm caused about 50% mortality in white shrimp, but this corresponded to an initial nominal crude oil concentration of 130 to 150 ppm – much less than observed on the killifish. Total hydrocarbon levels in water were not linearly proportional to the dosing levels of crude oil or dispersed oil. For example, a 10X increase in nominal crude oil concentration did not result in a 10X increase in total hydrocarbon level (Figures 2.15 and 2.16). Measured hydrocarbon concentrations in exposure water appeared to increase more as a function of time in the water, than with initial dosing concentration. Thus, the exposure time effect during the 96 h tests appeared to supercede the observed difference in total hydrocarbon concentrations associated with the differences in the nominal oil concentrations. Michel (1999) reported that the water-soluble fraction of hydrocarbon was less than 10 ppm for heavy oils (e.g., No. 6 Fuel oil, Banker C), characteristic of ANSC used in this study, and the water-soluble fraction ranged from 10 to 100 ppm for medium oils (most crude oils), characteristic of SLC. In this study, the weighted mean concentration of 7-9 ppm total hydrocarbons over 96 hours in both SLC and ANSC was

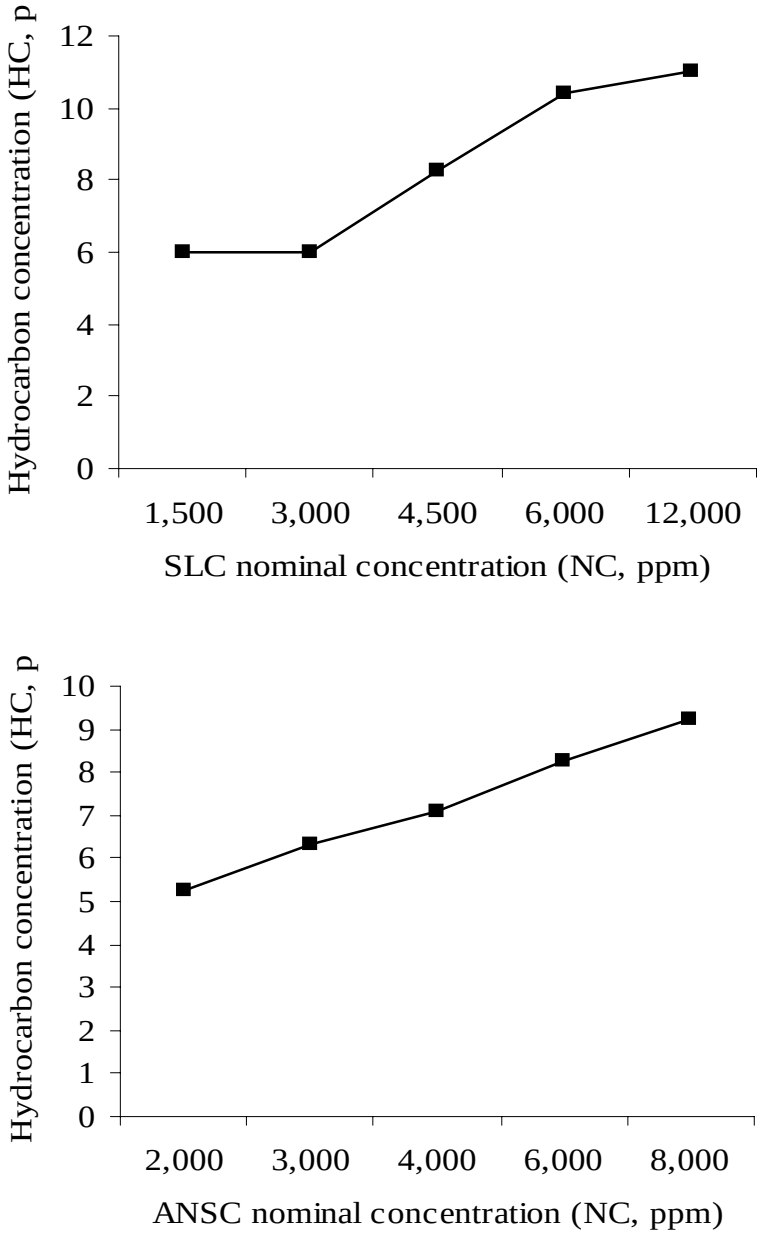

Figure 2.15. The measured hydrocarbon concentration (HC, ppm) change with the nominal concentration (NC, ppm) for South Louisiana crude oil (SLC) and Alaskan north slope crude oil (ANSC).

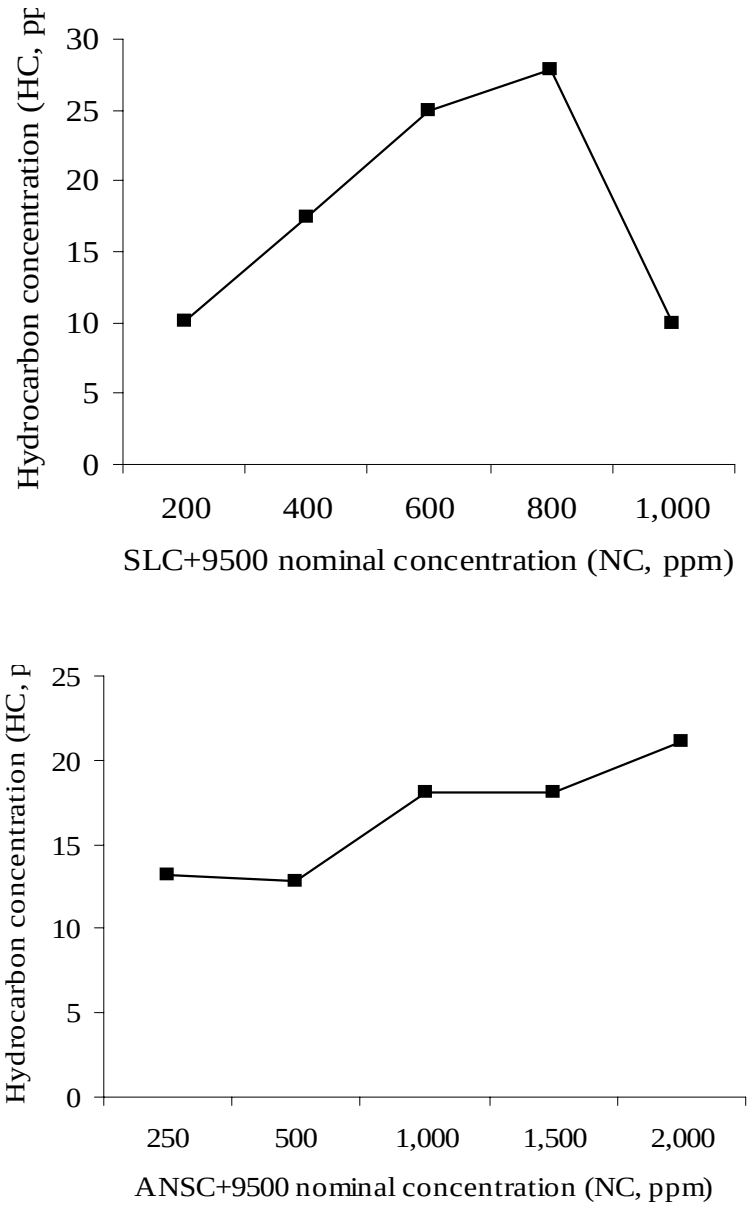

Figure 2.16. The measured hydrocarbon concentration (HC, ppm) change with the nominal concentration (NC, ppm) for dispersed South Louisiana crude oil (SLC+9500) and dispersed Alaskan north slope crude oil (ANSC+9500).

obtained at both relatively low (50 to 100 ppm) and high (8,000 to 12,000 ppm) nominal crude oil concentrations. Dispersion of crude oil by physical handling is not likely sufficient to allow the water-soluble fraction in crude oils to attain maximum solubility, especially for high nominal crude oil concentrations. Some investigators suggest that that an LL50 (lethal loading) should be used to express the effects of materials containing low solubility compounds (Peterson, 1994; Markarian et al., 1995). Loading (dosing) level of a low solubility material such as crude oil may be a better term than concentration, which often connotes the concentration soluble compounds in water. The toxicity of a material expressed as a loading value, has little value unless the method of preparation is identified (Rice et al., 1977; Singer et al., 1998).

In this study, soluble hydrocarbons were more toxic than the dispersant. The toxicity of hydrocarbons in dispersed oils and crude oils to white shrimp in this study were similar, but the toxicity of hydrocarbons in dispersed oils was less than the crude oils for Gulf killifish. Many earlier studies reported that dispersed oil was more toxic than crude oil, but this was not observed in this study. When crude oil was used to dose exposure containers in this study, the oil remained on the surface and only a small water-accommodated fraction (WAF) was dissolved in the water (as determined from total hydrocarbon levels). The WAF consists of both soluble and dispersed hydrocarbons in the water when the oil or dispersed oil is mixed in water following a standard protocol (Singer et al., 1997). When dispersant was added to the crude oil and then dosed, a higher concentration of soluble and dispersed oil components dissolved in the exposure water. The toxicity of the WAF of crude oil is nearly indistinguishable from the toxicity of the WAF of dispersed oil (NRC, 1989). It is possible to derive different conclusions from oil

and oil dispersant toxicity tests depending on the compound used to derive estimates of toxicity, for example, nominal concentrations versus hydrocarbon concentrations. The statistical methods used to estimate toxicity of a compound is often based on a dose-response curve of a toxicant or mixture of toxicants. Deriving definitive conclusions from specific types of oil or dispersed oil is difficult because modes of toxic action can be caused by both a combination of toxic water soluble compounds in the oil and by physical impairment by oil droplets coating the gills of aquatic animals and impairing gas exchange.

Species Tolerance

In this study, white shrimp was the most sensitive species of the three species tested to the crude oils, dispersed oils, and dispersant. Bioassays on adult shrimp Pandalus danae with dispersed oils showed that removal of monoaromatic chemicals reduced toxicity about sevenfold. It could be possible that aromatic hydrocarbons of oils or dispersed oils produced most of the toxicity to shrimp (Anderson et al., 1987). Juvenile Litopenaeus setiferus are more sensitive to No. 2 Fuel Oil than postlarvae (NAS, 1985), and postlarval shrimp have higher tolerance to dispersants and hydrocarbons than less advanced post-larvae. Shrimp are also more sensitive to oil and dispersed oil during molting phases (Fucik et al., 1995).

