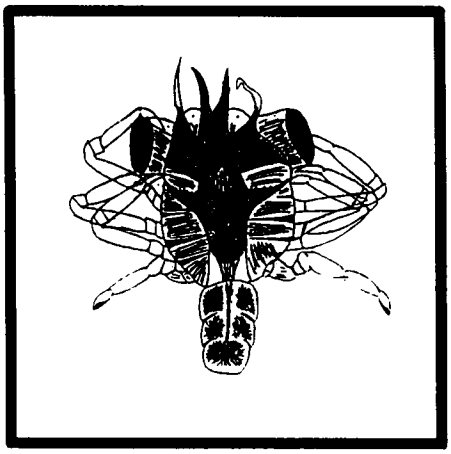
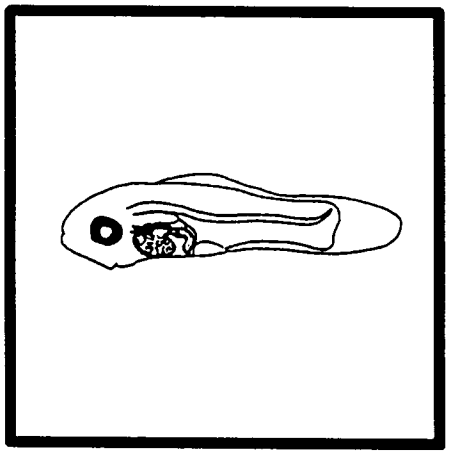
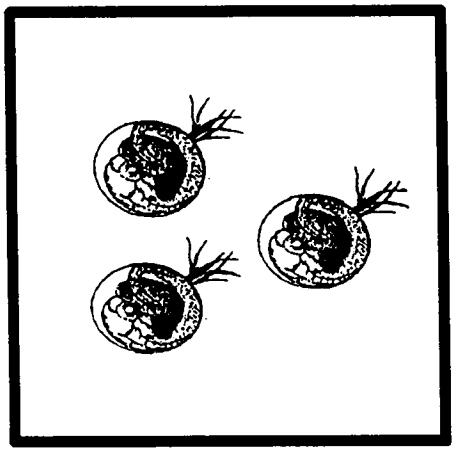
OCS Study
MMS 94-0021

Dispersed Oil Toxicity Tests with Biological Species Indigenous to the Gulf of Mexico







MMS U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

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Dispersed Oil Toxicity Tests with Biological Species Indigenous to the Gulf of Mexico

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COVER ARTWORK

The cover artwork is the result of the artistic efforts of two individuals. Justin Lane, age 15, of Denver, CO, created the megalopae larva using pen and ink. Suzi Short of Jupiter, FL, sketched the eggs and larva from several sources within the scientific literature. Reading top to bottom, the images are: blue crab (*Callinectes sapidus*) megalopae larva, spot (*Leiostomus xanthurus*) larva, and inland silverside (*Menidia beryllina*) eggs.

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ABSTRACT

Static and flowthrough aquatic acute toxicity testing protocols were utilized on egg and larval stages of seven commercially important invertebrate and fish species from the Gulf of Mexico. Test organisms were exposed to two different oils (from the Western and Central Gulf of Mexico), dispersed oil, and a single dispersant (Corexit 9527). Species evaluated included brown shrimp (*Penaeus aztecus*), white shrimp (*P. setiferus*), blue crab (*Callinectes sapidus*), eastern oyster (*Crassostrea virginica*), red drum (aka redfish or channel bass, *Sciaenops ocellatus*), inland silverside (aka silverside minnow, *Menidia beryllina*), and spot (*Leiostomus xanthurus*). In lieu of acute toxicity testing on gulf menhaden (*Brevoortia patronus*), which were unavailable, a congener (Atlantic menhaden, *B. tyrannus*) was evaluated using study-specific acute toxicity testing protocols for comparative purposes. Mysids (*Mysidopsis bahia*) were also evaluated as part of a chronic toxicity assessment. A total of 292 chemical analyses were conducted on oil (i.e., the water accommodated fraction, WAF) and dispersed oil at various phases of acute toxicity testing to characterize the degradation of oil and oil dispersant mixtures. Oil characterization tests were also conducted on both oils at the beginning and end of the study to determine if the oils changed significantly. Further, seven analyses were performed on a Corexit 9527 exposure to determine total petroleum hydrocarbon (TPH) and polynuclear aromatic hydrocarbon (PAH) background concentrations. The two oils showed minor chemical differences. Naphthalenes were present in the highest concentrations in both oils (i.e., 420 to 510 μg/g) and were generally several times greater than the other PAH compounds analyzed. For the acute toxicity testing, static tests tended to have the highest overall TPH concentrations with the dispersed oil levels being four to five times greater than that measured in the WAF. Flowthrough concentrations tended to be more variable without the clear distinctions seen in the static tests. Replication between the various flowthrough exposures was good, particularly with regard to hydrocarbons. Given that only a limited number of comparable study efforts have been completed on these early life stages, these results are increasingly important. The finding is particularly noteworthy in comparisons between various flowthrough tests, where total naphthalenes for both the Western and Central Gulf oils were approximately three times that of the WAF. Because a complete characterization of hydrocarbons in test media could not be accomplished for every toxicity test, this finding is quite important as it allows one to extrapolate between tests. Agreement in the static exposures was less evident, suggesting that greater variability is likely in toxicity results originating from this exposure method. The chemical characterizations may explain many of the anomalies observed in the toxicity data. Much of the variability that was seen in the fish tests may be attributed to the fact that most of these tests were run under static conditions, where some of the greatest variability in TPH concentrations was obtained. By comparison, the TPH, naphthalenes, and BTEX (benzene, toluene, ethylbenzene, xylene) concentrations were relatively uniform in flowthrough tests where most of the invertebrate exposures were completed. An important and historically-consistent finding in these tests was the lower sensitivity of the embryonic stages compared to the early larval stages. The overall sensitivity of the fish versus invertebrates appears to be similar. Invertebrates performed better as test organisms, as overall survival in controls was better. The naturally high mortality of the fish larvae compounded efforts to obtain acceptable test results and necessitated repeating several of the tests many times. The invertebrate tests, however, were generally accomplished with good control survival and results. BTEX compounds were a possible source of toxicity in the WAF exposures, whereas naphthalenes appeared to be the primary cause of toxicity in the dispersed oil exposures.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA - analysis of variance

ASTM - American Society for Testing and Materials

bbl - barrel(s)

BTEX - benzene, toluene, ethylbenzene, and xylenes

cm - centimeter(s)

°C - degrees Centigrade

EC_{50} - median effective concentration; concentration at which 50% of the test organisms are affected after a prescribed period of exposure

EPA - Environmental Protection Agency

FID - flame ionization detection/detector

g - gram(s)

GC - gas chromatography

HMM - Hawaiian Marine Mix

hr(s) - hour(s)

in. - inch(es)

IR - infrared spectroscopy

kg - kilogram(s)

l - liter(s)

LC_{50} - concentration at which 50% of the test organisms have succumbed after a prescribed period of exposure

LOEC - lowest observed effect concentration(s)

LOOP - Louisiana Offshore Oil Port

m - meter(s)

mg - milligram(s)

ml - milliliter(s)

mm - millimeter(s)

MMS - Minerals Management Service

MS - mass spectrometry

nm - nannometer(s)

NMFS - National Marine Fisheries Service

NOEC - no observed effects concentration(s)

NRC - National Research Council

OCS - outer continental shelf

PAH(s) - polynuclear aromatic hydrocarbon(s)

PL - post larva(e)

ppb - part(s) per billion

ppm - part(s) per million

ppt - part(s) per thousand

PVC - polyvinyl chloride

QA/QC - quality assurance/quality control

SIM - selected ion monitoring

TI - Toxicity Index

TPH(s) - total petroleum hydrocarbon(s)

μg - microgram(s)

μm - micrometer(s)

USDOI - U.S. Department of the Interior

UV - ultraviolet

WAF - water accommodated fraction

WSF - water soluble fraction

CHAPTER 1 - EXECUTIVE SUMMARY

1.1 OVERVIEW

Static and flowthrough aquatic acute toxicity testing protocols were utilized on egg and larval stages of seven commercially important invertebrate and fish species from the Gulf of Mexico. Species evaluated included brown shrimp (*Penaeus aztecus*), white shrimp (*Penaeus setiferus*), blue crab (*Callinectes sapidus*), eastern oyster (*Crassostrea virginica*), red drum (aka redfish or channel bass, *Sciaenops ocellatus*), inland silverside (aka silverside minnow, *Menidia beryllina*), and spot (*Leiostomus xanthurus*). Attempts were also made to secure eggs and larvae of gulf menhaden (*Brevoortia patronus*) from several sources, however, these attempts were unsuccessful. In lieu of acute toxicity testing on gulf menhaden, a congener (Atlantic menhaden, *Brevoortia tyrannus*) was evaluated using study-specific acute toxicity testing protocols for comparative purposes. Mysids (*Mysidopsis bahia*) were also evaluated as part of a chronic toxicity assessment.

Test organisms were exposed to 1) the water accommodated fractions (WAFs) of two different oils; 2) two oil-plus-dispersant mixtures (i.e., each oil separately treated with a chemical dispersant); and 3) one dispersant alone. The two test oils were acquired from the Western and Central Gulf of Mexico outer continental shelf, respectively, while the dispersant tested was Corexit 9527.

A total of 292 chemical analyses were conducted on oil (i.e., the WAF) and dispersed oil at various phases of toxicity testing to characterize the chemical changes which were expected to occur over the course of each test. In addition, oil characterization tests were conducted on both whole oils at the beginning (i.e., Day 1) and at the end of the study (i.e., Month 19) to determine if the oils changed significantly over the course of the project. Further, seven analyses were performed on a Corexit 9527 exposure to determine total petroleum hydrocarbon (TPH) and polynuclear aromatic hydrocarbon (PAH) background concentrations in the exposure medium. Specific chemical analyses and target compounds of interest included the following:

- Gas chromatography with flame ionization detection (GC-FID) to determine TPH;
- GC/mass spectrometry (GC/MS) to determine purgeable aromatic hydrocarbons (i.e., "volatiles," including benzene, toluene, ethylbenzene, and xylenes, jointly termed BTEX);
- GC/MS with selected ion monitoring (GC/MS-SIM) to determine polynuclear aromatic hydrocarbons (PAHs);
- Infrared spectroscopy (IR) as a cost-efficient method to determine TPH; and
- Ultraviolet (UV) spectrometry of the dispersant during test exposures for comparative measures of change.

A tabular summary of the life stages tested and chemistry completed is provided in **Table 1.**

Table 1. Summary of invertebrate and fish species tested with the water accommodated fractions (WAF) of Western and Central Gulf of Mexico oils, dispersed oils, and dispersant alone (Corexit 9527). Target chemical categories are noted parenthetically, where appropriate.