Gulf killifish showed increased sensitivity to crude oils and dispersed oils when dissolved oxygen level was low (< 2 mg/L). Anderson et al. (1987) reported that the aromatic hydrocarbons did not appear to be acutely toxic to sand lance below 3 ppm. It was possible oil droplets in the water column coated the gills of the killifish and impaired oxygen exchange and was a contributing factor in mortality. With restricted airflow at

150 bubbles per minute, fish were found to reside just below the oil surface. They opened and closed their gill covers widely and frequently after 24 hours or more of exposure, and this appeared to be a sign of clinical toxicity. The fish near the oil/water interface appeared to intake oil droplets into their mouth and then expurgate them. In dispersed oil, the dispersants reduced the effects of oil droplets and it is likely that dissolved hydrocarbons may have been a more important factor in the toxic action on killifish. Also, the surfactants in the dispersant decrease the interfacial tension between gill epithelial cells and the surrounding medium, thus reducing oxygen exchange and causing asphyxia and death (Singer, et al., 1994). Further studies on the oxygen consumption rates of killifish exposed to the oil and dispersed oil need be conducted to help explain the action of dispersant in the toxicity of dispersed oils.

Eastern oysters have the ability to close their valves and cease pumping water to protect them from environmental disturbances, including the presence of oils and hydrocarbons in the water. Shuba and Heikamp (1989) reported that Crassostrea virginica (mean length 3.3 cm) exhibited mortality only when exposed to concentrations of dispersed oil of 2,500 ppm. Bivalves are reported to bioaccumulate contaminants including the oil hydrocarbons at relatively high concentrations (Kennedy et al., 1996). It is possible that physical coating of oil on the gill epithelium resulting in asphyxia might be in part responsible for the toxic action of oil and dispersed oil on oysters. A relationship was not established between dissolved hydrocarbon concentrations and oyster mortality and the levels evaluated in this study. Clearly, exposure to higher water temperature (> 28 ^{o}C on average) increased the toxicity of oils, dispersant, and dispersed oils to oysters, which was not unexpected. Sub-lethal toxicity tests should be conducted

with SLC and ANSC and the dispersed oils to determine their effects on the water pumping function of oysters in order to help to explain modes of toxic action.

Joint Toxicity

The nominal crude oil and dispersed oil concentrations are not representative of the exposure concentration of dissolved hydrocarbons to which the test organisms were exposed. An effective dispersant should mobilize more hydrocarbon components from crude oils into the water thus exposing test organisms to potentially higher levels of toxicant (Mackay et al., 1982). In this study, the joint action of crude oil and dispersant, based on the nominal concentration, was found to be synergistic in Gulf killifish and additive in white shrimp. Researchers have stated that the higher toxicity of chemically dispersed oil to aquatic organisms is generally a reflection of exposure to higher levels of hydrocarbons rather than a higher inherent toxicity (Peakall et al., 1987; NRC, 1989). Wells and Harris (1980) evaluated the responses of fish to dispersant, oil, and dispersed oil, and they reported that the interaction between the crude oils and dispersed oils were additive in their toxicity. Thus, with use of an effective but low-toxicity dispersant, the acute toxicity of the dispersed oil should reflect the toxicity of the oil-derived hydrocarbons (NRC, 1989).

In contrast to the results on nominal concentrations of crude oil and dispersed oil in this study, the joint toxicity action of crude oil hydrocarbons and dispersant indicated there was an antagonistic effect for Gulf killifish, but the joint effect on white shrimp was additive, as was observed for the nominal concentration. Different aquatic species may have a different response to a single toxicant or a toxicant mixture. Based on the findings of this study, COREXIT 9500 could reduce the toxicity of dissolved hydrocarbons to

Gulf killifish. However, the concentration of dissolved hydrocarbons in water between dispersed oil and crude oil were similar, although slightly higher for dispersed oil. The total hydrocarbon analysis used in this study did not quantify the various types or components with the total hydrocarbon fraction. It is possible that the dispersant increased the concentration of non-toxic hydrocarbons dissolved in the water from the dispersed oil; however, further chemical analysis of the specific hydrocarbon types dissolved in water from dispersed oil would be necessary. However, the results of this study do indicate that dispersed oil did not result in a synergistic increase in toxicity of crude oil for both Gulf killifish and white shrimp.

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Chapter 3

Short-term Field Investigation on the Toxicity of Alaskan North Slope Crude Oil (ANSC) and Dispersed Alaskan North Slope Crude Oil (ANSC+9500) to Gulf Killifish, White Shrimp, and Eastern Oyster

Objectives

The objective of this study was to determine the toxic effects of Alaskan North Slope Crude oil (ANSC) and Alaskan North Slope Crude oil plus Exxon COREXIT 9500 (ANSC+9500) to Gulf killifish, white shrimp, and Eastern oysters in short-term field exposures.

Materials and Methods

Species

The species used in this field study were same as those used in laboratory tests: Gulf killifish Fundulus grandis, Eastern oyster Crassostrea virginica, and white shrimp Litopenaeus setiferus. All three species are abundant in the sensitive inshore coastal ecosystems of Louisiana. The Eastern oysters and white shrimp are highly valued components of Louisiana's commercial marine fisheries, and Gulf killifish are an important prey species for sport fishes. Adult white shrimp Litopenaeus setiferus (7-8 cm TL) were collected from the Louisiana Universities Marine Consortium (LUMCON, Chauvin, Louisiana) in April, 2002 and juvenile Litopenaeus setiferus (2-3 cm TL) were obtained from the Gulf Coast Research Laboratory (Ocean Springs, Mississippi) in November, 2002. Juvenile Eastern oysters Crassostrea virginica (average total weight of 100 g) were collected from Grand Isle, Louisiana. Juvenile Gulf killifish Fundulus grandis were purchased from Gulf Coast Minnows Company (Thibodaux, Louisiana, USA) and averaged 7 cm total length (TL). These fish were larger than those used in the

laboratory toxicity trials to prevent escape from live cages used to hold the animals during the field trials. All test organisms were held for 48 hrs in synthetic seawater with a salinity of 8 - 9 ppt to acclimate the organisms before they were transported to the test site. The organisms were transported in 80-L containers (coolers) and the water was aerated continuously with an aeration pump. Organisms that appeared to be stressed from visual observation were discarded and not used in the tests.

Chemicals

The oil dispersant COREXIT 9500 (9500) (Nalco/Exxon Energy Chemicals, L.P., Sugar Land, Texas) and fresh Alaska North Slope Crude oil (ANSC) were used as test chemicals. COREXIT 9500 is a relatively new dispersant designed to treat higher viscosity oils and emulsions (Singer et al. 1996). This dispersant has not been used extensively to treat oil spills because of uncertain environmental acceptability. The ANSC is more viscous than South Louisiana crude oil (SLC) and has not been sufficiently tested in Louisiana's coastal zone. The COREXIT 9500 and ANSC used in these two field tests came from the same source as those used in acute toxicity laboratory trials, and their physical and chemical characteristics were described in Chapter 2. The dispersed oil (ANSC+9500) was made by physically mixing ANSC and 9500 in an 8-L plastic container at a recommended oil:dispersant ratio of 20:1 (v:v) (NRC, 1989) immediately prior dosing.

Test Site

Field tests were conducted in April and the November, 2002. The study site was located in an intermediate/brackish water marsh, representative of coastal Louisiana, at the Pointe Au Chien State Wildlife Management Area (WMA) in Terrebonne Parish near

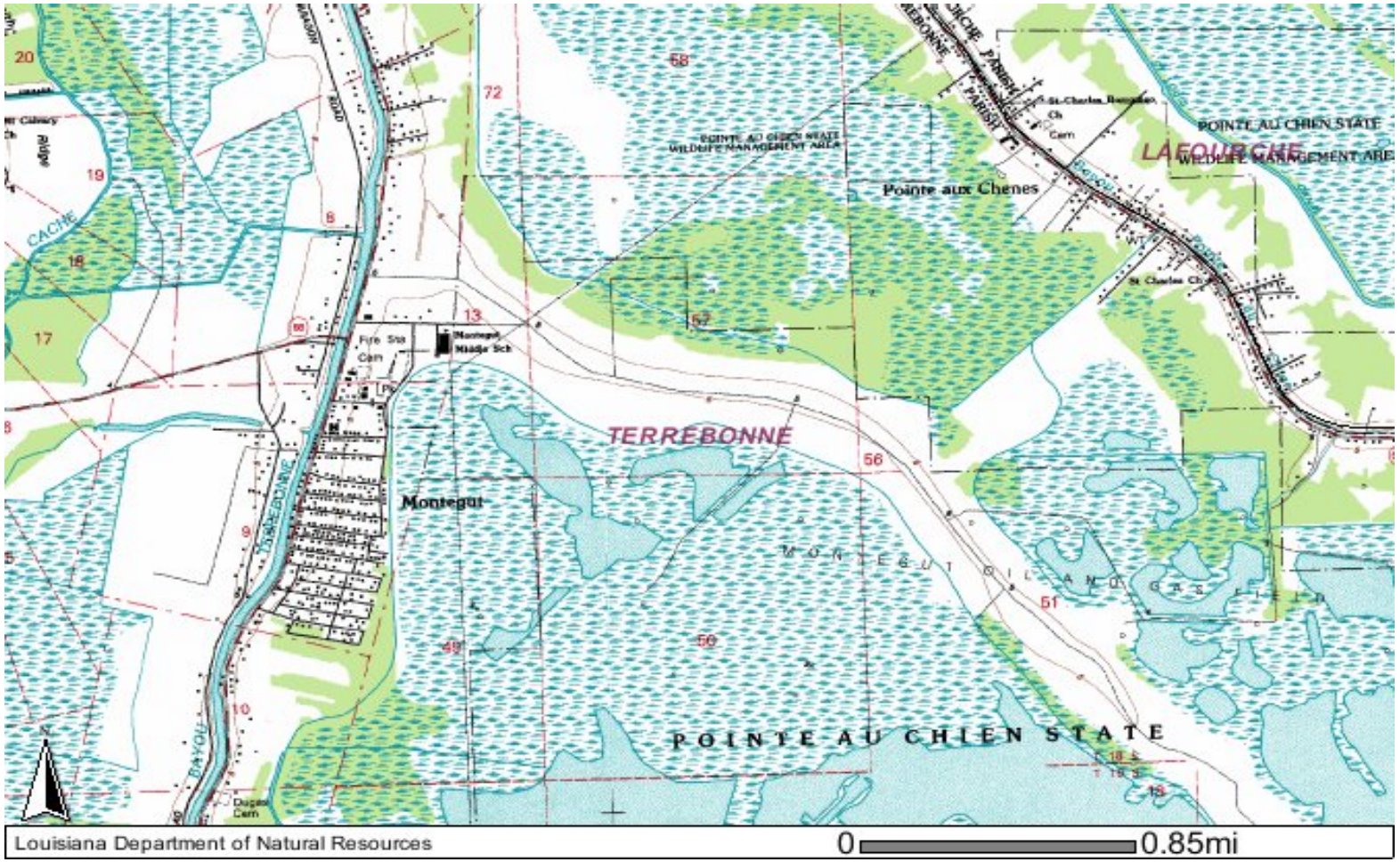

Figure 3.1. Oil (ANSC) and dispersed oil (ANSC+9500) field test site. Point-aux-Chien, Louisiana, about 50 miles Southeast of New Orleans, Louisiana.

Montegut, Louisiana (Figure 3.1). The source water was Humble Canal, about 1.6 km south of the Bayou Terrebonne/Humble Canal intersection (DeLaune et al., 2000). The water depth at the test site was during the studies was 50 cm deep.

Exposure Protocol

Three wooden enclosures (1.80 cm L × 90 cm W × 60 cm D) were used as test systems. A boom was placed on one side of each enclosure with a removable wall that allowed oil and dispersed oil to flush with tidal action and wind-driven water flow (Figure 3.2). Four experimental treatments were evaluated and the exposure trials were conducted for 24 hours. The experimental treatments were as follows: (1) Inside Control (inside wooden enclosure, no oil, dispersed oil, or dispersant; 0 ppm), (2) Outside Control (outside wooden enclosures immediately adjacent to the study site; 0 ppm), (3) ANSC (inside enclosure, 30 ppm nominal dosing concentration); and (4) dispersed oil mixture, ANSC+9500 (inside enclosure, 30 ppm nominal dosing concentration of ANSC). Three replicate cylindrical plastic mesh cages (58-cm H, 23-cm dia, and 1.2-cm mesh) contained each of the three test species (9 cages per treatment) and were placed inside each of the three enclosures and outside of the "inside control" enclosure. Each cage was placed on the bottom of the canal, with the top protruding above the water's surface. The cages werestabilized with a metal rod to prevent them from toppling over. Three small cages (2

Figure 3.2. Pouring ANSC+9500 into an experimental test enclosure.

mm mesh size) were placed inside the larger cages to contain the smaller juvenile shrimp used in the November field trial. Twelve to 18 organisms were randomly assigned to the three cages assigned for each species (4 to 6 organisms per cage) (Figure 3.3).

After placement of the test organisms in the cages, the crude oil and dispersed oil (ANSC and ANSC+9500) were applied to their respective enclosure by gently and evenly pouring the chemicals onto the water surface (Figure 3.2). After a few minutes, the side of each enclosure adjacent to the boom was removed to allow for normal tidal action and flushing within the enclosure. Mortality of test organisms in each exposure cage was recorded at 24 h.

Figure 3.3. Setting the cages with organisms into the enclosure.

Water and Sediment Analysis

Water within the enclosures and outside the enclosures was analyzed at 0 and 24 h for dissolved oxygen (YSI Model 58 oxygen meter, Yellow Springs Instrument Company, Inc., Yellow Springs, Ohio), salinity (YSI Model 33, Yellow Springs Instrument Co., Inc., Yellow Spring, Ohio), pH (glass electrode) and un-ionized ammonia and nitrite (HACH Chemical Company, Loveland, Colorado). Water temperature was measured *in situ* with the dissolved oxygen (DO) meter. A water sample from each treatment was collected for total hydrocarbon analysis at 0, 3 and 24 h by fluorometry. Sample bottles were placed 20 cm under the water surface, the caps were removed until

the bottles were filled, and the caps were placed back on the bottles before removing them from the water to prevent direct oil contamination of the water sample. Samples were stored on ice until returned to the laboratory for analysis of total hydrocarbons following analytical procedures described in Chapter 2. Selected water samples at 0 and 24 h were analyzed by a gas chromatograph determine the total petroleum hydrocarbons (TPH) concentrations and to compare them with total hydrocarbon concentrations as determined by fluorometry (Lindau, et. al., 2003; Lessard, Demarco, 2000).

Sediment samples were taken randomly at each of three different locations of each treatment at 24 h by hand. They were stored on ice until returned to the laboratory for analysis of the total petroleum hydrocarbons (TPH) concentration by chromatographic analysis

Data Analysis

Both survival and mortality were used to describe the toxicity response of the test organisms in each treatment. Survival rate was determined by the percentage of organisms alive after 24 h based on the total number of organisms used in each treatment. Mortality was calculated as the percentage of organisms dead after 24 h based on the total number of organisms used in each cage during the tests. The analysis of variance (ANOVA) was used to determine if differences existed in the mortality differed among treatments (petroleum exposure) and among species. Treatment means were declared to be significant at $\alpha < 0.05$. All statistical analyses were conducted using Statistical Analysis System (SAS) software (Statistical Analysis System software version 8.2 for Windows, SAS Institute, Inc., Gary, NC).

Results

Hydrocarbon Analysis

Total hydrocarbons concentration (HC) as determined by fluorometery showed that COREXIT 9500 allowed more ANSC fractions to dissolve and disperse into the water (Figure 3.4 - 3.5). At a dosing level of 30 ppm, total hydrocarbon concentrations in water at 0 h ranged from 14 to 24 ppm with dispersed oil and only 10 ppm with ANSC. The HC concentrations decreased rapidly in 3 hours and were near 0 ppm after 24 hours. Chromatographic analysis showed the TPH of ANSC+9500 in the water decreased from 8,390 μg/L at 0 h to 485 μg/L at 24 h. The TPH of ANSC decreased from 1,870 μg/L at 0 h to 587 μg/L at 24 h. The TPH concentration in the outside control at 0 h was 556 μg/L. The HC inside and outside controls was less than 0.6 ppm, and TPH values were less than 600 μg/L.

The analysis of sediments showed TPH present in the sediment of all treatments including the controls (Figure 3.6), averaging 20,000 to 25,000 μg/kg. It is possible that the sediment could have been contaminated with petroleum from a nearby oil production storage facility.

Water Quality

Temperature, salinity, nitrite, ammonia and pH showed little change over 24 h in each field test (Table 3.1). The salinity averaged 11.5 ppt in April trial and 5.0 ppt in the November trial. Un-ionized ammonia (NH_3-N) was below 0.17 mg/L, DO exceeded 3 mg/L and pH averaged 8.0. Water temperature ranged from 24 ^{o}C to 30 ^{o}C and with

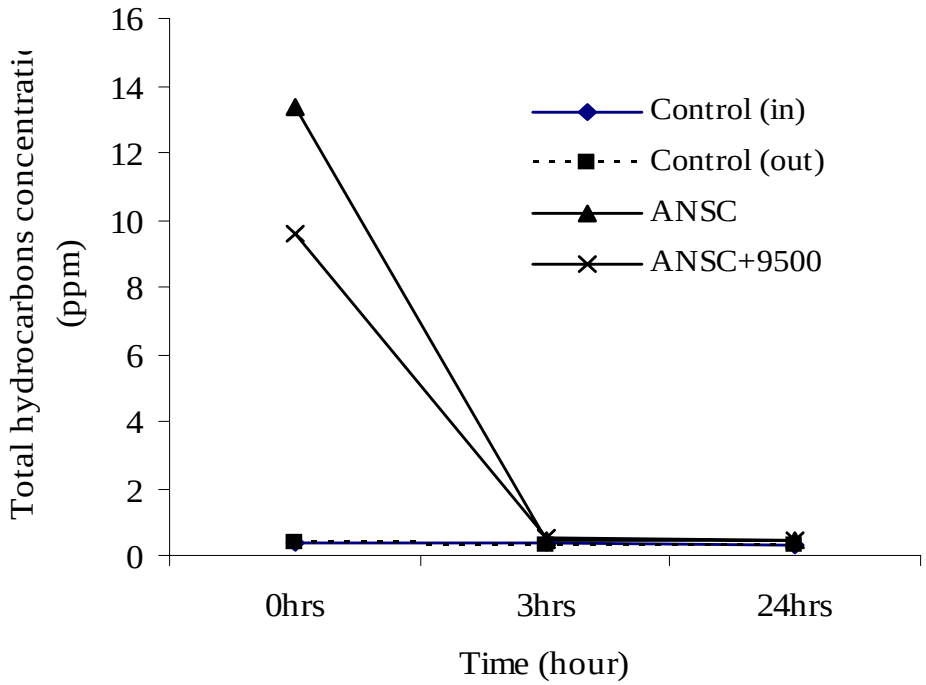

Figure 3.4. Hydrocarbon concentration of Alaskan north slope crude oil (ANSC) and dispersed Alaskan north slope crude oil (ANSC+9500) in water over time in the April field trial.

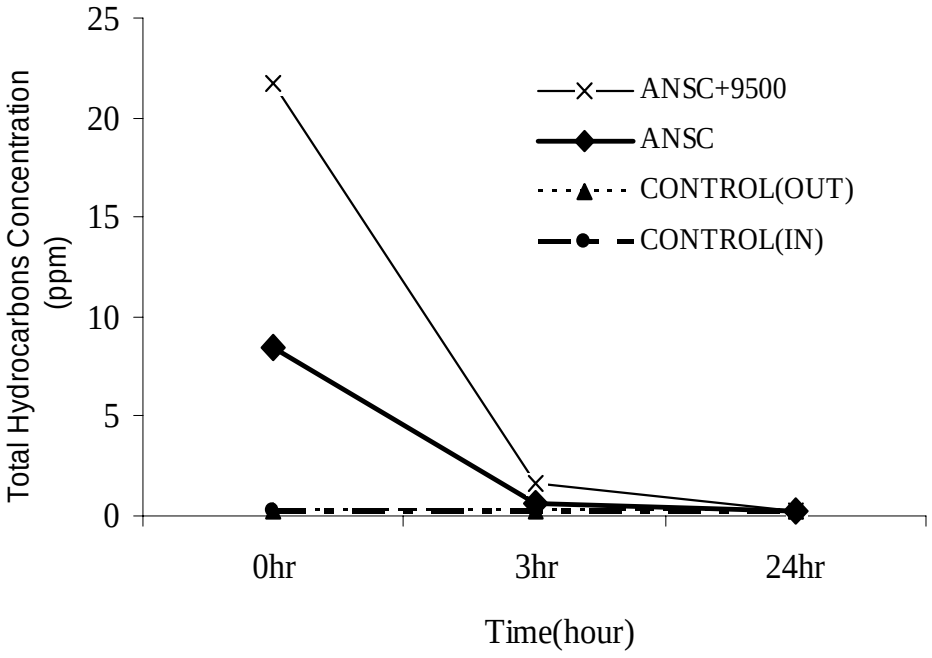

Figure 3.5. Hydrocarbon concentration of Alaskan north slope crude oil (ANSC) and dispersed Alaskan north slope crude oil (ANSC+9500) in water over time in the November field trial.