Species	Test[b]	Western Gulf of Mexico Oil[a]		Central Gulf of Mexico Oil[a]		Dispersant
		WAF[c] of Oil	Oil + Dispersant	WAF[c] of Oil	Oil + Dispersant	
Brown shrimp (*Penaeus aztecus*)	FT/A	post larvae tested	post larvae tested	Not tested	Not tested	Not tested
White shrimp (*Penaeus setiferus*)	FT/A	PL22 tested (PAH)[d]	PL22 tested (PAH); mysis stage tested (TPH)	PL15 tested (PAH)	PL15 tested (PAH)	PL15 tested (PAH); PL22 tested (PAH); mysis stage tested (TPH)
Blue crab (*Callinectes sapidus*)	FT/A	megalopae tested (TPH)	megalopae tested (TPH)	megalopae tested (BTEX)	megalopae tested (BTEX)	megalopae tested (BTEX); megalopae tested (TPH)
Eastern oyster (*Crassostrea virginica*)	S/A	Not tested	embryos tested (TPH); embryos tested (TPH)	Not tested	embryos tested (TPH)	embryos tested (TPH); embryos tested (TPH)
Red drum (Redfish) (*Sciaenops ocellatus*)	S/A, FT/A	larvae tested (BTEX)	larvae tested (BTEX)	eggs/larvae[e] tested (PAH); larvae tested (PAH)	eggs/larvae[e] tested (PAH); larvae tested (PAH)	eggs/larvae[e] tested (PAH)
Inland silverside (Silverside minnow) (*Menidia beryllina*)	S/A, FT/A	eggs, larvae tested (PAH); eggs, larvae tested (TPH, BTEX)	eggs, larvae tested (PAH); eggs, larvae tested (TPH, BTEX)	eggs, larvae tested (TPH, BTEX)	eggs, larvae tested (TPH, BTEX)	eggs, larvae tested (PAH); eggs, larvae tested (TPH, BTEX)
Spot (*Leiostomus xanthurus*)	S/A	eggs/larvae[e] tested (TPH, BTEX)	eggs/larvae[e] tested (TPH, BTEX)	eggs/larvae[e] tested (TPH, BTEX)	eggs/larvae[e] tested (TPH, BTEX)	eggs/larvae[e] tested (TPH, BTEX)

Table 1. Summary of invertebrate and fish species tested with the water accommodated fractions (WAF) of Western and Central Gulf of Mexico oils, dispersed oils, and dispersant alone (Corexit 9527). Target chemical categories are noted parenthetically, where appropriate (continued).

Species	Test[b]	Western Gulf of Mexico Oil[a]		Central Gulf of Mexico Oil[a]		Dispersant
		WAF[c] of Oil	Oil + Dispersant	WAF[c] of Oil	Oil + Dispersant	
Mysid (*Mysidopsis bahia*)	S/C	larvae tested	Not tested	Not tested	Not tested	Not tested
Atlantic menhaden (*Brevoortia tyrannus*)	S/A	eggs/larvae[e] tested (PAH)	eggs/larvae[e] tested (PAH)	eggs/larvae[e] tested (PAH)	eggs/larvae[e] tested (PAH)	eggs/larvae[e] tested (PAH)

[a] oil characterization of both test oils conducted via GC/MS-SIM in November 1991 (initial) and June 1993 (19-month).
[b] FT/A = flowthrough, acute exposure; S/A = static and/or static renewal, acute exposures; S/C = static renewal, chronic exposure testing; flowthrough acute exposures were considered optimal; static acute testing was used to minimize handling and increase control survival.
[c] water accommodated fraction, prepared per the protocol of Anderson *et al.* (1974).
[d] analytical methodologies used included GC/MS-SIM for PAHs, GC-FID for TPH, and GC/MS for BTEX.
[e] indicates a species-specific egg stage of short duration where larval hatching occurs during the 96-hr acute toxicity test.

Artificial seawater was employed (i.e., Hawaiian Marine Mix [HMM]) as the basic exposure media. Control water was the untreated HMM seawater. The dispersed oil mixture was prepared via hand-shaking one part dispersant to 10 parts oil. Dispersed oil mixtures were mixed and used at concentrations of 100, 50, 25, 12.5, and 6.25 ppm. The WAF was prepared by adding one part oil to nine parts HMM. This mixture was stirred for 24 hrs at room temperature. The WAF was drained from below the oil layer and used at concentrations of 100, 50, 25, 12.5, and 6.25%. In all exposures, temperature, salinity, dissolved oxygen, and pH were measured daily in each concentration. Larval fish were fed rotifers while invertebrates were fed brine shrimp daily during the tests. Feeding in this manner was intended to minimize stress for the animals during particularly sensitive life stages.

Static exposures were conducted in glass fingerbowls. Fish exposures used 5 replicates with 10 individuals per replicate or 10 replicates with 5 individuals each. A separate set of test chambers was established for the sole purpose of collecting samples for chemical analyses. This minimized the need to disturb test animals and to reduce sample volumes during the tests. Test containers were placed in an incubator at 20°C under a daily (i.e., 24-hr) light regime of 16 hrs of light and 8 hrs of darkness.

Flowthrough tests were conducted in a specially-designed acute toxicity test chamber to accommodate the small size of the test animals. The basic test chamber was comprised of five 250-ml glass beakers (i.e., exposure beakers), each of which had two 2.5-cm holes located opposite one another near the bottom of each beaker. Each hole was covered with 350-μm Nitex mesh, allowing for the retention of test animals in each beaker and a free exchange with the surrounding test medium. Exposure beakers were placed in a Plexiglas chamber which held approximately 10 l of test medium. An adjustable powerhead circulated the test medium through a 0.5-in. polyvinyl chloride (PVC) pipe which had been drilled with small holes. These holes were directed towards the sides of the tank, minimizing current energy in the tank but allowing for adequate circulation and mixing. Flowrates were checked before and during tests to maintain consistent exchange rates in the various exposure chambers.

Clean artificial seawater was added at a flowrate of approximately five liters every 24 hrs (i.e., 50% of the volume of the Plexiglas tank). Clean seawater, provided from several saltwater reservoirs, was pumped to a headtank located above the test chambers. From the headtank, water was gravity fed to each test chamber through PVC pipe and controlled through the use and adjustment of needle valves. Flow rates were checked daily and readjusted as necessary to maintain adequate flow. Overflow water drained from each test chamber through a 3-in. hole into a trough where it was collected and treated with a carbon filter before release. Each test chamber was equipped with 1) a submersible heater to control temperature; and 2) overhead lights, controlled by timer, which provided 16 hrs of light and 8 hrs of darkness.

Three separate banks, each holding seven test chambers (i.e., 21 test chambers total), were available for concurrent testing of dispersant, dispersed oil, and WAF mixtures. For the seven test chambers contained within each bank, acute toxicity testing protocols called for the creation of 1) five chambers which contained different concentrations of test media; 2) a control chamber; and 3) a chamber for collecting chemistry samples.

Static renewal exposures were utilized for the chronic toxicity testing phase. Mysids (*Mysidopsis bahia*) were exposed to 20, 10, 5, 2.5, and 1.3% WAF and a control. Exposures included eight replicates containing five individuals each. All chronic exposures were conducted at 26°C under a daily regime of 14 hrs of light and 10 hrs of darkness.

1.2 RESULTS

1.2.1 Oils and Dispersant

Based on results of the initial GC/MS-SIM analyses (i.e., in Month 1), the two oils showed minor chemical differences. The Central Gulf oil exhibited higher concentrations of chrysenes, phenanthrenes, fluorenes, and dibenzothiophenes. Naphthalenes were present in the highest concentrations in both oils (i.e., 420-510 μg/g) and were generally several times greater than the other PAH compounds analyzed. Concentrations for all of the PAH compounds showed a "bell curve" distribution with the C_1-, C_2-, and C_3-methylated compounds exhibiting the highest concentrations.

Concentrations of the hydrocarbons within each whole oil at the end of the study (i.e., in Month 19) revealed several basic differences from the initial analyses. The naphthalenes and fluorenes, for the most part, were higher while the phenanthrenes, dibenzothiophenes, and chrysenes exhibited lower or similar concentrations when compared to the earlier measurements. Within a class of compounds, a "bell curve" distribution was consistent between the two analyses. Differences in concentrations may suggest variability within the testing method rather than being indicative of any real change in the oils over time.

When the dispersant alone was analyzed by GC/MS-SIM, only trace levels of naphthalene (0.16 μg/g) and phenanthrene (0.012 μg/g) were detected.

1.2.2 Chemical Analyses

1.2.2.1 Total Petroleum Hydrocarbons

Average concentrations of TPHs were determined for all acute toxicity tests (i.e., integrated over the 96-hr exposure period). Static tests tended to have the highest overall TPH concentrations with the dispersed oil levels being four to five times greater than that measured in the WAF. Flowthrough concentrations tended to be more variable without the clear distinctions seen in the static tests. The Western Gulf oil had similar average concentrations in the WAF and dispersed oil mixtures. By comparison, the WAF of the Central Gulf oil had much lower TPH concentrations than did the dispersed oil.

The distribution of TPHs in the WAF and dispersed oil exposures over time was determined. The two oils showed similar patterns of hydrocarbon loss and decline over the 96-hr period. This was true for both the WAF and the dispersed oil. In comparing the WAF and the dispersed oil, however, higher loss rates were evident in the dispersed oil mixture. At the end of the 96-hr period, it was noteworthy that the final concentrations of TPH in the dispersed oil mixtures and in the WAF did not markedly differ.

In a comparison of Western and Central Gulf oils, initial TPH concentrations were found to be similar in both flowthrough and static exposure systems. Final concentrations tended to be slightly higher in the static system. With time, the greatest loss of hydrocarbons in the flowthrough system occurred within the first 24 hrs, particularly in the dispersed oil mixture.

1.2.2.2 BTEX Compounds

BTEX concentrations integrated over 96-hr exposures did not differ markedly between the two oils tested. BTEX concentrations in the static system were at least an order of

magnitude higher than in the flowthrough system. Similarly, concentrations in the WAF were an order of magnitude greater than in the dispersed oil mixtures. The range of variation between samples was generally less than 30%. In spite of the order of magnitude differences in concentrations, the majority of the BTEX determinations noted for both the dispersed oil mixtures and the WAF in the flowthrough system were lost within the first 6 hrs, with concentrations close to detection limits within 24 hrs.

1.2.2.3 Polynuclear Aromatic Hydrocarbon Distributions

In the determination of total naphthalenes, fairly similar results were obtained for both Western and Central Gulf oils. Naphthalene concentrations were two to three times higher in the dispersed oil when compared to the WAF. Variability between exposures was generally between 30-50%. Initial concentrations were higher in the dispersed oil compared to the WAF, however, after 96 hrs similar levels had been reached. A considerable amount of the PAHs had been lost within the first 24 hrs, particularly in the dispersed oil mixture. The rate of decrease could not all be accounted for by flow rates in the flowthrough system, suggesting that volatilization was a major contributor to losses during the exposure periods.

Other than the naphthalenes, the hydrocarbons with the highest initial concentrations were dibenzothiophenes, phenanthrenes, and fluorenes, all of which were an order of magnitude less than was measured for the naphthalenes. These hydrocarbons were generally at or near detection limit levels within 24 hrs and mostly absent from the exposures at 96 hrs.

1.2.2.4 Corexit 9527 Test Concentrations

Dispersant concentrations were measured by UV-spectrophotometric methods in a flowthrough test. Beginning with an initial measured concentration of 82 mg/l (100 mg/l nominal), the concentration after the 96-hr exposure was approximately 32 mg/l. The fact that the final concentration was higher than expected (i.e., the final concentration should have been approximately 5 mg/l after 96 hrs) suggests that flow rates were less than estimated or that mixing was not homogeneous. The latter hypothesis is supported by the fact that hydrocarbons in most tests were lost at rates greater than that which could be accounted for solely by dilution and flow rates.

1.2.3 Acute Toxicity Testing

A summary of the LC_{50} determinations (i.e., concentration at which 50% of the test organisms have succumbed after a prescribed period of exposure) for all vertebrate and invertebrate tests is included in **Table 2**. **Table 2** also compares these results based on a toxicity index (TI) which uses ppm- or ppb-hrs as a measure of toxicity. Several TI determinations were calculated using the product of total naphthalenes, TPH, or total BTEX exposure and time of exposure. Total naphthalenes are used for comparison because these have been shown to be the most toxic components of oil (Anderson *et al.* 1974). Given the extent of prior research using TI calculations based on TPH, this index was calculated for comparative purposes. Similarly, TI determinations for BTEX were calculated because of the relatively high BTEX measurements encountered in the exposures. The TI approach, as described by Anderson *et al.* (1984), is based on the premise that toxicity is a function of not only concentration but also duration of exposure.

Table 2. Summary of LC_{50} and Toxicity Index determinations for invertebrate and vertebrate species exposed to the water accommodated fractions (WAF) of Western (W) and Central (C) Gulf oils, dispersed oil mixtures, and dispersant alone (Corexit 9527).

Species	LC_{50}			Toxicity Index for Naphthalenes (ppb-hrs)		Toxicity Index for TPH (ppm-hrs)		Toxicity Index for BTEX (ppb-hrs)	
	WAF W/C (%)	Dispersant (mg/l)	Oil & Dispersant W/C (mg/l dispersant)	WAF W/C	Disp. Oil W/C	WAF W/C	Disp. Oil W/C	WAF W/C	Disp. Oil W/C
Brown shrimp (*Penaeus aztecus*)	59.9/ND[a]	ND/ND	52.7/ND	1,971/ND	4,382/ND	291/ND	222/ND	26,489/ND	1,432/ND
White shrimp (*Penaeus setiferus*)	>100/30.2	11.9/11.9	18.6/13.8	>3,290/457	1,547/692	>486/10	78/147	>44,222/ 14,246	505/678
Blue crab (*Callinectes sapidus*)	>100[b]/70.7	81.2/77.9	90.8/19.8	>3,290/1,070	7,550/992	>486/24	383/210	>44,222/ 33,351	2,467/973
Eastern oyster (*Crassostrea virginica*)	ND/ND	4.9[c]	11.2/4.0[c]	ND/ND	930/200	ND/ND	288/92	ND/ND	304/197
Inland silverside larvae (*Menidia beryllina*)	66.4/59.1	42.5/46.7	59.4/>100	1,974/895	4,689/5,012	326/322	572/>2,288	414,277/ 404,527	13,411/ 29,495
Inland silverside embryos (*Menidia beryllina*)	>100/>100	>100/>100	>100/>100	>3,290/1,514	>8,315/5,012	>544/>572	>2,570/ >2,288	>690,462/ >684,479	25,399/ >29,495
Atlantic menhaden[d] (*Brevoortia tyrannus*)	64.1/42.1	42.4/42.4	22.2/64.6	1,557/507	1,260/2,575	267/163	341/1,014	>283,043/ 178,751	2,639/ 11,101
Spot[d] (*Leiostomus xanthurus*)	>100/70.7	27.4/27.4	68.2/50.3	>2,429/852	3,870/2,005	>417/273	1,046/790	>441,564/ 300,183	10,587/ 8,644
Red drum[d] (*Sciaenops ocellatus*)	>100/74.0	52.6/>100	>100/>100	>2,429/892	>2,429/ >3,986	>417/286	>1,534/ >1,570	>441,564/ 314/194	>15,523/ >17,184

[a] ND = not determined.
[b] 64% survival in 100% concentration.
[c] represents an extrapolated EC_{50} value.
[d] indicates 48-hr test results only.

1.2.3.1 Brown Shrimp (*Penaeus aztecus*)

Brown shrimp were obtained on only one occasion during the course of the study and only in sufficient numbers to allow testing with the Western Gulf WAF and dispersed oil. The TI for total naphthalenes for this species was 1,971 ppb-hrs for the WAF and 4,382 ppb-hrs for the dispersed oil (**Table 2**). TI values for TPH were 291 ppm-hrs for the WAF and 222 ppm-hrs for the dispersed oil. The TI for total BTEX was 26,489 ppb-hrs for the WAF and 1,432 ppb-hrs for the dispersed oil. All of the toxicity in the exposures occurred within the first 24 hrs with little to no toxicity observed in subsequent days, consistent with hydrocarbon patterns where the majority of the materials were lost within the first 24 hrs of exposure.

1.2.3.2 White Shrimp (*Penaeus setiferus*)

White shrimp tests were conducted with both the Western and Central Gulf oils using postlarvae at ages of 15 days (Central Gulf oil) and 22 days (Western Gulf oil). In these tests, the Central Gulf oil produced TI values for total naphthalenes of 457 ppb-hrs for the WAF and 692 ppb-hrs for the dispersant/oil mixture, whereas the Western Gulf oil produced TI values of >3,290 ppb-hrs for the WAF and 1,547 ppb-hrs for the dispersed oil (**Table 2**). TI values for TPH of Central Gulf oil were 10 and 147 ppm-hrs for the WAF and dispersed oil, respectively; TI values for the Western Gulf oil were >486 and 78 ppm-hrs for the WAF and dispersed oil. The TI values for total BTEX of the Central Gulf oil were 14,246 ppb-hrs for the WAF and 678 ppb-hrs for the dispersed oil; by comparison, the Western Gulf oil produced TI values of >44,222 ppb-hrs for the WAF and 505 ppb-hrs for the dispersed oil. The dispersant itself had a measured LC_{50} of 11.9 mg/l (**Table 2**). As with the oil exposures, the majority of the toxicity occurred within the first 24 hrs.

After the 96-hr exposures were completed, surviving shrimp were transferred to clean artificial seawater and grown for an additional 30 days. Weights of the animals at the end of this period were taken. Control weights in the two sets of test were consistent but slightly higher in the Central Gulf oil exposures, suggesting effects of stress. Effects on growth from the exposures themselves were noticeable in the surviving shrimp at the highest concentration in the Western Gulf dispersed oil and to a lesser extent in WAF. No effects were seen on growth in the 50-ppm concentration of the dispersant. These results are consistent with the findings from the acute toxicity testing. In the Central Gulf exposures, reduced growth was noted in the 25-ppm dispersant concentration and in the 100% WAF. Slightly lower (though not significant) weights were observed in the 25-ppm concentration of the dispersed oil. Again, these results closely paralleled the results of the toxicity tests.

1.2.3.3 Blue Crab (*Callinectes sapidus*)

Blue crab exposures were conducted with both Western and Central Gulf oils. Based on results of the dispersant only tests, similar sensitivities to the Corexit 9527 were obtained in the two tests (**Table 2**). Similarly, the results of exposure to the WAFs did not differ greatly. In these tests, the Central Gulf oil produced TI values for total naphthalenes of 1,070 ppb-hrs for the WAF and 992 ppb-hrs for the dispersant/oil mixture, whereas the Western Gulf oil produced TI values of >3,290 ppb-hrs for the WAF and 7,550 ppb-hrs for the dispersed oil (**Table 2**). TI values for TPH of Central Gulf oil were 24 ppm-hrs for the WAF and 210 ppm-hrs for the dispersed oil; TI values for the Western Gulf oil were >486 ppm-hrs for the WAF and 383 ppm-hrs for the dispersed oil. TI values for total BTEX of the Central Gulf oil were 33,351 ppb-hrs for the WAF and 973 ppb-hrs for the dispersed oil; the Western Gulf oil produced

TI values of >44,222 ppb-hrs for the WAF and 2,467 ppb-hrs for the dispersed oil. In both sets of tests, the megalopae larvae were metamorphosing to the crab stage while the test was ongoing. While most of the toxicity in the dispersed oil tests appeared to occur within the first 24 hrs, mortalities in the WAF and dispersant mixtures were most evident after the first 24 hrs. This suggests a greater sensitivity to the dispersed oil based on test results. However, it may also be that the timing of molts was a particularly critical period with regard to the severity of effects and may explain the differences observed between the two oils.

1.2.3.4 Eastern Oyster (*Crassostrea virginica*)

The oysters were exposed in static tests to Corexit 9527 and to the dispersed oil mixtures. No tests were conducted with WAFs due to difficulties in getting animals to spawn. An EC_{50} value (i.e., the median effective concentration) was extrapolated for this species because effects were evident at the lowest exposure concentrations tested. Further, lowest observed effect concentrations (LOEC) and no observed effects concentrations (NOEC) were calculated using the Kruskal-Wallis method. The dispersant was particularly toxic to the embryo/larval stages, resulting in an EC_{50} of less than 6.25 ppm. Similarly, the dispersed oil mixtures had statistically significant effects at the lowest concentrations tested (6.25 ppm). In exposures to the Central Gulf dispersed oil, the EC_{50} was less than 6.25 ppm dispersed oil. The Western Gulf dispersed oil had a lower toxicity than the Central Gulf dispersed oil with an NOEC of 6.25 ppm and an LOEC of 12.5 ppm.

1.2.3.5 Inland Silverside (*Menidia beryllina*)

Tests with inland silverside were conducted under both static and flowthrough exposures. Test data were developed for both embryo and larval exposures (**Table 2**). The numbers of surviving embryos and larvae were similar after 96 hrs in the three different exposure media (i.e., WAF, dispersant, and dispersed oil). The 96-hr toxicity tests were followed by recovery to Day 9, at which time the fewest survivors overall were found in the WAFs and dispersant exposures. When compared to the controls, the 50% and 50 mg/l concentrations showed a similar rate of mortality in the three exposures, suggesting a delayed response. A subsequent flowthrough test confirmed the relative insensitivity of the fish when the exposure was begun in a late embryonic stage. In these tests, equally high hatching rates were measured in all of the exposures. No effects on fry survival were measured in the three exposures at the end of the 96-hr tests. No attempt was made to extend these tests beyond this period. By comparison, a static test exposure had effects in reducing hatching rates in a 75 and 50% WAF, possibly due to the effects of higher exposure concentrations than those observed in flowthrough tests. Tests which had begun with the larvae produced similar results during exposures to Western and Central Gulf oils.

1.2.3.6 Atlantic Menhaden (*Brevoortia tyrannus*)

Atlantic menhaden were obtained on only one occasion during the course of the study. This species was tested as an alternative to gulf menhaden (*B. patronus*), the latter of which was unavailable. Toxicity testing was conducted under static exposure conditions because of the sensitivity of the animals. Test specimens were received as embryos within approximately 36 hrs after their release from females. Tests were initiated with embryos and hatching occurred within the first 24 hrs of the test. Results were obtained in 48-hr exposures because of the significant mortalities which occurred in the controls (**Table 2**). However, the test results provided clear patterns of toxicity during these exposures.

Two oils were tested and produced similar results in a comparison of the WAF and dispersed oil. Only one acceptable test was achieved in the dispersant exposures. The sensitivity of this species was comparable to that observed for spot (*Leiostomus xanthurus*).

1.2.3.7 Spot (*Leiostomus xanthurus*)

Toxicity testing of spot was conducted under similar conditions to those realized for Atlantic menhaden. Spot appeared to be more sensitive to the Central Gulf oil compared to the Western Gulf oil (**Table 2**), with consistently higher TI values evident for the Western Gulf oil. As with Atlantic menhaden, test results were obtained for 48-hr exposures due to significant control mortality. An 80% mortality rate is considered normal for this species during a spawn. Hatching success and fry survival were evaluated in various exposures to Western and Central Gulf oils. In both sets of tests, similar results were obtained, indicating that hatching success was similar between WAFs and dispersed oil exposures and that effects were most evident in only the highest exposure concentrations after 48 hrs. In both cases, 96-hr survival was lowest in the dispersed oil mixtures.