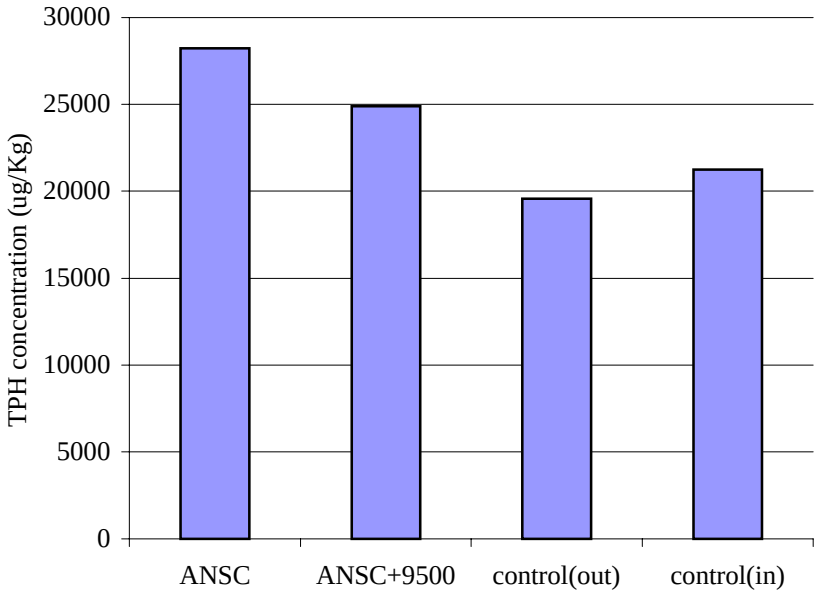

Figure 3.6. The total petroleum hydrocarbons concentrations (µg/Kg wet W) of the sediments in four petroleum exposure regimes.

mean of 27 ^{o}C during the April trial, and averaged 20 ^{o}C in the November trial. Water quality was acceptable for the physiological health of the test organisms in each trial, and no difference among the four treatments was observed in any of the water quality parameters measured (P>0.05) for each field test (Table 3.1).

Mortality

Survival was relatively high for all three species during the 24 h exposure, generally exceeding 83% (Figure 3.7). Average mortalities in all controls were less than 10% which showed the short-term environmental conditions were suitable for test organisms during the two field toxicity tests. No significant difference in 24-h mortality of white shrimp, Eastern oysters, and Gulf killifish was observed between the two 0 ppm controls (inside and outside enclosures) and oil and dispersed oil at an application rate of 30 ppm ($P > 0.05$) (Figure 3.7). No difference in mortality was observed between Gulf killifish and Eastern oysters ($P>0.05$) but mortality was higher for white shrimp ($P < 0.05$; Figure 3.7).

Discussion

Oil and Dispersed Oil

Under the laboratory conditions, both dissolution and evaporation of dissolved hydrocarbons were restricted when oil or dispersed oil was physically mixed into the limited volume of water. The mixing would have likely increased the rate of dissolution of hydrocarbons into the water column but would have also exposed test organisms to a higher concentration of toxic aromatic compounds. In the field trials, a large water mass appeared to facilitate the dissolution of the hydrocarbons resulting in higher concentrations of hydrocarbons in exposure water. Most oil spills occur in the open

Table 3.1. Water quality in two petroleum field toxicity trials.

Water Parameters	April Field Toxicity Trial		November Field Toxicity Trial	
	0 h	24 h	0 h	24 h
DO (mg/L)	3.3-4.0	7.0-7.5	6.3-6.8	6.8-7.5
Salinity (ppt)	11	12	5.0	5.0
Un-Ionized Ammonia (mg/L)	0.17	0.13	0.03	0.03
Nitrite-Nitrogen (mg/L)	0-0.2	0	0.1-0.2	0.1-0.3
pH	8.0	7.5-8.0	8.0	8.0
Water Termperature (^{o}C)	27.2	27.2-28.0	19.8-19.9	19.0-19.1

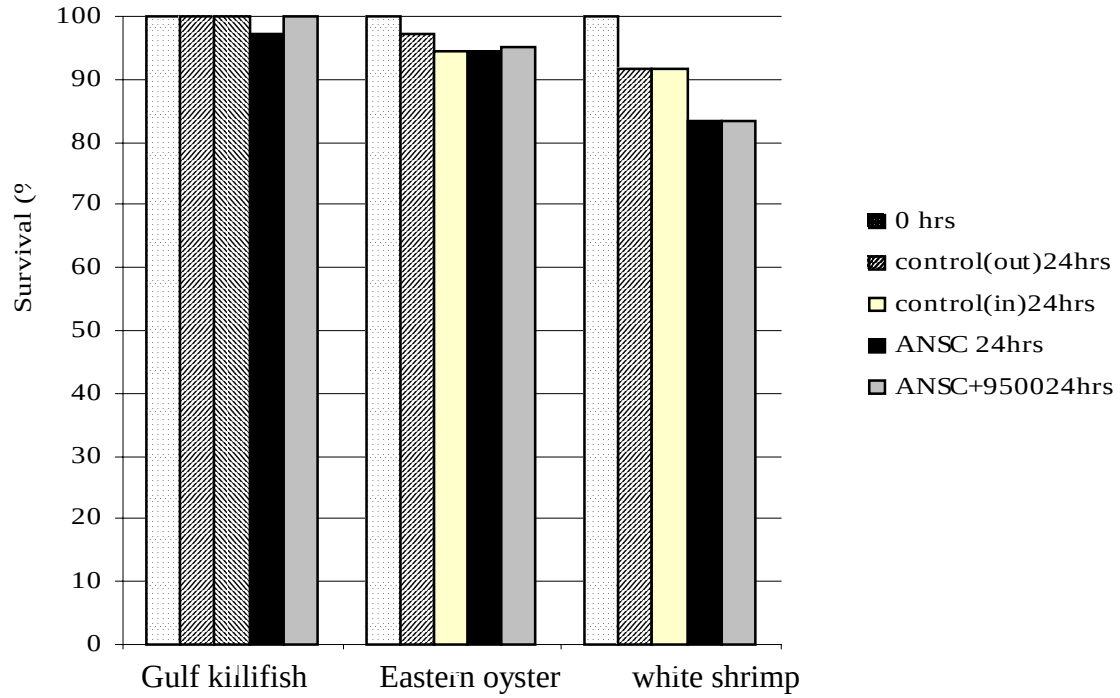

Figure 3.7. Survival (%) of Gulf killifish Fundulus grandis, white shrimp Litopenaeus setiferus and Eastern oyster, Crassostrea virginica with ANSC only, dispersed ANSC (ANSC+9500) and two controls (outside and inside).

ocean and are treated with chemical dispersant. Exxon has determined in field studies that a rate of 30 ppm of dispersed oil can be considered representative of the concentration that would likely occur in coastal environments (J. P. Canevari, Engineering advisor, Exxon Research and Engineering, personal communication, 2001). Based on a dosing rate of 30 ppm used in this study, more than 25% of hydrocarbons in ANSC and more than 33% of ANSC+9500 entered the water column shortly after the enclosures were dosed (Figure 3.4 – 3.5). However, most of the water-accommodated hydrocarbons were diluted rapidly through the tidal flushing and wind-driven water flow and approached concentrations near 0 ppm within 3 hours of dosing. The dissolved toxic compounds could not be concentrated to a certain level to cause significant mortality in the test organisms. In addition, the larger surface area in field trials likely enhanced the rate of evaporation and loss of light-weight and potential toxic aromatic hydrocarbons to the atmosphere, although this might be inhibited in dispersed oil. One example is from a paper prepared for the 1997 International Oil Spill Conference by Alun Lewis:

> "The wreck of the barge Noth Cape on the coast of Rhode Island in January 1996 led to the loss of nearly 20,000 barrels of home heating oil, similar to No. 2 fuel oil in physical and chemical properties. The oil was highly dispersible and weather conditions were extreme, with winds up to 60 mph. NOAA (OSIR, 1996) estimates were that as much as 80 percent of the oil was dispersed within the first eight hours after release. The maximum TPH level in water samples taken from the vicinity of the wreck two days later was about 6 ppm, and oil was evenly distributed throughout the water column within five days. TPH values were below the detection limit (0.1 ppm) (Research Planning, Inc., 1996)"

This study in a low tidal action Louisiana coastal marsh demonstrated that crude oil and dispersed oil can rapidly be diluted and depleted within 24 hours.

The rapid decline in hydrocarbon concentration observed within 3 hours in this study might be related to the increased vertical mixing (Mcauliffe, 1989). Theoretically,

if 80 ppm ANSC+9500 (concentration based on 0.1 meter water column) was applied to the water surface and if 50 ppm of hydrocarbons were dispersed and mixed into the top 0.1 meter water column, normal vertical mixing would decrease the concentration of hydrocarbons in a system 1 meter deep to 5 ppm, assuming even distribution, and the hydrocarbon concentration would decline to 1 ppm if mixing occurred down to a depth of 5 meters (Figure 3.8). This illustration based on the data published in Lewis and Aurand (1997). The concept would also apply to horizontal mixing in a shallow water system by dispersion of oil or dispersed oil over wide area and increased water volume.

The losses of hydrocarbons from each enclosure in the oil and dispersed oil treatments can be compared to each other after the concentrations are converted to mass values (Page, et al., 1998). In both treatments, it took 3 hours for most of the hydrocarbons to be depleted to near 0 ppm levels. However, the loss in dispersed oil was about 1.5 to 2.5 times higher than the crude oil (non-dispersed) treatment because of the higher initial levels of dissolved hydrocarbons in dispersed oil. Page, et al. (1998) reported losses in dispersed Arabian crude oil were 80 times higher than Arabian crude oil during the first 30 minutes, whereas there was more floating oil unaccounted for in non-dispersed crude oil treatment. We estimated the amount of oil adhered to the wooden enclosure and boom in this study was likely less than 10% of the total oil in non-dispersed crude oil treatment and much less in dispersed oil treatment. Most of the dispersed oil was flushed from the enclosures from tidal flushing and wave actions.

Toxicity Evaluation

No noticeable toxicity was observed in the two field trials under the conditions of this study. Toxicity could vary significantly under different conditions including the

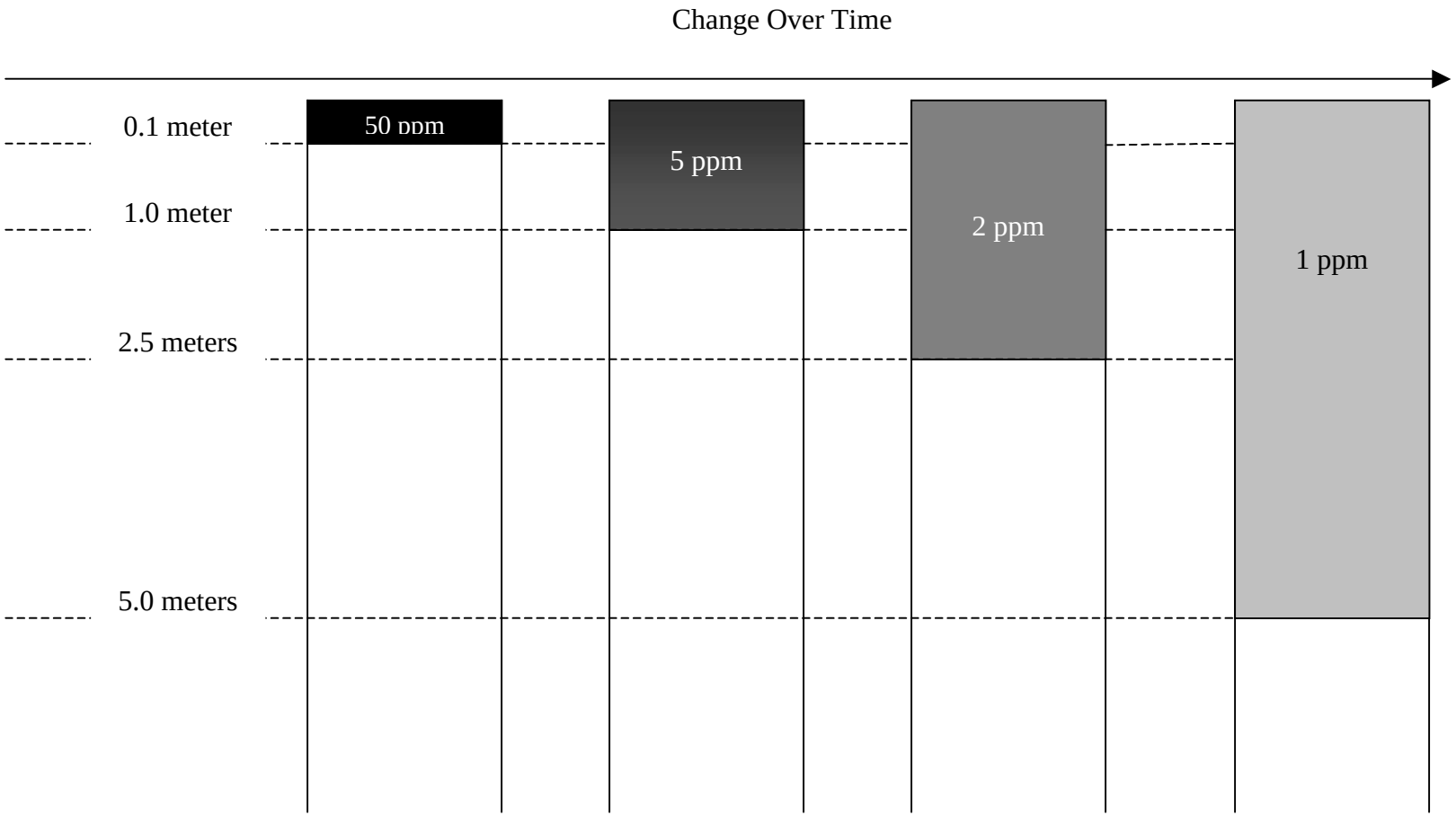

Figure 3.8. Total hydrocarbons concentration (ppm) changes of the dispersed oil in the water column through the vertical mixing over time (after Lewis and Aurand, 1997).

Table 3.2. Survival rate (%) of Gulf killifish, Eastern oyster, and white shrimp after 24 hours exposure to two controls, Alaskan north slope crude oil (ANSC) and dispersed Alaska north slope crude oil (ANSC+9500) in two field toxicity tests.

Species	Survival rate (%)							
	April Trial				November Trial			
	Control (in)	Control (out)	ANSC	ANSC+9500	Control (in)	Control (out)	ANSC	ANSC+9500
Gulf killifish	77.8	100	100	100	100	100	94.4	100
Eastern oyster	94.4	88.9	88.9	94.4	100	100	100	95.8
White shrimp	0*	44.4	83.3	50	91.7	91.7	83.3	83.3

*: Shrimp stressed during handling.

exposure concentration, time of exposure, and changes in environmental conditions or species used in the tests. The more practical estimates of oil toxicity might be associated with the field trials, and the laboratory toxicity tests should ideally represent conditions that could likely occur under field conditions. The exposure time in the field study (24 hours) was much shorter than in laboratory trials. The hydrocarbon concentrations were comparatively constant in the laboratory acute toxicity tests, and wave and tidal action rapidly reduced levels of potentially toxic hydrocarbons in the field trials.

High survival rates for Gulf killifish, white shrimp, and Eastern oysters in the field that exposed to concentrations of hydrocarbons that initially ranged from 8-21 ppm in both field trials and laboratory acute toxicity tests, were likely attributed to two major factors: (1) tidal flushing and vertical mixing (for dispersed oil) that reduced hydrocarbon concentrations to less than 1 ppm within 3 hours; and (2) short time of exposure to potentially toxic levels of hydrocarbons minimized the lethal effect on the organisms. Accordingly, spiked exposure in laboratory toxicity studies might be a superior method to resemble and simulate the gradual, but rapid decline in toxic hydrocarbon concentrations which were observed in the field.

White shrimp were clearly more sensitive to oil and dispersed oil exposure than Gulf killifish and Eastern oysters. A significant amount of oil following the dispersant applied in a shallow water with low tide could cause the short-term toxicity to sensitive aquatic species including white shrimp. Problems were encountered in the April field trials when 100% mortality of Litopenaeus setiferus occurred in the inside control enclosure, which appeared to be associated with handling stress (Table 3.2). In the November field trial, two of the small cages containing juvenile Litopenaeus setiferus

were jarred loose and floated to the water's surface resulting in additional mortality in the crude oil treatment. Short-term oil exposure field tests provide an approximate assessment of potential acute toxicity of hydrocarbons to aquatic organisms during changing environmental conditions but do not address long-term acute or chronic issues associated with crude oil or dispersed oil exposure to aquatic organisms (Rice et al., 1977).

The hydrocarbon levels in sediments, dispersed oil and oil enclosures and the controls did not differ ($P > 0.05$). All sediments had a concentration of hydrocarbons of about 20-25 ppm (20,000 to 25,000 μg/kg), and there was a likelihood sediments could have been contaminated by a nearby oil production facility. This could have accounted for the slight mortality observed in Eastern oysters and white shrimp. The species are benthic and were placed in cages whose bottoms were in contact with the sediment. Although this can be verified through the control treatments, it does suggest that benthic communities could be potentially impacted.

When the toxicity values generated from laboratory tests are applied to field conditions, a number of physicochemical and biological factors should be considered (Wells, 1985). Estimates of toxicity are relative numbers that can and do change depending on the environmental conditions and biological populations being exposed (NRC, 1989). Organisms generally show higher sensitivity to oil toxicants in warm waters compared to cool or colder waters (NRC, 1989). Mortality of Easter oysters in these studies was higher at warmer temperatures, in both the laboratory and field trials. In the second field trial, juvenile Crassostrea virginica showed almost 100% survival rate at an average temperature below $20^{o}C$ (Table 3.2). Temperature had a profound

influence on oil and dispersant toxicity because increased metabolism usually results in an increase in the uptake of potentially toxic chemicals.

In addition, biological factors such as life stage, physiological condition, and habitat preferences of organisms affect toxicity estimates. Many crustaceans, particularly those in advanced pre-molt, and molting stages are particularly sensitive to dissolved and physically dispersed hydrocarbons as well as to dispersant (NRC, 1985; Sprague et al., 1982). Wilson (1977) reported that starvation of fish larvae could increase their susceptibility to oil toxicants. Species living near the surface might be exposed to higher concentrations of hydrocarbons after oil is chemically dispersed on the surface. Many species in the littoral and shallow sub-littoral zones are particularly susceptible to potential oil spills (NRC, 1989). An ecosystem study was beyond the scope of this research project, but valuable information was produced on the potentially toxicity of oils and dispersed oils to economically and ecologically important brackish and marine species in Louisiana's coastal zone.

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Chapter 4

Summary and Conclusions

1. Louisiana has a significant portion of the USA's riverine, estuarine, and coastal ecosystems. A significant amount of petroleum is refined, stored, or transported through these areas. Nearly 15% of USA crude oil production is located in Louisiana, and 20% of the nation's oil flows through Louisiana's coastal marshes. Louisiana is also a national leader in commercial and recreational fisheries production and its coastal wetlands contribute 28 percent to the total volume of domestic fisheries harvest. These wetlands, that provide essential habitat for fisheries species, are vulnerable to oil spills.
2. The objective of this study was to evaluate the acute toxicity of one oil dispersant, Corexit 9500 (9500), two crude oils, South Louisiana Crude oil (SLC) and Alaskan North Slope Crude oil (ANSC), and two dispersed oils, dispersed South Louisiana Crude oil (SLC+9500) and dispersed Alaskan North Slope Crude oil (ANSC+9500) to three commercially and ecologically important species indigenous to the Gulf of Mexico: Gulf killifish Fundulus grandis, Eastern oyster Crassostrea virginica and white shrimp Penaeus setiferus. Joint (mixture) toxicity was examined to describe the effects of crude oil (SLC or ANSC) and 9500 as either additive, synergistic or antagonistic.
3. Static non-renewal exposure regime was used to evaluate the lethal effect of five chemicals: SLC, ANSC, SLC+9500, ANSC+9500 and 9500 alone to each of three test species over an exposure period of 96 hours in the laboratory. Organisms were exposed to non-toxicant control (dilution water only) and either five or six

toxicant concentrations. The exposure solutions were made by adding the oil, dispersed oil, or dispersant directly in the exposure water. Water quality was monitored daily. Total hydrocarbons were measured for crude oils and dispersed oil solutions at dosing and at 24-hour intervals by fluorometery. The 96-h LC50 were determined for each of the five chemical toxicants for each species using both nominal (dosed, NC) concentrations of the oils, dispersed oils, and dispersant, and analytical (measured) concentration of total hydrocarbons (HC) for oils and dispersed oils. The joint action (mixture) toxicity of dispersant when combined with crude oil was determined from an isobole, and chemical mixture toxicity of crude oil and the dispersant mixture was interpreted as being additive (AD), synergistic (SY), or antagonistic (AN) to the aquatic animals tested.