1.2.3.8 Red Drum (*Sciaenops ocellatus*)

Tests with red drum were attempted on numerous occasions during the course of the study, however this species proved to be the most difficult in attempts to obtain acceptable test results. In those tests where acceptable results were obtained for the two oils, this species proved to be relatively insensitive (i.e., high TI values) to the exposures (**Table 2**). Hatching success realized by test organisms exposed to the Western Gulf oil in a flowthrough system was also evaluated. Hatching success was affected only at the highest concentration of the dispersed oil and in the higher concentrations of the dispersant. The WAF did not appear to significantly affect hatching success.

1.2.4 Chronic Toxicity Testing with Mysid (*Mysidopsis bahia*)

The WAF at the highest concentration (i.e., 20%) was not toxic to the animals at 48 hrs, however, after seven days an LC_{50} of 17.9% WAF was measured. The NOEC for survival after seven days was 5% WAF. However, growth showed an NOEC of <1.25% WAF. The fecundity NOEC was 1.25% WAF.

1.3 DISCUSSION

1.3.1 Oil Characterization

Replication between the various exposures was good, particularly with regard to hydrocarbon exposures. This finding is particularly noteworthy in comparisons between various flowthrough tests, where total naphthalenes for both the Western and Central oils were approximately three times that of the WAF. Because a complete characterization of hydrocarbons in test media could not be accomplished for every toxicity test, this finding is quite important as it allows one to extrapolate between tests. Further, if it is assumed that PAHs represent the toxic components of the oils tested, these data can also be used to support the hypothesis that the toxicity attributed to dispersed oil should have been three times greater than that attributed to the WAF. Agreement in these ratios was also evident for TPH and BTEX concentrations. This is further evidence for the consistency of test exposures and comparability of associated toxicity test data.

Agreement in the static exposures was less evident, suggesting that greater variability is likely in toxicity results originating from this exposure method. This variability could be a function of the ways that tests were conducted and the difficulty in reproducing an oyster embryo/larval test in such a way as to be comparable to a fish exposure (i.e., where different types of test containers may be used). Similarly, variability between test results may also reflect differences in the solubility and rates of volatilization of the TPH fraction as opposed to the BTEX compounds, the latter of which tend to be more uniformly soluble as well as volatile.

With only minor exception, it was possible to obtain fairly constant exposures between the flowthrough and static treatments, regardless of whether the test medium was dispersed oil or the WAF. Anomalous results were obtained, however, for the TPHs in the Western Gulf dispersed oil and the WAF of the Central Gulf oil. The ratios of the dispersed oil were approximately half that of the WAF when the flowthrough and static systems were compared. This may suggest that the overall volatility of the dispersed oil is greater than that of the WAF. This could also be suggested by the fact that BTEX were found at much higher concentrations in the WAF than in the dispersed oil mixtures.

1.3.2 Acute Toxicity Characterizations

The chemical characterizations may explain many of the anomalies observed in the toxicity data. Much of the variability that was seen in the fish tests may have been attributed to the fact that most of these tests were run under static conditions, where some of the greatest variability in TPH concentrations were obtained. By comparison, the TPH, naphthalenes, and BTEX concentrations were relatively uniform in flowthrough tests where most of the invertebrate exposures were completed.

Another source of variability in the tests may arise from the organisms themselves. A significant problem encountered during the study was the difficulty in achieving good control and animal survival during the exposures. This was particularly true for the larval fish, where an 80% control survival was nearly impossible to attain. Problems with control survival were not unique to this study; naturally high mortality is characteristic of several of these species.

Because the goal of this study effort was to use the youngest animals possible, better results might have been obtained with slightly older organisms. For example, the EPA protocols for *Menidia*, a commonly used aquatic toxicity test species, recommend the use of nine-day-old fish for testing. Prior to this age, the fish are difficult to keep alive and to transition through feeding stages. This project experienced difficulty in keeping newborn *Menidia* alive in tests although the eggs were generally insensitive. If such species are to be used in the future, test protocols should consider the use of older animals which have already gone through life stages where mortality is naturally high.

The invertebrates were less sensitive than the fish to such handling and generally provided acceptable control results. However, it was difficult to duplicate invertebrate results which may suggest a wide variation in sensitivity in a population. It was also difficult to obtain these animals. As a consequence, it was not possible to complete replicate tests which could narrow statistical limits.

Levels of total naphthalenes were typically three times higher in the dispersed oil exposures than in the WAF. This would suggest that the dispersed oil should also be three times as toxic as the WAF, assuming that napthalenes are the primary cause of toxicity. However, when the TI ratios are compared for the dispersed oil versus the WAF, a much

different picture emerges. On the basis of the TI determinations, dispersed oil toxicity was approximately 20 to 70% that of the WAF. An obvious question arises: Do these results suggest that the dispersed oil is less toxic than the WAF? The answer is no, as in many of the tests, a higher toxicity was frequently measured in the dispersed oil exposures compared to the WAF. However, on the basis of the measured hydrocarbon exposures, it would appear that the dispersed oil is proportionally less toxic than the WAF.

One explanation for this apparent anomaly in the measured toxicities could be related to the actions of the oil following treatment with the dispersant. The term "water accommodated fraction" or WAF has been utilized to account for the combination of small droplets and dissolved hydrocarbons that are found in the exposure medium. Similarly, when the oil is dispersed, a large proportion of this oil is in the form of small droplets and/or emulsions. Given the size of the animals used in the tests, it is likely that such particles would not be available for ingestion by the animals and would eventually be lost to the system. Similarly, in the closed and semi-enclosed systems used in these tests, solubility limits could have been reached so that the dissolved hydrocarbon concentrations would have been similar in both the WAF and dispersed oil exposures.

Another possible explanation for the differences observed may involve the rate at which the hydrocarbons were lost from the system. As seen with the dispersed oil mixtures, BTEX hydrocarbons were initially present in much lower concentrations than in the WAF. Anderson *et al.* (1981) has previously reported a similar effect in dispersed oil exposures. The loss of these hydrocarbons almost immediately from the system can probably be accounted for by an "explosive-like" volatilization. This effect may arise due to the increased surface area that would result when oil droplets form following dispersion. However, that would assume that these droplets must come into contact with the atmosphere where BTEX compounds can escape. Given the rate at which this would need to occur to account for the rapid loss, it is likely that the methods used to mix the dispersed oil may have had an influence on the rates of volatilization of BTEX. Shaking, as was used in the present set of tests, would not likely occur in the environment.

The rate of loss of hydrocarbons from the dispersed oil exposures was greater than could be accounted for solely by flow rates. This also indicates volatilization was an important factor in the fate of the hydrocarbons. It was also apparent that most of the toxicity in the dispersed oil exposures occurred within the first 24 hrs. If the total period during which toxic levels are present in the dispersed oil medium is shorter than with the WAF, a proportionately lower toxicity could be expected.

Comparisons of toxicity index values produced interesting results. When TPH TI ratios were compared, considerable variability was evident, suggesting that TPH ratios alone could not adequately explain the toxicity.

BTEX in the dispersed oil mixtures resulted in <1-8% of the toxicity evident in the WAF. By comparison, BTEX levels in the dispersed oil mixtures were 4-17% of their concentrations in the WAF. This might suggest that the observed toxicity in WAF exposures was due largely to effects from the BTEX levels rather than from the naphthalenes or TPH.

Identification of BTEX as the primary contributor to toxicity in these tests is consistent with the rapid loss of these compounds from test media and the fact that most of the toxicity occurred within the first 24 hrs of the test. This conclusion has implications with regard to toxic effects following oil spills and the use of dispersants. The data suggest that toxicity to

the larval animals may occur very rapidly. It also suggests that the WAF may be as toxic as the dispersed oil because of the difference in BTEX levels that are attained in the WAF versus the dispersed oil mixture. Whether the necessary conditions to produce toxicity in an actual spill condition can be attained (i.e., sufficiently high exposure for the needed duration) will probably vary with environmental conditions at the time of a spill.

An important and historically-consistent finding in these tests was the lower sensitivity of the embryonic stages compared to the early larval stages. The investigations of Sharp *et al.* (1979) indicated that the timing of exposure was significant in influencing toxicity. The most sensitive period seemed to coincide with a period of organogenesis within the embryo. Similarly, Fisher and Foss (1993) found a correlation between toxicity and the stage of egg development in the grass shrimp, *Palaemonetes pugio*. The embryos of spot, Atlantic menhaden, and red drum were equally insensitive to the hydrocarbon exposures. These fish had short incubation periods of between 24 and 48 hrs. These embryos generally reached the laboratory when they were approximately 24 to 30 hrs old. Therefore, exposures probably did not begin until after the most sensitive periods for the embryos had passed. Somewhat greater sensitivity may have been obtained if the embryos had been immediately exposed to the hydrocarbons upon release from the females.

The overall sensitivity of the fish versus invertebrates appears to be similar. The invertebrates performed better as test organisms, as overall survival in controls was better. The naturally high mortality of the fish larvae compounded efforts to obtain acceptable test results and necessitated repeating several of the tests many times. The invertebrate tests, however, were generally accomplished with good control survival and results.

A question that has often arisen with regard to this type of testing is the applicability of test results between geographic regions and species. McAuliffe (1987) has compared the relative sensitivities of marine fish and invertebrates using ppm-hrs (i.e., TI). Many of the species compared by McAuliffe (1987) are from colder environments than those used in the present study. Nevertheless, the results reported by McAuliffe (1987) for both the fish and invertebrates are variable but within the ranges found in this investigation.

In other acute toxicity tests conducted on a tropical Australian shrimp species (*Penaeus monodon*) and a penaeid shrimp cultured in south Texas (*P. vannemai*), LC_{50} determinations for exposures to Corexit 9527 ranged from 35 to 45 ppm for both species (The SeaCrest Group 1993). This is less toxic than the approximately 12 ppm determined for white shrimp (*P. setiferus*) in the present study but is probably within the ranges of variability observed in these types of tests.

Finally, it was an objective of this study to compare the results of the embryo-larval testing with those obtained by Shuba and Heikamp (1989) for juvenile and adult fish and invertebrates of the same species. Comparisons of the results of Shuba and Heikamp (1989) with those realized during the present study indicated general consistency. As was evident in this study, toxicity occurred rapidly within the tests (i.e., within 24 hrs, the lowest LC_{50} value had been reached and did not differ markedly from the 96-hr LC_{50} values). This was consistent with the chemical analyses of Shuba and Heikamp (1989) in which the hydrocarbon concentrations had largely been lost from the system within the first 24 hrs. Overall, the trends appeared to be consistent between the two studies and suggest that the juvenile and adult invertebrates were only slightly less sensitive than the larval organisms. Comparable fish data were available only for the red drum (redfish); in this case, no clear distinctions were possible although this species

appeared to be more sensitive than the invertebrates tested. These results are largely consistent with the existing literature.

There are some obvious difficulties evident when comparing the data of Shuba and Heikamp (1989) with the current study results. Chief among these are the different oil types used in the exposures, as well as different methodologies for measuring the hydrocarbon concentrations during exposures. Shuba and Heikamp (1989) note that the methodologies used to measure the PAHs caused problems in obtaining satisfactory results in their analyses. This was evident in their test results which showed inconsistency between exposures.

By comparison, the methodologies used in the present set of tests gave fairly consistent results in terms of initial exposure concentrations. The only exception to this trend was evident in several of the TPH determinations from static test exposures.