4. Dispersed oils were more toxic than crude oils based on nominal concentrations (NC). For Gulf killifish, the mean 96-h NC LC50 of SLC and ANSC were 4,457 ppm and 4,492 ppm, respectively, 11.5 times and 3.5 times higher than the 96-h NC LC50 of SLC+9500 and ANSC+9500. The 96-h NC LC50 of SLC and ANSC for white shrimp was 132.6 ppm and 145.4 ppm, 2.7 times and 2.5 times higher than the 96-h NC LC50 of SLC+9500 and ANSC+9500, respectively. No difference was observed in the toxicity of SLC and ANSC, or the dispersed oils, for either Gulf killifish or white shrimp. The mean 96-h HC LC50s of SLC and ANSC were 6.49 ppm and 6.59 ppm for white shrimp and 8.30 ppm and 7.67 ppm for Gulf killifish, respectively. The mean 96-h HC LC50 values of dispersed oils (SLC+9500 or ANSC+9500) were about twice that of the crude oils for Gulf killifish and about the same for white shrimp. Mortality in oysters was not

positively correlated with increasing levels of crude oil, or dispersed oil. Dispersed oils were more toxic than crude oils based on nominal concentrations, but no difference in toxicity of crude oils and dispersed oils was observed based on HC concentrations. No synergistic toxicity was observed between SLC or ANSC and dispersant Corexit 9500.

5. Two 24-hour field exposures were conducted to determine the toxic effects of ANSC and ANSC+9500 to Gulf killifish, white shrimp, and Eastern oyster in a brackish water Louisiana coastal marsh. Three wooden enclosures were used as exposure systems. Four experimental treatments were evaluated: (1) Inside control (inside wooden enclosure, no oil, dispersed oil, or dispersant; 0 ppm), (2) Outside control (outside wooden enclosures immediately adjacent to the study site; 0 ppm), (3) ANSC (inside enclosure, 30 ppm nominal dosing concentration); and (4) Dispersed oil mixture, ANSC+9500 (inside enclosure, 30 ppm nominal dosing concentration). Test organisms were placed in cages inside the enclosures and outside the control enclosure prior to dosing. After dosing, one side of each enclosure was removed to allow for normal tidal action and flushing within the enclosure.
6. At a dosing level of 30 ppm, total hydrocarbon concentrations in water at 0 h ranged from 14 to 24 ppm with dispersed oil and only 10 ppm with ANSC. The HC concentrations decreased rapidly in 3 hours and were near 0 ppm after 24 hours. Survival was relatively high for all three species during the 24 h exposure, generally exceeding 83%. Average mortalities in all controls were less than 10% which showed the short-term environmental condition were suitable for test

organisms during the two field toxicity tests. No significant difference in 24-h mortality of white shrimp, Eastern oysters, and Gulf killifish was observed between the two 0 ppm controls (inside and outside enclosures) and oil and dispersed oil at an application rate of 30 ppm. No difference in mortality was observed between Gulf killifish and Eastern oyster but mortality was slightly higher for Gulf white shrimp.

7. High survival rates for killifish, shrimp, and oysters in the field that were exposed to concentrations of hydrocarbons that initially ranged from 8-21 ppm is likely attributed to tidal flushing and vertical mixing (for dispersed oil) that reduced hydrocarbon concentrations to less than 1 ppm within 3 h, and the short time of exposure to potentially toxic levels of hydrocarbons minimized the lethal effect on the organisms. White shrimp were more sensitive to oil and dispersed oil exposure than Gulf killifish and the Eastern oyster. The findings of the laboratory and field studies indicate that short-term exposure to nominal concentrations of crude oil or dispersed oil of 30 ppm or less are not likely to have an acute toxic effect on Gulf killifish, white shrimp, and the Eastern oyster.

Appendix: Raw Data

Appendix Table 1. Mortality of Gulf killifish Fundulus grandis at different nominal concentrations (NC, ppm) of South Louisiana crude oil (SLC) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	No. Org.	Cumulative Mortality				% Mortality
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	8	0	0	0	0	0
500	8	0	0	0	0	0
1000	8	0	0	0	0	0
2000	8	0	0	0	0	0
4000	8	0	0	2	2	25
8000	8	0	0	8*	8*	100
0	8	0	0	0	0	0
1500	8	0	0	0	0	0
3000	8	0	0	2	2	25
6000	8	1	5	6	6	75
12000	8	8	8	8	8	100

*: the glass pipet was floated on the surface and stop the air flow.

Appendix Table 2. Hydrocarbon concentrations (HC, ppm) change with time at different nominal concentrations (NC, ppm) of South Louisiana crude oil (SLC) in 96-h static nonrenewal tests for Gulf killifish Fundulus grandis.

Nominal conc. (NC, ppm)	Hydrocarbon concentration (HC, ppm)					Weighted mean (HC)
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	0	0.56	0.12	0.71	0.86	0.46
1500	2.94	3.94	6.27	9.38	10.85	6.02
3000	4.26	5.59	6.69	8.11	8.16	6.05
6000	6.86	7.37	11.05	15.14	17.10	10.79
12000	10.26	9.85	12.22	13.94	13.25	11.34

Appendix Table 3. Mortality of Gulf killifish Fundulus grandis at different nominal concentrations (NC, ppm) of Alaskan north slope crude oil (ANSC) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	No. Org.	Cumulative Mortality				% Mortality
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	8	0	0	0	0	0
500	8	0	0	0	0	0
1000	8	0	0	0	0	0
2000	8	0	0	0	0	0
4000	8	0	0	0	0	0
8000	8	0	0	0	0	0
0	8	0	0	0	0	0
1500	8	0	0	0	0	0
3000	8	0	0	2	2	25
6000	8	1	5	6	6	75
12000	8	8	8	8	8	100
0	10	0	0	0	0	0
2000	10	0	0	0	0	0
3000	10	0	0	1	2	20
4000	10	0	0	7*	7*	70*
6000	10	0	2	6	6	60
8000	10	3	6	7	7	70

*: the glass pipet was floated on the surface and stop the air flow.

Appendix Table 4. Hydrocarbon concentration (HC, ppm) change with time at different nominal concentrations (NC, ppm) of Alaskan north slope crude oil (ANSC) in 96-h static nonrenewal tests for Gulf killifish Fundulus grandis.

Nominal conc. (NC, ppm)	Hydrocarbon concentration (HC, ppm)					Weighted mean (HC)
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	0	0.56	0.12	0.71	0.86	0.46
1500	1.52	3.91	5.39	9.07	8.86	6.02
3000	1.82	5.32	7.09	10.33	11.94	6.05
6000	2.75	7.68	8.63	11.45	13.55	10.79
12000	4.66	9.54	12.31	14.65	18.76	11.34
0	0	0.71	0.63	0.79	0.82	0.64
2000	1.84	3.41	5.83	8.89	10.07	6.02
3000	1.64	3.48	6.79	11.59	12.05	7.18
4000	2.16	4.46	7.94	11.88	12.57	7.91
6000	2.57	4.46	8.75	13.67	16.19	9.07
8000	3.04	4.88	8.72	16.27	18.23	10.13

Appendix Table 5. Mortality of Gulf killifish Fundulus grandis at different nominal concentrations (NC, ppm) of South Louisiana crude + COREXIT 9500 (SLC + 9500) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	No. Org.	Cumulative Mortality				% Mortality
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	8	0	0	0	0	0
125	8	0	0	0	0	0
250	8	0	0	0	0	0
500	8	0	0	0	0	0
1000	8	0	0	0	0	0
2000	8	0	0	0	0	0
0	8	0	0	0	0	0
250	8	0	3	3	3	37.5
500	8	0	6	6	6	75
1000	8	6	7	7	7	87.5
2000	8	8	8	8	8	100
0	10	0	0	0	0	0
200	10	0	0	0	0	0
400	10	0	4	4	5	50
600	10	0	6	8	8	80
800	10	1	9	9	9	90
1000	10	4	10	10	10	100

Appendix Table 6. Hydrocarbon concentration (HC, ppm) change with time at different nominal concentrations (NC, ppm) of dispersed South Louisiana crude oil (SLC+9500) in 96-h static nonrenewal tests for Gulf killifish Fundulus grandis.

Nominal conc. (NC, ppm)	Hydrocarbon concentration (HC, ppm)					Weighted mean (HC)
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	0	0.56	0.12	0.71	0.86	0.46
250	27.23	19.62	13.21	13.32	12.65	16.52
500	36.32	28.75	22.16	20.85	19.34	24.90
1000	42.35	35.65	26.98	22.35	22.62	29.37
2000	45.83	39.86	33.17	29.71	26.77	34.76
0	0	0.71	0.63	0.79	0.82	0.64
200	20.73	10.36	10.26	11.22	10.07	11.81
400	31.21	19.33	15.92	16.93	14.31	18.74
600	34.06	28.52	21.21	24.45	23.71	25.77
800	43.31	32.78	25.48	25.77	22.91	29.29
1000	39.61	22.24	15.36	17.52	19.50	21.17

Appendix Table 7. Mortality of Gulf killifish Fundulus grandis at different nominal concentrations (NC, ppm) of Alaskan north slope crude oil + COREXIT 9500 (ANSC + 9500) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	No. Org.	Cumulative Mortality				% Mortality
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	8	0	0	0	0	0
125	8	0	2*	2*	2*	25*
250	8	0	0	0	0	0
500	8	0	0	0	0	0
1000	8	0	0	0	0	0
2000	8	0	8	8	8	100
0	8	0	0	0	0	0
250	8	0	0	0	0	0
500	8	0	0	0	0	0
1000	8	0	0	0	0	0
2000	8	7	8	8	8	100
0	10	0	0	0	0	0
250	10	0	0	0	0	0
500	10	0	0	0	0	0
1000	10	0	2	2	3	30
1500	10	0	4	6	7	70
2000	10	8	10	10	10	100

*: the glass pipet was floated on the surface and stop the air flow

Appendix Table 8. Hydrocarbon concentrations (HC, ppm) change with time at different nominal concentrations (NC, ppm) of dispersed Alaskan north slope crude oil (ANSC+9500) in 96-h static nonrenewal tests for Gulf killifish Fundulus grandis.