A considerable effort has been expended over the years in evaluating the effects of oil and dispersed oil on marine organisms. Yet, the question still arises as to the value of these tests in making decisions concerning the use of dispersants during actual spill conditions. In all cases, such decisions regarding the use or non-use of dispersants will come down to a tradeoff between the natural resource damages that occur by dispersing oil versus oil-associated impacts to sensitive coastal and shoreline habitats. It can certainly be asserted that dispersed oil would have more significant impacts during periods of the year when larval fish and invertebrate stages are present in the water column. However, the high natural mortalities experienced by many of these species may overshadow any effects from exposure to dispersed oil.

There can be little question that dispersed oil can have effects on larval organisms given significant exposure. However, attempts to measure actual effects on larval organisms in the field following oil spills and dispersant usage is difficult if not impossible to obtain in a scientifically valid manner. While one can project toxic levels of oil from laboratory data and chemical concentrations can be measured in the field, it is much more difficult to get accurate pictures of larval distributions. This is because of the patchy distributions that are generally present and the need for multiple samples to obtain statistically valid numbers.

The present study effort extends our quantification of the effects of oil and dispersed oil on marine organisms. Data acquired do not differ appreciably from those previously obtained in other dispersed oil effects studies. In essence, results from this effort support the contention that dispersed oil can have effects on larval marine organisms. Data indicate that the toxicity occurred rapidly and that complete, 96-hr exposures were not required to induce lethal effects. The possible contribution of BTEX to total toxicity was a surprise given the fact that it is rapidly lost from the system. However, the dispersed oil was not markedly more toxic than the undispersed oil. While this may be an unexpected result in some regards, it would appear that an explanation based on the fate of hydrocarbons in the exposure system can account for the observations.

The TI determinations were useful in explaining these effects and should be considered in future field programs as a means to provide comparative data with laboratory investigations. Singer *et al.* (1990) measured toxicity of Corexit 9527 to the early life stages of four marine species. Concentrations of Corexit producing LC_{50}s were similar to those reported in this study. However, these authors also concluded that the use of a Toxicity Index may not provide truly comparable values across species. They suggested that this may be due to the fact that the index may overlook complex physiological and biochemical processes. However, our data suggest that the index should be calculated on the basis of the concentrations of the

causative agent in a product. For instance, BTEX may have been a major contributor to toxicity in the test animals. A TI based on naphthalene or TPH concentrations alone could not wholly account for the observed toxicity. Standardization of the TI approach (i.e., use of total naphthalenes, TPH, total BTEX, etc.) would allow more comparative evaluations between species and geographic regions where oil effects are measured.

The present study effort provided repeatable testing conditions from which credible toxicity data were produced. More importantly, this study provided toxicity information for previously untested early life stages and underscored the difficulties inherent in the use of eggs and larvae in acute toxicity testing.

CHAPTER 2 - INTRODUCTION

2.1 HISTORICAL PERSPECTIVE

2.1.1 Fate and Effects of Oil and Dispersed Oil

Testing on the fate and effects of oil and dispersed oil on the marine environment can be traced back to the early 1960's. However, it was the 1968 *TORREY CANYON* spill and the 1969 Santa Barbara blowout which truly spurred activities along these lines of investigation. Much of the early research focused on arctic and subarctic marine organisms because of an emphasis on Alaskan and Canadian oil exploration. Malins (1977) produced a volume which summarized the available information to date. Other comprehensive reviews on the effects of oil in the marine environment have been developed by Neff (1979) and the National Research Council (NRC 1985). The effects of dispersed oil have also been summarized in NRC (1989).

Throughout the 1970's, the largest body of information on Gulf of Mexico species and the effects of oil were being generated at Texas A&M University in the laboratory of Anderson and Neff. The information generated during this period continues to be the most comprehensive for Gulf of Mexico species. One of the most frequently quoted articles in oil spill research over the last two decades has been Anderson *et al.* (1974). The significance of this work was that it identified the low molecular weight hydrocarbons, particularly the aromatic hydrocarbons, as the primary contributors to toxicity from oil. It also described a method by which a water soluble fraction (WSF) of the oil could be prepared for testing the effects of oil. The term "water accommodated fraction" (WAF) has also been employed by numerous researchers (e.g., Johns and Pechenik 1980; Bokn *et al.* 1993) to reflect not only those hydrocarbon fractions which are soluble in water, but also the microscopic droplets found in suspension in water.

The complexity and physical behavior of petroleum contribute to the difficulty in designing laboratory studies which can simulate the real-world effects of oil in the environment (Neff 1989). Over the years, numerous approaches have been used to conduct oil exposures in the laboratory including the introduction of oil as 1) a WSF; 2) an oil-in-water dispersion; 3) a surface slick; 4) chemically-dispersed oil; 5) oil-contaminated food; and 6) oil-contaminated sediments. The current study effort requires a comparison of toxicity associated with oil and dispersed oil. The NRC (1989) concluded that the optimum method for comparing oil alone versus dispersed oil effects was to use a WSF of the whole oil in concert with a chemically-dispersed oil mixture.

One of the biggest concerns in conducting laboratory studies is to simulate as closely as possible concentrations of oil and dispersed oil which will occur in a real spill situation. The 1989 Oil and Dispersant Toxicity Testing Workshop, sponsored by the MMS, included two findings: 1) tests need to be consistent with the manner in which organisms are exposed in the environment; and 2) tests should include concentrations of dispersant, oil, and dispersed oil that would be found in the environment (Duke and Petrazzuolo 1989).

With regard to these issues, various approaches have been used for toxicity testing in the laboratory. These have included static bioassays in which a one time exposure is provided and the oil or dispersed oil is allowed to degrade over the course of the test. This generally simulates an actual spill event but does not allow for dilution effects. This methodology was frequently used in early oil toxicity testing but has realized less frequent use in

recent times. One of the biggest complaints with this test has been that it does not allow for a good assessment of exposure concentrations over time.

A second method of testing involves a static renewal system in which exposure medium is renewed every 24 hrs during a test. This is a commonly used approach in current toxicity testing and one that appears to be well accepted by the regulatory agencies. This method produces an exposure which is a cross between the static test and flowthrough test. While the goal of this procedure is to produce a constant level of exposure, in reality it allows contaminant concentrations to peak at every water renewal.

Most flowthrough exposures have been designed to maintain a contaminant at a constant level. At low concentrations, this is consistent with a chronic exposure but is the least realistic in terms of simulating a real world spill scenario. However, for purposes of calculating an LC_{50} or EC_{50} value, it will provide the most accurate estimate. (LC_{50} is defined as the lethal concentration for 50% of the test organisms; EC_{50} is defined as the median effective concentration.)

Anderson *et al.* (1984) investigated the issue of using diluting concentrations versus constant levels for exposing marine organisms to dispersed oil. In this test, dispersed oil was delivered to produce diluting concentrations that reached zero in 8 or 24 hrs. The tests employed a shrimp (*Pandalus danae*) and the Pacific sand lance (*Ammodytes hexapterus*). A toxicity index (TI) measured in ppm-days was used to compare different species and exposure conditions which included constant concentrations.

Close agreement was found between the toxicity indices of shrimp exposed to constant concentrations of dispersed oil and those exposed to diluting concentrations. The authors concluded that it was not necessary to conduct diluting exposures to assess the sensitivity of an organism to a specific oil or dispersant.

The relative toxicities of an oil, a dispersant, or dispersed oil can be determined using LC_{50} values. Controversy over the use of this measure and its applicability to real world prediction of impacts has always been present. The NRC (1989) has stated that laboratory tests are poor simulations of natural conditions because they are conducted under standard, controlled conditions. While the laboratory tests involve exposing animals over four days to more or less constant concentrations, ocean exposures would involve progressive and generally rapid dilution. Nevertheless, these shortcomings do not devalue the importance of such toxicity tests.

Responsible agencies are being increasingly faced with decisions on how to respond to oil spills. It is clear that most mechanical methods of cleanup range from ineffective to minimally effective at best. On massive spills, booms and skimmers can cover only limited areas. By comparison, aircraft can be used to treat large areas of a spill with dispersants. However, a decision to use dispersants can result in tradeoffs that must weigh the amount of oil which will be dispersed into the water column where it can impact pelagic organisms. In a commercially-important fishery area, such decisions can have environmental, economic, and political implications. Therefore, it is important to be able to compare the relative risks involved with decisions related to dispersant usage. That is where measures such as an EC_{50} or an LC_{50} value can be valuable.

The study effort and results outlined in the following report provide a comparative measure of the potential toxicity associated with dispersed and nondispersed oil to various

commercial species. Such data could be utilized in current spill modeling exercises (e.g., see Trudel *et al.* 1989) to provide a decision-making tool on whether to disperse or not disperse during a spill. At the same time, it will enhance the decision-making process by establishing the concentrations that will be required to produce mortalities to these species.

The species which have been selected for testing are those which have some of the highest commercial value not only for the Gulf of Mexico but for the rest of the U.S. as well. All of the proposed species, except for the blue crab (*Callinectes sapidus*), were recommended for testing of various life stages (Duke and Petrazzuolo 1989). Yet, it is noteworthy that few of these species have seen extensive laboratory testing with regard to the effects of oil. Some of this is undoubtedly related to the difficulty of maintaining some of these species in the laboratory. For example, experience has demonstrated the difficulty in maintaining blue crab larvae in the laboratory. Costlow (1979) studied only the megalops stage in his investigations with Dimilin because of the high variability in survival during the 7 to 8 zoeal stages. Costlow and Clare (1989) recommended using *Rithropanopeus harrisii* as a surrogate species for dispersed oil studies. Neff (personal communication 1993) has reported on his difficulties in using redfish (red drum) eggs and larvae for toxicity testing. Similar difficulties in keeping menhaden alive for tests has also been noted (W. Hettler, National Marine Fisheries Service [NMFS], Beaufort, NC, personal communication 1993).

While no studies appear to have been completed using the early life stages of Gulf of Mexico crabs and dispersed oil, there is existing information on the effects of oil on at least two species. *R. harrisii* was favored by Laughlin and Neff (1980) and Laughlin *et al.* (1978) for their studies to measure the lethal and sublethal effects of oil on survival, respiration, growth, and development rate. Neff *et al.* (1976) also studied the effects of oil on *R. harrisii* and found the larvae to be more sensitive than those of the horseshoe crab (*Limulus polyphemus*). Laughlin and Neff (1977) described the interactive effects of temperature, salinity, and chronic exposure to No. 2 fuel oil on survival, development rate, and respiration of *Limulus*. Lee *et al.* (1977) investigated the metabolism of petroleum hydrocarbons in adult blue crabs.

A number of studies have been conducted on the effects of oil and dispersed oil on shrimp. White and brown shrimp (*Penaeus setiferus* and *P. aztecus*) were studied extensively in acute and chronic studies with oil in Anderson and Neff's laboratory at Texas A&M University and the results reported in Cox (1974), Anderson *et al.* (1974), and Neff *et al.* (1976). Edwards (1978) investigated the effects of WSFs of oil on metabolism and growth in the shrimp *Crangon crangon*. These studies concentrated on the juvenile and adult stages of these organisms. Broderson *et al.* (1977) focused their studies on the effects of WSFs of Cook Inlet crude to larval and adult stages of Alaskan shrimp and crabs. Shuba and Heikamp (1989) conducted acute studies in which they compared the effects of oil and dispersed oil on the penaeid species but the studies were again limited to adult organisms. Anderson *et al.* (1981, 1984, 1987) used the Pacific coonstripe shrimp (*Pandalus danae*) in their studies with dispersed oil and a WSF of Prudhoe Bay crude oil; however, only adult organisms were tested.