Nominal conc. (NC, ppm)	Hydrocarbon concentration (HC, ppm)					Weighted mean (HC)
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	0	0.56	0.12	0.71	0.86	0.455
250	5.64	9.13	13.97	15.66	21.84	13.13
500	6.21	8.32	11.39	17.98	22.13	12.97
1000	10.80	15.35	18.18	20.32	22.54	17.63
2000	18.37	21.56	27.69	26.40	28.11	24.72
0	0	0.71	0.63	0.79	0.82	0.635
250	4.90	9.11	12.54	16.56	31.92	11.81
500	6.05	8.82	13.89	19.38	20.75	14.16
1000	9.75	17.05	19.08	24.74	22.61	19.26
1500	18.45	13.67	17.91	28.25	16.71	19.35
2000	14.11	19.57	19.06	27.90	27.85	21.88

Appendix Table 9. Mortality of Gulf killifish Fundulus grandis different nominal concentrations (NC, ppm) of COREXIT 9500 (9500) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	No. Org.	Cumulative Mortality				% Mortality
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	8	0	0	0	0	0
25	8	0	0	0	0	0
50	8	0	0	0	0	0
100	8	0	0	0	0	0
200	8	0	1	3	5	25
400	8	8	8	8	8	100
0	10	0	0	0	0	0
100	10	0	0	0	0	0
150	10	0	2	2	3	30
200	10	3	9	10	10	100
300	10	8	10	10	10	100
400	10	10	10	10	10	100
0	10	0	0	0	0	0
250	10	0	1	1	1	10
500	10	0	0	0	0	0
1000	10	0	0	0	0	0
1500	10	0	0	3	6	60
2000	10	2	7	8	8	80

Appendix Table 10. Mortality of Eastern oyster Crassostrea virginica at different nominal concentrations (NC, ppm) of South Louisiana Crude oil (SLC) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	No. Org.	Cumulative Mortality	% Mortality
	0 hr HC	96 hr HC	
0	10	0	0
100	10	2	20
1000	10	6	60
5000	10	5	50
10000	10	2	20
0 (ctrl)	10	0	0
1000	10	4	40
2000	10	5	50
5000	10	5	50
10000	10	0	0
0 (ctrl)	10	0	0
1000	10	3	30
2000	10	2	20
5000	10	4	40
10000	10	3	30

Appendix Table 11. Hydrocarbon concentrations (HC, ppm) change with time at different nominal concentrations (NC, ppm) of South Louisiana crude oil (SLC) in 96-h static nonrenewal tests for Eastern oyster Crassostrea virginica.

Nominal conc. (NC, ppm)	Hydrocarbon concentration (HC, ppm)					Weighted mean (HC)
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	0.86	1.33	1.33	1.33	1.45	1.29
100	1.76	3.05	1.92	2.35	2.74	2.39
1000	2.97	5.44	4.30	4.81	5.36	4.68
5000	9.43	8.57	7.90	8.29	10.25	8.65
10000	15.45	16.98	14.59	14.44	14.32	15.22
0	2.39	2.92	3.19	3.27	3.54	3.09
1000	9.29	9.82	13.01	--	22.26	14.06
2000	11.68	12.04	20.00	--	40.30	29.02
5000	18.23	16.99	20.97	--	38.92	33.27
10000	24.16	21.68	19.65	--	33.97	35.20
0	0.54	0.51	0.48	0.52	0.52	0.51
1000	9.27	7.57	7.72	9.35	13.89	9.06
2000	14.16	10.56	10.43	13.15	15.43	12.23
5000	19.25	15.66	14.62	17.28	20.15	16.82
10000	24.30	21.84	19.51	23.58	20.71	21.86

-- : no observation

Appendix Table 12. Mortality of Eastern oyster Crassostrea virginica at different nominal concentrations (NC, ppm) of Alaskan North Slope Crude oil (ANSC) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	No. Org.	Cumulative Mortality	% Mortality
	0 hr	96 hr	
0	10	0	0
100	10	1	10
1000	10	2	20
5000	10	4	40
10000	10	3	30
0	10	0	0
1000	10	8	80
2000	10	2	20
5000	10	0	0
10000	10	0	0
0	10	0	0
1000	10	0	0
2000	10	0	0
5000	10	1	10
10000	10	1	10

Appendix Table 13. Hydrocarbon concentrations (HC, ppm) change with time at different nominal concentrations (NC, ppm) of Alaskan north slope crude oil (ANSC) in 96-h static nonrenewal tests for Eastern oyster Crassostrea virginica.

Nominal conc. (NC, ppm)	Hydrocarbon concentration (HC, ppm)					Weighted mean (HC)
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	0.86	1.33	1.33	1.33	1.45	1.29
100	1.60	2.11	2.66	4.03	4.77	3.00
1000	1.92	2.43	3.56	4.23	4.77	3.39
5000	2.27	2.43	4.26	4.23	4.54	3.58
10000	2.46	3.09	5.13	6.14	5.91	4.64
0	2.39	2.92	3.19	3.27	3.54	3.09
1000	6.46	8.41	14.34	--	31.54	16.17
2000	7.96	11.59	23.10	--	48.73	24.74
5000	9.29	13.36	21.86	--	37.97	22.19
10000	8.85	11.59	16.81	--	29.22	17.61
0	0.54	0.51	0.48	0.52	0.52	0.51
1000	7.67	7.04	9.13	9.04	12.68	8.85
2000	8.78	8.87	11.78	14.24	16.63	11.90
5000	9.04	10.40	14.57	17.94	20.71	14.45
10000	9.16	10.83	16.17	13.48	16.74	13.36

-- : no observation

Appendix Table 14. Mortality of Eastern oyster Crassostrea virginica at different nominal concentrations (NC, ppm) of dispersed South Louisiana Crude oil (SLC + 9500) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	No. Org.	Cumulative Mortality	% Mortality
	0 hr	96 hr	
0	10	0	0
100	10	1	10
500	10	5	50
1000	10	7	70
2000	10	10	100
4000	10	10	100
0 (ctrl)	10	0	0
100	10	0	0
500	10	10	10
1000	10	8	80
1500	10	8	80
2000	10	4	40
0 (ctrl)	10	0	0
100	10	0	0
500	10	0	0
1000	10	9	90
1500	10	8	80
2000	10	2	20

Appendix Table 15. Hydrocarbon concentrations (HC, ppm) change with time at different nominal concentrations (NC, ppm) of dispersed South Louisiana crude oil (SLC+9500) in 96-h static nonrenewal tests for Eastern oyster Crassostrea virginica.

Nominal conc. (NC, ppm)	Hydrocarbon concentration (HC, ppm)					Weighted mean (HC)
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	0.86	1.33	1.33	1.33	1.45	1.29
100	11.54	11.93	9.74	4.93	4.93	8.71
500	60.02	39.51	21.87	13.34	10.84	27.54
1000	69.84	62.40	48.00	33.88	23.83	47.78
2000	68.27	70.50	56.10	35.60	20.31	51.62
4000	71.17	53.52	53.99	59.35	54.10	57.37
0	2.39	2.92	3.19	3.27	3.54	3.09
100	32.04	7.96	9.47	10.27	11.59	12.38
500	96.81	25.66	6.19	14.42	16.55	25.74
1000	111.15	43.27	15.93	15.75	16.46	34.69
1500	102.92	57.96	17.35	16.90	18.76	38.26
2000	89.91	57.96	23.36	26.73	29.73	41.97
0	0.54	0.51	0.48	0.52	--	0.51
100	28.90	13.26	11.09	11.74	--	14.89
500	103.48	40.22	18.13	13.56	--	38.96
1000	151.30	109.13	38.30	23.10	--	78.21
1500	135.22	95.70	46.45	23.15	--	73.78
2000	124.65	93.48	52.02	30.43	--	74.35

-- : no observation

Appendix Table 16. Mortality of Eastern oyster Crassostrea virginica at different nominal concentrations (NC, ppm) of dispersed Alaska north slope Crude oil (ANSC + 9500) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	No. Org.	Cumulative Mortality	% Mortality
	0 hr	96 hr	
0 (ctrl)	10	0	0
100	10	0	0
500	10	4	40
1000	10	10	100
2000	10	10	100
4000	10	10	100
0 (ctrl)	10	0	0
100	10	0	0
500	10	0	0
1000	10	1	10
1500	10	8	80
2000	10	6	60
0 (ctrl)	10	0	0
100	10	0	0
500	10	6	60
1000	10	5	50
1500	10	2	20
2000	10	8	80

Appendix Table 17. Hydrocarbon concentrations (HC, ppm) change with time at different nominal concentrations (NC, ppm) of dispersed Alaskan north slope crude oil (ANSC+9500) in 96-h static nonrenewal tests for Eastern oyster Crassostrea virginica.

Nominal conc. (NC, ppm)	Hydrocarbon concentration (HC, ppm)					Weighted mean (HC)
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	0.86	1.33	1.33	1.33	1.45	1.29
100	6.30	13.26	15.96	27.66	37.48	19.69
500	7.08	11.46	16.55	21.64	32.67	17.38
1000	4.30	8.06	16.94	20.85	26.29	15.29
2000	4.62	7.47	14.55	13.26	22.03	12.15
4000	4.19	6.18	9.70	8.96	11.97	8.23
0	2.39	2.92	3.19	3.27	3.54	3.09
100	9.73	7.88	8.94	10.97	14.07	9.92
500	16.02	11.95	12.21	14.16	17.08	13.72
1000	23.54	22.92	14.34	16.19	19.20	18.71
1500	28.14	33.10	18.23	17.79	21.86	23.53
2000	27.79	29.73	22.48	24.87	26.73	26.09
0	0.54	0.51	0.48	0.52	--	0.51
100	16.30	9.78	10.07	14.57	--	11.76
500	25.87	21.30	23.48	23.26	--	23.12
1000	43.04	36.52	42.30	30.43	--	38.52
1500	47.17	51.74	55.22	35.00	--	49.35
2000	56.30	61.52	65.00	46.09	--	59.24

-- : no obeservation

Appendix Table 18. Mortality of Eastern oyster Crassostrea virginica at different nominal concentrations (NC, ppm) of COREXIT 9500 (9500) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	Cumulative Mortality		% Mortality
	0 hr	96 hr	
0 (ctrl)	10	2	20
20	10	4	40
100	10	5	50
150	10	0	0
200	10	1	10
400	10	0	0
0 (ctrl)	10	0	0
100	10	0	0
150	10	3	30
200	10	10	100
300	10	10	100
400	10	10	100
0 (ctrl)	10	0	0
120	10	1	10
140	10	0	0
160	10	0	0
180	10	6	60
200	10	8	80

Appendix Table 19. Mortality of white shrimp Penaeus setiferus at different nominal concentrations (NC, ppm) of South Louisiana Crude oil (SLC) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	No. Org.	Cumulative Mortality				% Mortality
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	8	0	0	0	0	0
100	8	1	1	3	3	37.5
1000	8	2	7	8	8	100
5000	8	8	8	8	8	100
10000	8	8	8	8	8	100
0	8	0	0	0	0	0
250	8	2	3	4	6	75
500	8	3	5	8	8	100
750	8	3	6	8	8	100
1000	8	4	6	8	8	100

Appendix Table 20. Hydrocarbon concentrations (HC, ppm) change with time at different nominal concentrations (NC, ppm) of South Louisiana crude oil (SLC) in 96-h static nonrenewal tests for white shrimp Penaeus setiferus.

Nominal conc. (NC, ppm)	Hydrocarbon concentration (HC, ppm)					Weighted mean (HC)
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	0.75	0.75	0.90	0.98	0.98	0.87
100	2.17	4.52	7.69	10.03	9.95	7.08
1000	5.56	6.71	12.14	13.50	15.08	10.67
5000	10.16	12.75	13.60	14.86	16.44	13.63
10000	14.43	19.16	17.29	18.30	16.22	17.52
0	0.20	0.35	0.31	0.29	0.39	0.31
250	2.69	5.12	7.47	10.41	11.55	7.53
500	3.24	5.35	8.02	11.53	12.26	8.16
750	2.96	5.26	7.95	11.91	13.74	8.37
1000	2.96	6.13	11.95	13.33	16.40	10.27

Appendix Table 21. Mortality of white shrimp Penaeus setiferus at different nominal concentrations (NC, ppm) of Alaskan north slope Crude oil (ANSC) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	No. Org.	Cumulative Mortality				% Mortality
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	8	0	0	0	0	0
100	8	1	1	3	3	37.5
1000	8	0	6	8	8	100
5000	8	6	8	8	8	100
10000	8	8	8	8	8	100
0	8	0	0	0	0	0
250	8	3	4	4	5	62.5
500	8	4	6	7	8	100
750	8	4	7	8	8	100
1000	8	5	7	8	8	100

Appendix Table 22. Hydrocarbon concentrations (HC, ppm) change with time at different nominal concentrations (NC, ppm) Alaskan north slope Crude oil (ANSC) in 96-h static nonrenewal tests for white shrimp Penaeus setiferus.

Nominal conc. (NC, ppm)	Hydrocarbon concentration (HC, ppm)					Weighted mean (HC)
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	0.75	0.75	0.90	0.98	0.98	0.87
100	2.37	5.43	7.69	8.07	9.43	6.77
1000	2.57	7.84	11.09	15.46	18.63	11.25
5000	5.01	8.30	13.35	14.88	17.94	12.00
10000	5.69	7.16	11.67	14.56	18.66	11.39
0	0.20	0.35	0.31	0.29	0.39	0.31
250	2.96	6.85	8.32	10.08	11.90	8.17
500	4.17	7.38	10.12	12.39	12.52	9.56
750	4.35	7.30	9.88	12.65	14.39	9.80
1000	4.81	10.77	12.65	14.93	17.16	12.33

Appendix Table 23. Mortality of white shrimp Penaeus setiferus at different nominal concentrations (NC, ppm) of dispersed South Louisiana crude oil (SLC + 9500) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	No. Org.	Cumulative Mortality				% Mortality
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	8	0	0	0	0	0
50	8	0	1	2	2	25
100	8	0	7	7	7	87.5
500	8	6	8	8	8	100
1000	8	8	8	8	8	100
0	8	0	0	0	0	0
50	8	2	4	6	6	75
75	8	4	5	6	7	87.5
100	8	5	8	8	8	100
250	8	8	8	8	8	100

Appendix Table 24. Hydrocarbon concentrations (HC, ppm) change with time at different nominal concentrations (NC, ppm) of dispersed South Louisiana crude oil (SLC+9500) in 96-h static nonrenewal tests for white shrimp Penaeus setiferus.

Nominal conc. (NC, ppm)	Hydrocarbon concentration (HC, ppm)					Weighted mean (HC)
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	0.75	0.75	0.90	0.98	0.98	0.87
50	4.67	4.90	4.83	5.73	6.41	5.25
100	10.16	6.86	4.83	6.18	6.94	6.61
500	33.88	24.81	10.94	9.35	8.67	16.59
1000	44.92	27.60	10.56	10.63	8.67	18.90
0	0.20	0.35	0.31	0.29	0.39	0.31
50	6.20	6.98	6.35	7.12	7.61	6.84
75	7.22	8.59	7.97	8.84	8.96	8.37
100	8.89	10.35	11.31	9.67	10.00	10.19
250	21.76	16.37	15.46	12.28	11.44	15.18

Appendix Table 25. Mortality of white shrimp Penaeus setiferus at different nominal concentrations (NC, ppm) of dispersed Alaska north slope crude oil (ANSC + 9500) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	No. Org.	Cumulative Mortality				% Mortality
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	8	0	0	0	0	0
50	8	1	1	3	3	37.5
100	8	0	0	3	3	37.5
500	8	8	8	8	8	100
1000	8	8	8	8	8	100
0	8	0	0	0	0	0
50	8	2	4	4	5	62.5
75	8	3	5	6	6	75
100	8	5	7	7	8	100
250	8	7	7	8	8	100

Appendix Table 26. Hydrocarbon concentrations (HC, ppm) change with time at different nominal concentrations (NC, ppm) of dispersed Alaska north slope crude oil (ANSC + 9500) in 96-h static nonrenewal tests for white shrimp Penaeus setiferus.

Nominal conc. (NC, ppm)	Hydrocarbon concentration (HC, ppm)					Weighted mean (HC)
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	0.75	0.75	0.90	0.98	0.98	0.87
50	2.85	6.49	7.69	11.61	11.54	8.25
100	3.39	6.33	9.13	10.63	10.71	8.29
500	7.50	9.88	8.97	9.58	8.67	9.13
1000	10.03	12.37	10.41	10.78	10.65	10.98
0	0.20	0.35	0.31	0.29	0.39	0.31
50	3.61	5.89	7.98	10.95	10.32	7.95
75	3.89	5.92	8.94	9.26	11.79	7.99
100	4.72	6.35	9.87	11.39	12.94	9.11
250	7.96	10.21	12.37	13.41	12.26	11.53

Appendix Table 27. Mortality of white shrimp Penaeus setiferus at different nominal concentrations (NC, ppm) of COREXIT 9500 (9500) in 96-h static nonrenewal tests.

Nominal conc. (NC, ppm)	No. Org.	Cumulative Mortality				% Mortality
	0 hr	24 hr	48 hr	72 hr	96 hr	
0	8	0	0	0	0	0
20	8	0	1	2	3	37.5
50	8	0	2	3	5	62.5
100	8	1	3	5	6	75
200	8	2	3	4	7	82.5
400	8	8	8	8	8	100
0	8	0	0	0	0	0
10	8	0	0	1	2	25
20	8	1	1	3	3	37.5
40	8	0	2	4	4	50
100	8	3	5	7	7	87.5

Appendix Table 28. Water quality in oil toxicity field test in April Trail.

Water parameter	0 hrs				24 hrs			
	Control (in)	Control (out)	ANSC	ANSC+9500	Control (in)	Control (out)	ANSC	ANSC +9500
DO (mg/L)	3.3	3.4	3.5	4.0	7.0	7.5	7.0	7.5
Salinity(ppt)	11	11	11	11	12	12	12	12
Ammonia(mg/L)	2.7	2.7	2.7	2.7	1.9	2.0	1.9	2.0
PH	8.0	8.0	8.0	8.0	7.5	8.0	8.0	8.0
Temp (^{o}C)	27.2	27.2	27.2	27.2	28.0	27.7	27.7	28.0
Nitrite (mg/L)	0	0	0.2	0	0	0	0	0

Control (in): Control inside
Control (out): Control outside
ANSC: Alaskan north slope crude oil
ANSC+9500: dispersed Alaskan north slope crude oil

Appendix Table 29. Survival in oil toxicity field test in April Trail

species	Number of Survival animals after 24 hrs (total 6 each cage)											
	Control (in)			Control (out)			ANSC			ANSC+9500		
Minnows	4	5	5	6	6	6	6	6	6	6	6	6
Oyster	5	6	6	5	5	6	6	5	5	5	6	6
Shrimp	0	0	0	1	4	3	6	3	6	4	2	3

Appendix Table 30. Water quality in oil toxicity field test in November Trial

Water parameter	0 hrs				24 hrs			
	Control (in)	Control (out)	ANSC	ANSC+9500	Control (in)	Control (out)	ANSC	ANSC +9500
DO (mg/L)	6.3	6.5	6.8	6.8	6.8	7.0	7.0	7.5
Salinity(ppt)	5	5	5	5	5	5	5	5
Ammonia(mg/L)	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Toxic NH_3	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
PH	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Temp (^oC)	19.9	19.8	19.8	19.9	19.0	19.0	19.1	19.0
Nitrite (mg/L)	0.1	0.2	0.2	0.1	0.1	0.3	0.1	0.1

Appendix Table 31. Survival in oil toxicity field test in November Trial

species	Number of Survival animals after 24 hrs (total 6 each cage) Number of Survival animals after 24 hrs (total 6 minnows, 8oysters and 4 shrimps each cage)											
	Control (in)			Control (out)			ANSC			ANSC+9500		
Minnows	6	6	6	6	6	6	6	6	5	6	6	6
Oyster	8	8	8	8	8	8	8	8	8	8	7	8
Shrimp	4	4	3	4	3	4	3	4	3	3	3	4

Vita

Bo Liu was born on March 3, 1975, in Jiaozuo, Henan, People's Republic of China. He attended Shanghai Fisheries University in Shanghai, People's Republic of China, and graduated July 1998 with a Bachelor of Science degree in hydrobiology and the title of his Bachelor of Science thesis was "The Effect of Photo-cycle period to Artemia sp. on Feeding, Growth, Survival and Spawn".

He was admitted into the Institute of Water Environmental Science, Shanghai Fisheries University, as a graduate student in 1998; and he worked in an environmental chemistry lab as a research assistant until November 1999.

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