Mackin and Hopkins (1961) conducted some of the earliest field studies which looked at the effects of oil on the oyster *Crassostrea virginica*. Anderson and Anderson (1975) compared the effects of salinity and oil on the chloride and osmotic regulation of this species. Lund (1957), Chipman and Galtsoff (1949), and Stegeman and Teal (1973) all investigated the effects of oil on the filtration and feeding response of the oyster. Renzoni (1975) looked at the effects of oil to the sperm, eggs, and larvae of three bivalve species including *C. virginica*. His earlier work (Renzoni 1973) with dispersants and oil had found that fertilization was more

sensitive to oil than early larval development of *C. angulata* and *Mytilus galloprovincialis*. Legore (1974) showed oil to affect the early development of the larvae of *C. gigas*.

Eggs and larvae of fish as well as the adults have been used to investigate the effects of petroleum products. Kuhnhold (1969) and Kuhnhold (1970) examined the acute effects of oil on the eggs and larvae of cod (*Gadus morhua*) and herring (*Clupea harengus*). Craddock (1977) summarized data on the acute effects from oil on eggs and larvae and found that the range of toxicities for crude oils ranged from 0.1 to 100 ppm. For soluble hydrocarbons, the range was 0.1 to 1.0 ppm; for a No. 2 fuel oil, toxicities ranged from 0.1 to 4.0 ppm.

Linden (1974) and Wilson (1976) found that fish embryos were less sensitive than the larvae to oil. Wilson (1977) and Lonning and Falk-Petersen (1978) reported that the transition from yolk sac to feeding is a particularly sensitive time for exposures. Sharp *et al.* (1979) exposed eggs of *Fundulus heteroclitus* to WSFs of No. 2 fuel oil and found that survival was influenced by the timing of exposure in relation to the stage of development.

The NRC (1989) concluded that a generalized hazard assessment for dispersed oil could not be made for ichthyoplankton without additional research on the topic. Only one recorded study was available which compared measured concentrations of oil and dispersed oil (Borseth *et al.* 1986). This study used eggs of plaice (*Pleuronectes platessa*) exposed to a WSF of oil and an oil dispersed with Finasol OSR-5. Other studies on the effects of dispersed oil on eggs and larvae of fish have been conducted but only reported the nominal concentrations of the oil (Linden 1975, 1976; Mori *et al.* 1983, 1984).

2.1.2 Dispersant Research

The first use of dispersants to control an oil spill occurred during the *TORREY CANYON* spill in 1968. These first generation dispersants proved to be inherently more toxic than the spilled oil and resulted in a general reluctance to accept dispersants as a viable alternative for treating oil spills throughout the 1970's. Over the years since the *TORREY CANYON* spill, however, dispersant technology has improved to the point where the products do not exhibit the toxicity of the early generation dispersants.

Nevertheless, through the 1980's, dispersant research and testing in the U.S. lagged behind that of the Canadians who were interested in the use of dispersants in arctic regions. The laboratories of Mackay and Wells have generated, by far, the bulk of our knowledge on the fate and effects of dispersed oil (e.g., Mackay *et al.* 1982, Wells *et al.* 1982, 1985, Peakall *et al.* 1985, 1987, Abernethy *et al.* 1986). As a result, much of the existing knowledge on the effects of dispersant usage in the marine environment has been done using mostly species from colder climes. Tropical and temperate studies were conducted by researchers such as Loya in Eilat, Israel and Bak and Elgershuizen from the Netherlands Antilles using second and third generation dispersants.

The current focus within dispersant research appears to be on dispersant effectiveness, with a limited emphasis on dispersant toxicity. By understanding how the variables (which control dispersant effectiveness) function, overall effectiveness may be improved. For the past several years, studies designed to characterize the mechanisms by which dispersants function have been undertaken by various researchers. One cooperative government effort has been supported jointly by the U.S. Department of the Interior (MMS) and Environment Canada, as summarized in Fingas *et al.* (1990) and Fingas and Tennyson (1991).

Technical seminars and oil spill conferences have also been utilized extensively for more than a decade as a forum for researchers to summarize the results of dispersant effectiveness and toxicity tests (e.g., Mackay *et al.* 1983; Nes and Norland 1983; Fingas 1988; Brown and Goodman 1988; Fingas *et al.* 1990).

In the early 1980's, the American Petroleum Institute funded fate studies of dispersed oil off California under the direction of McAuliffe. Another field program was undertaken at about the same time by Gilfillan and Page who have published extensively on the results of their tests off Maine (e.g., Gilfillan *et al.* 1985). An Exxon subsidiary funded coral and dispersant studies in the Middle East during this period. Since then, attempts to conduct field experiments in this country have been mostly discouraged. However, U.S. researchers were funded by the MMS to conduct studies in Panama in the late 1980's which looked at seagrass, mangrove, and coral environments following a refinery spill (e.g., Burns and Knap 1988; Garrity and Levings 1990, 1993; Burns and Yelle-Simmons 1994; Burns *et al.* 1994; Garrity *et al.* 1994; Levings *et al.* 1994).

Because of the lack of acceptance of dispersants as a viable treatment alternative during a spill, funding for laboratory studies over this time period has been lacking. Anderson received funding for toxicity studies during the early to mid-1980's. Shuba and Heikamp (1989) have described the results of their studies on five Gulf species as part of the work done for the LOOP (Louisiana Offshore Oil Port) site. More recently, a joint effort between Exxon Biomedical Science Inc. (EBSI), the Marine Spill Response Corporation (MSRC), and the University of California, Santa Cruz evaluated various aspects of dispersant and dispersed oil toxicity. This ongoing program has several objectives, including: 1) evaluation of a flowthrough toxicity testing system developed by the University of California, Santa Cruz for sensitive, early life stages; 2) determination of the repeatability of acute LC_{50} tests completed in different laboratories; and 3) evaluation of differences between test results of dispersant toxicity tests conducted using more conventional methods and test species. To date, final LC_{50} results are available for continuous 96-hr acute toxicity exposures (i.e., via ASTM and flowthrough protocols) to several test organisms; species tested included *Menidia beryllina* (inland silverside) and *Mysidopsis bahia* (mysid) (Pace and Clark 1994). In related dispersant toxicity research, Singer *et al.* (1990) evaluated the toxicity of an oil dispersant to the early life stages of four California marine species. Species and lifestages tested included zoospores of giant kelp (*Macrocystis pyrifera*), embryos of red abalone (*Haliotis rufescens*), juveniles of the mysid (*Holmesimysis costata*), and larvae of the topsmelt (*Atherinops affinis*). Toxicity tests using most or all of these species and several dispersants have also been summarized by Singer *et al.* (1991, 1993). Abalone embryos revealed the greatest sensitivity to dispersant exposure, while the mysid and topsmelt showed intermediate to low sensitivity.

With large oil spills in U.S. waters in recent years, the lack of effective and suitable alternative control strategies has opened up a greater acceptance for the possible use of dispersants in coastal and offshore waters. This led to the publication in 1987 by the American Society for Testing and Materials (ASTM) of dispersant use guidelines and subsequent modeling efforts which evaluated dispersant usage (e.g., S.L. Ross Model for dispersant usage in the Gulf of Mexico).

Some information needs still exist, however, with regard to decision-making for the use of dispersants in the waters of the Gulf of Mexico. For this reason, the U.S. Department of the Interior (USDOI), Minerals Management Service (MMS) funded a study to develop additional data on the effects of dispersed oil on early life stages of seven important commercial and recreational species from the Gulf of Mexico. Entitled "Dispersed Oil Toxicity Tests with Biological Species Indigenous to the Gulf of Mexico," the 33-month study effort was completed

by Continental Shelf Associates, Inc. (CSA) of Jupiter, FL and Ventura, CA, with the support of The SeaCrest Group and VISTA Laboratories, Inc., of Broomfield, CO. Expert technical support was also provided by Dr. Jack Anderson of Columbia Aquatic Sciences and Dr. Jerry Neff of Battelle Ocean Sciences.

2.2 STUDY OBJECTIVES

The objectives of the current study include:

- To expose the eggs and/or larvae of seven Gulf of Mexico marine fish and shellfish species to 1) the WAFs of two different Gulf of Mexico oils; 2) the dispersed oil mixtures of the same oils; and 3) a single dispersant. All exposures were to be conducted in controlled, flowthrough or static 96-hr acute toxicity tests.

- To characterize the chemical composition of the test oils and exposure media (i.e., WAF; dispersed oil) during various phases of acute toxicity testing.

- To summarize the results of acute toxicity testing and parallel chemical analyses of exposure media on each of the seven commercially-important fish and shellfish species using gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS), GC/MS with selected ion monitoring (GC/MS-SIM), and infrared (IR) spectroscopy.

In pursuit of these objectives, it was as desirable to develop appropriate flowthrough exposure methodologies which were reproducible, taking into consideration the results of recent study efforts (e.g., Shuba and Heikamp 1989).

The following sections summarize the results of static and flowthrough aquatic acute toxicity testing protocols utilized on egg and/or larval stages of seven commercially-important invertebrate and fish species. Species evaluated included blue crab (*Callinectes sapidus*), eastern oyster (*Crassostrea virginica*), brown shrimp (*Penaeus aztecus*), white shrimp (*Penaeus setiferus*), red drum (aka redfish, *Sciaenops ocellatus*), inland silverside (aka silverside minnow, *Menidia beryllina*), and spot (*Leiostomus xanthurus*). Unsuccessful attempts were made to secure eggs and larvae of an eighth commercially-important fish species, gulf menhaden (*Brevoortia patronus*); in lieu of tests on this species, acute toxicity testing was conducted on a congener, Atlantic menhaden (*Brevoortia tyrannus*), while chronic toxicity testing was undertaken on mysids (*Mysidopsis bahia*).

Two test oils were acquired from the western and central Gulf of Mexico outer continental shelf (OCS), respectively, while the dispersant tested was Corexit 9527. Oils chosen were commingled crude oils found in two major Gulf of Mexico pipeline systems. Oil samples were taken at a shore terminal for the Central Gulf oil and at a final offshore pumping station for the Western Gulf oil. The two pipeline systems from which representative oils were drawn carry oil from a large number of producing wells spread over large areas of the Gulf. Commingled oils were chosen rather than crude oils from individual fields because the risk of spills occurring from pipeline systems in recent years has been much greater than the risk of spills occurring from OCS production or drilling facilities. There have been no spills greater than 1,000 bbl from OCS production or drilling operations since 1980. Furthermore, the risk of contact with coastal resources is much greater for pipeline systems that eventually make landfall.

The two commingled oils were also characterized in the MMS report "Adaptation of the MMS Oil-Weathering Model for Use in the Gulf of Mexico Region," in detail (Kirstein 1992). Weathering results for these two oils were provided. The current study effort was completed so that, if a real spill from either pipeline system were to occur, spill response efforts would have information both on the effects of dispersant usage and on the weathering characteristics of each crude oil mixture.

Multiple chemical analyses were conducted on both the WAF and dispersed oil mixtures at various phases of biomonitoring to characterize the chemical composition of the exposure medium, as well as to document the chemical changes which were expected to occur over the course of each test. Chemical components determined included total petroleum hydrocarbons (TPH), purgeable aromatic hydrocarbons (e.g., benzene, toluene, ethylbenzene, and xylenes, or BTEX), and polynuclear aromatic hydrocarbons (PAH).

Data derived from this study should be useful in several ways, including serving as reference material in the preparation of environmental impact statements for offshore oil and gas leasing and development and for review of oil spill contingency plans. These data will also be useful in assessing the potential damage associated with the use of dispersants. Finally, study results will provide a sound basis for comparison with past and future acute toxicity testing efforts conducted using egg and larval stages of marine species.

CHAPTER 3 - MATERIALS AND METHODS

3.1 ACUTE TOXICITY TESTING

All acute toxicity tests were conducted for 96 hrs using two separate oil samples representative of Western and Central Gulf of Mexico oil production. Tests were conducted using a WAF, a dispersed oil mixture, and the dispersant (Corexit 9527) alone. Representative oils were obtained through a coordinated effort between the USDOI, MMS, Gulf of Mexico OCS Region and industry. The Corexit 9527 was provided by Exxon Corporation. Artificial seawater (i.e., Hawaiian Marine Mix [HMM]) was employed as the basic exposure medium. Subsequent dilutions were then made to expose the test organisms at the appropriate concentrations (i.e., 100, 50, 25, 12.5, and 6.25 ppm dispersed oil or 100, 50, 25, 12.5, and 6.25% WAF). Control water was the untreated HMM seawater.

The WAF was prepared by adding one part oil to nine parts HMM. This mixture was slowly stirred on a magnetic stirplate for 24 hrs at room temperature. The WAF was drained from below the oil layer after allowing the mixture to stand for one hour; the WAF was used at concentrations of 100, 50, 25, 12.5, and 6.25%.

The dispersed oil mixture was prepared by hand-shaking (i.e., moderate agitation) one part dispersant to 10 parts oil to obtain a cloudy mixture. This mixture was then weighed to produce a concentration of 100 ppm dispersed oil when added to the HMM seawater in the exposure chambers. Gently mixing of the mixture in the flowthrough chambers was provided by the powerheads which circulated the water. Tests were run at concentrations of 100, 50, 25, 12.5, and 6.25 ppm dispersed oil.

In all exposures, temperature, salinity, dissolved oxygen, and pH were measured daily in each concentration. Test organisms were provided either by commercial suppliers or academicians and regulatory agencies conducting research. Larval fish were fed rotifers while invertebrates were fed brine shrimp daily during the tests. Feeding in this manner was intended to minimize stress for the animals during particularly sensitive life stages.

3.1.1 Testing Protocol by Species

The testing for all species, with the exception of oysters (*Crassostrea virginica*), followed generalized procedures as described in Peltier and Weber (1985). The oyster embryo/larval test followed ASTM Method E 724-89. Tests on shrimp (*Penaeus aztecus, P. setiferus*) and crab (*Callinectes sapidus*) were conducted under flowthrough exposures, while static test conditions were used on oysters because of the size of the embryos/larvae. Fish were run under both static and flowthrough conditions. Data for the inland silverside (*Menidia beryllina*) were obtained under flowthrough conditions. Attempts to conduct red drum (*Sciaenops ocellatus*) tests under flowthrough conditions were unsuccessful and were eventually completed using static exposures. The spot (*Leiostomus xanthurus*) and Atlantic menhaden (*Brevoortia tyrannus*) were completed under static conditions because of a need to minimize handling.

Although tests were conducted for 96 hrs for all species, acceptable test data for the fish could only be obtained from 48-hr exposures. This was due to the naturally high mortality that pervaded the fish tests. No standard reference toxicant tests were conducted because additional test organisms could not be secured in sufficient quantities.

3.1.2 Static Exposures

Static exposures were conducted in glass fingerbowls. Fish exposures used 5 replicates with 10 individuals per replicate or 10 replicates with 5 individuals each. A separate set of test chambers was established for the sole purpose of collecting samples for chemical analyses. This minimized the need to disturb test animals and to reduce sample volumes during the tests. Test containers were placed in an incubator at 20°C under a daily (i.e., 24-hr) light regime of 16 hrs of light and 8 hrs of darkness.

3.1.3 Flowthrough Exposures

Flowthrough tests were conducted in a specially-designed acute toxicity test chamber to accommodate the small size of the test animals (**Figure 1**). The basic test chamber was comprised of five 250-ml glass beakers (i.e., exposure beakers, **Figure 1**), each of which had two 2.5-cm holes located opposite one another near the bottom of each beaker. Each hole was covered with 350-μm Nitex mesh, allowing for the retention of test animals in each beaker and a free exchange with the surrounding test medium. Exposure beakers were placed in a Plexiglas chamber which held approximately 10 l of test medium. An adjustable powerhead circulated the test medium through a 0.5-in. polyvinyl chloride (PVC) pipe which had been drilled with small holes. These holes were directed towards the sides of the tank, minimizing current energy in the tank but allowing for adequate circulation and mixing. Flow rates were checked before and during tests to maintain consistent exchange rates in the various exposure chambers.

Clean artificial seawater was added at a flow rate of approximately five liters every 24 hrs (i.e., 50% of the volume of the Plexiglas tank). Clean seawater, provided from several saltwater reservoirs, was pumped to a headtank located above the test chambers. From the headtank, water was gravity fed to each test chamber through PVC pipe and controlled through the use and adjustment of needle valves. Flow rates were checked daily and readjusted as necessary to maintain adequate flow. Overflow water drained from each test chamber through a drain hole whose centerpoint was 7.6 cm above the bottom of the test chamber; drained water flowed into a trough where it was collected and treated with a carbon filter before disposal. Each test chamber was equipped with 1) a submersible heater to control temperature; and 2) overhead lights, controlled by timer, which provided 16 hrs of light and 8 hrs of darkness.

Three separate banks, each holding seven test chambers (i.e., 21 test chambers total), were available for concurrent testing of dispersant, dispersed oil, and WAF mixtures. For the seven test chambers contained within each bank, acute toxicity testing protocols called for the creation of 1) five chambers which contained different concentrations of test media; 2) a control chamber; and 3) a chamber for collecting chemistry samples.

It became apparent during several of the invertebrate tests that cannibalism could become a problem during exposures. Subsequently, sealable 30-ml plastic beakers were used. These small beakers were punctured on the sides to allow water exchange to and from the Plexiglas chamber; single animals were subsequently placed in each beaker. The beakers were filled with the exposure medium and allowed to float freely within the Plexiglas chamber.

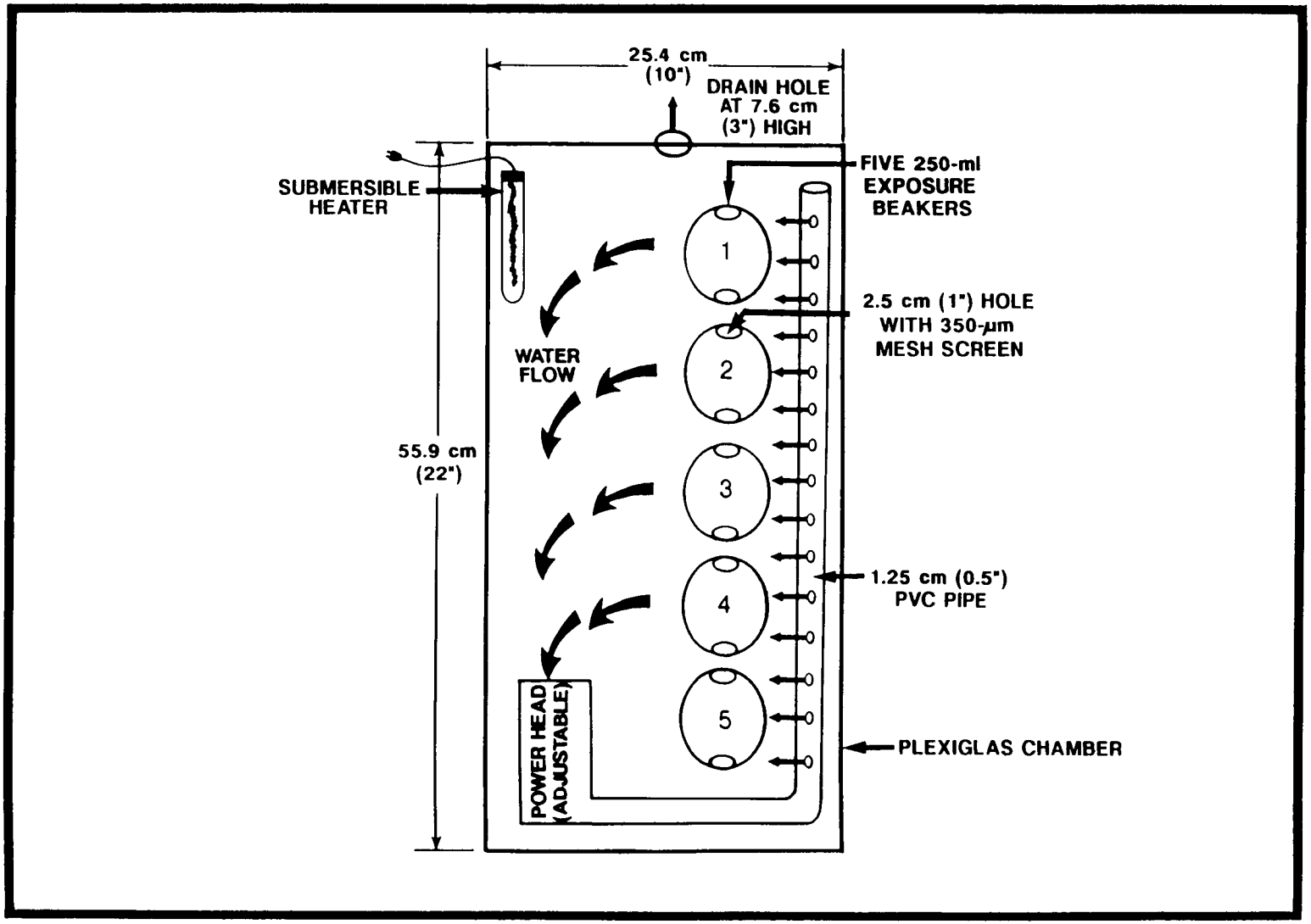

Figure 1. Modified acute toxicity flowthrough test chamber.

3.2 CHRONIC TOXICITY TESTING

The mysid *Mysidopsis bahia* was selected for chronic toxicity testing. Chronic tests used 7-day old animals exposed to a WAF of the Western Gulf oil for 7 days. Tests were conducted at concentrations of 20, 10, 5, 2.5, and 1.3% WAF and a control of HMM artificial seawater. Solutions were renewed daily and at the end of the exposure. Exposures included eight replicates containing five individuals each. Test containers (i.e., glass fingerbowls) were placed in an incubator at 26°C under a daily regime of 14 hrs of light and 10 hrs of darkness. Surviving animals were observed for fecundity and weighed. Statistical differences were determined between the controls and exposed animals for survival, growth, and fecundity. LC_{50} determinations were developed from Spearman-Karber analyses. No observed effects concentrations (NOEC) were calculated using ANOVA with Dunnett's test, per EPA's chronic toxicity testing protocols (i.e., Method 1007; Weber *et al.* 1988).

3.3 CHEMICAL ANALYSES

Chemical analyses were conducted at various stages of toxicity testing to measure TPH, purgeable aromatic hydrocarbons (benzene, toluene, ethylbenzene, and xylenes [BTEX]), and PAH. The methods for conducting these analyses are described below.

Table 3 provides a summary of the individual tests and the total numbers of each test completed during the program. In all, a total of 292 individual analyses were performed for exposures using the two oils. Four additional analyses were also completed on the whole oils to characterize PAH concentrations at the beginning and end of the acute toxicity testing phase. In addition, seven analyses were performed on a Corexit 9527 exposure to determine TPH background concentrations in the exposure medium.

Initially, it was proposed that a modified random process be used for collection of chemistry (i.e., water) samples during the acute toxicity testing phase. However, it soon became apparent that the volume of water required for testing would preclude complete hydrocarbon characterization with each test. This was due to the fact that each analysis generally required one liter of sample, however flow rates were less than what would be required to generate this amount. It was subsequently decided that instead of sampling only in selected tests, chemical samples would be collected and some level of chemical analysis would be conducted for each test. Therefore, for each test, samples were collected for determination of either BTEX, TPH, or PAH.

In addition to analysis of the water samples during the test exposures, each of the oils was characterized at the beginning (i.e., in Month 1) and at the end of the acute toxicity testing phase of the study (i.e., in Month 19). Initially, each oil was slated for testing using GC/MS at Day 1 and at Day 30, per contract requirements. Based on the results of the initial oil characterization, however, the analytical methodology was altered. Subsequent analyses were performed on the oils using GC/MS-SIM techniques. This analysis minimized the difficulties encountered when trying to analyze whole oils by standard GC/MS techniques and allowed for a characterization of the PAHs in the oils.

3.3.1 Gas Chromatography with Flame Ionization Detection

TPHs were determined by the protocols outlined in the State of California Leaking Underground Fuel Tank (LUFT) manual. This methodology, also referred to as a "Modified

Table 3. Total numbers of chemical analyses for each type of oil and analytical type.

Type Analysis	Central Gulf Oil			Western Gulf Oil			Dispersant	Total Analyses
	Water Accommodated Fraction	Dispersed Oil	Oil	Water Accommodated Fraction	Dispersed Oil	Oil		
TPH[a]	21	30	--	33	39	--	6	129
PAH[b]	17	17	2	22	17	2	1	78
BTEX[c]	22	22	--	26	26	--	--	96
Total Analyses	60	69	2	81	82	2	7	303

[a] TPH via GC-FID Method 8015.
[b] PAH intially determined via GC/MS Method 625; subsequently replaced by GC/MS-SIM.
[c] BTEX (purgeable aromatic hydrocarbons) via GC/MS Method 624.

8015," consisted of extraction of the sample with hexane and subsequent analysis of the extract utilizing a gas chromatograph with a flame-ionization detector (FID). Individual components were separated by the GC capillary column to obtain a pattern which was characteristic of the petroleum fraction. Quantitative analysis of the TPH was done by comparison of the chromatographic pattern obtained from the sample against one obtained from a standard of the particular petroleum hydrocarbon; in this case, the standard was crude oil. Comparison of the chromatograms provided an accurate value of the TPH present in the sample. Using this method, a detection limit of 10 mg/kg, or 10 ppm, was attained in a clean water sample. A Perkin-Elmer Model 8500 gas chromatograph with FID was used for these analyses.

3.3.2 Gas Chromatography/Mass Spectrometry

3.3.2.1 Purgeable Aromatic Hydrocarbons

Purgeable aromatic hydrocarbons (volatiles) were determined by GC/MS Method 624. Volatile compounds were purged from the sample by helium gas. This carrier gas was then introduced into the GC where the individual components were separated by the GC capillary column. Each compound was then ionized in a quadrapole unit and recorded as mass spectra. Data from all volatile compounds present in the sample were then reconstructed into an ion chromatogram. The chromatogram peak obtained due to elution from the GC column combined with the mass spectra "fingerprint" provided a combination which is unique to a compound. GC/MS thus provided state-of-the-art identification of organic compounds and was the method of choice to resolve compounds of interest such as BTEX from the interference of other volatile organic compounds present in petroleum fractions.

A Finnigan OWA with a 16-unit Tekmar Purge and Trap Autosampler was used to provide analysis of volatiles by Method 624. This method was selected for use because of its better resolution in samples with high background contaminants, allowing for a distinction between individual compounds.

3.3.2.2 Polynuclear Aromatic Hydrocarbons

PAHs were determined by GC/MS Method 625. This method is analytically similar to Method 624, except that Method 624 specifies injection of an extract containing the compounds of interest directly into the instrument rather than using a purge and trap technique. It was the method of choice for resolving specific organic compounds such as PAHs in the presence of high levels of interference from other components of the crude oil. However, the method proved to be unsatisfactory with regard to detection limits and interference and was replaced by the GC/MS-SIM technique.

Two separate extractions were used to obtain the semivolatiles of interest. The first extraction was performed at an acidic pH and isolated compounds such as phenols. The second extraction was performed at a neutral to basic pH and isolated other semivolatile compounds such as the PAHs. Together, the base/neutral fraction and the acid fraction (sometimes called BNA) were analyzed to determine Method 625 semivolatiles. For this current study effort, only analysis of the base/neutral fraction was undertaken to determine PAHs. A research-grade Finnigan 4000 GC/MS with an autosampler was employed to complete the analyses.

3.3.3 Gas Chromatography with Selected Ion Monitoring

Because of the relative insensitivity of the Method 625 approach, it was decided to utilize a modified GC/MS technique referred to as GC/MS selected ion monitoring (GC/MS-SIM). This method is similar to Method 625 but focuses on a limited number of ions and their alkyl homologues rather than a broad spectrum of hydrocarbons. For the present set of tests, the ions chosen for analysis included naphthalenes (including the C_0 to C_4 naphthalenes), fluorenes (including C_0 to C_3 fluorenes), phenanthrenes (including C_0 to C_4 phenanthrenes), anthracene, dibenzothiophenes (including C_0 to C_3 dibenzothiophenes), and chrysenes (including C_0 to C_4 chrysenes). This method allowed analyses down to the parts per trillion level.

3.3.4 Infrared Spectroscopy

IR spectroscopic analyses were initially scheduled as a measure of TPHs in the test exposures. A Buck Scientific IR was employed during the preliminary sample analyses. However, this method was eventually replaced by the methods noted previously when it became apparent that sufficient sample material would not be available from ongoing acute toxicity testing.

3.3.5 Ultraviolet Spectrometry

Ultraviolet (UV) spectrometry was employed to measure Corexit 9527 during test exposures. A standard curve was prepared using a dilution method series beginning with a 25 mg/l sample which was then scanned for absorbance at wavelengths from 200 to 700 nm. A peak absorbance was obtained at 231 nm and subsequent readings were made at this wavelength. Absorbance values taken from samples collected at various time periods were compared to the sample curve and concentrations calculated in mg/l.

3.4 PHOTODOCUMENTATION

Analytical equipment used in the analyses and exposure setups were documented with 35-mm slides. An Olympus microscope equipped with a camera attachment was also used to obtain pictures of several of the test organisms.

3.5 QUALITY ASSURANCE/QUALITY CONTROL

Quality assurance was provided for chemical analyses through the use of Environmental Protection Agency (EPA) protocols for quality assurance/quality control (QA/QC). This included the use of spikes, duplicates, and blank samples. QA/QC data accompanied the submission of each set of analytical results. Chain of custody procedures were followed in transferring samples between the toxicity laboratory and the chemical laboratory.

It had been anticipated that reference toxicant tests would be conducted on each test species. However, this proved to be unfeasible because of the difficulty in obtaining adequate numbers of test organisms. For all species except inland silverside and red drum, it was possible to secure only enough organisms to conduct single exposures with each of the test species. However, all other test protocols followed the recommended procedures in EPA and ASTM guidelines.

CHAPTER 4 - RESULTS

4.1 OILS AND DISPERSANT

The two Gulf of Mexico oils were initially analyzed by GC/MS, however few detectable PAHs were discernible. Such findings were attributed to limitations in the methods rather than a lack of hydrocarbons in each respective oil.

Application of the GC/MS-SIM technique resulted in significantly different findings (**Table 4**). **Figures 2** and **3** depict the PAH characteristics of each oil using GC/MS-SIM. As evident in these graphics, the two oils showed minor differences initially. The Central Gulf oil had higher concentrations of chrysenes, phenanthrenes, fluorenes, and dibenzothiophenes than the Western Gulf oil. Naphthalenes were the PAH compounds present in the highest concentrations in both oils and were generally several times greater than the other compounds. Concentrations for all of the compounds showed a "bell curve" distribution with the C_1-, C_2-, and C_3-methylated compounds exhibiting the highest concentrations.

Concentrations of the hydrocarbons within each whole oil at the end of the study (i.e., in Month 19) revealed several basic differences from the initial analyses. The naphthalenes and fluorenes, for the most part, were higher while the phenanthrenes, dibenzothiophenes, and chrysenes exhibited lower or similar concentrations when compared to the earlier measurements. Within a class of compounds, the "bell curve" distribution was consistent between the two analyses. Differences in concentrations may suggest variability within the testing method rather than being indicative of any real change in the oils over time.

When the dispersant alone was analyzed by GC/MS-SIM, only minimal levels of naphthalene (0.16 μg/g) and phenanthrene (0.012 μg/g) were detected.

4.2 CHEMICAL ANALYSES

The following subsections outline the analytical results for TPH (total petroleum hydrocarbons), BTEX (i.e., benzene, toluene, ethylbenzene, xylenes) compounds, PAH (polynuclear aromatic hydrocarbons) compounds, and the dispersant, Corexit 9527. Tabular summaries of the analytical chemistry results are presented in **Appendix A**.

4.2.1 Total Petroleum Hydrocarbons

TPH concentrations have been summarized for both static and flowthrough tests. **Table 5** provides average starting concentrations for all tests of the various hydrocarbon types measured during the exposures. **Table 6** summarizes the average concentrations for all tests using the highest concentration integrated over the 96-hr exposure periods (i.e., concentration x hours). This provides a measure of the total hydrocarbons to which the animals were exposed during the course of a test. This measure was used in order to compare exposure conditions between different types of test (e.g., static vs. flowthrough, Western Gulf oil vs. Central Gulf oil). The static tests tended to have the highest overall TPH concentrations with the dispersed oil levels being four to five times greater than that measured in the WAF. Concentrations for the two whole oils were similar.

Table 4. Concentrations (μg/g) of PAHs measured in the Western and Central Gulf oils at the beginning and end of the present study.

Analyte	Western Gulf Oil		Central Gulf Oil	
	Initial	Final	Initial	Final
Naphthalene	440	510	420	ND[a]
C_1-Naphthalene	1,800	1,300	1,600	1,040
C_2-Naphthalene	1,100	1,500	1,100	1,700
C_3-Naphthalene	790	1,500	1,000	1,600
C_4-Naphthalene	420	850	630	1,100
Fluorene	29	27	9.9	26
C_1-Fluorene	83	200	190	250
C_2-Fluorene	150	280	340	350
C_3-Fluorene	130	230	280	330
Phenanthrene	68	6	110	22
C_1-Phenanthrene	150	89	340	190
C_2-Phenanthrene	170	84	410	190
C_3-Phenanthrene	130	74	350	140
C_4-Phenanthrene	96	69	97	54
Anthracene	ND	11	ND	16
Dibenzothiophene	91	27	75	38
C_1-Dibenzothiophene	110	70	210	130
C_2-Dibenzothiophene	180	99	380	190
C_3-Dibenzothiophene	170	63	310	120
Chrysene	8.7	ND	13	ND
C_1-Chrysene	16	8	34	32
C_2-Chrysene	18	11	52	36
C_3-Chrysene	14	6	44	19
C_4-Chrysene	ND	4	14	7

[a] ND = not detected at a detection limit of 0.01 μg/g.

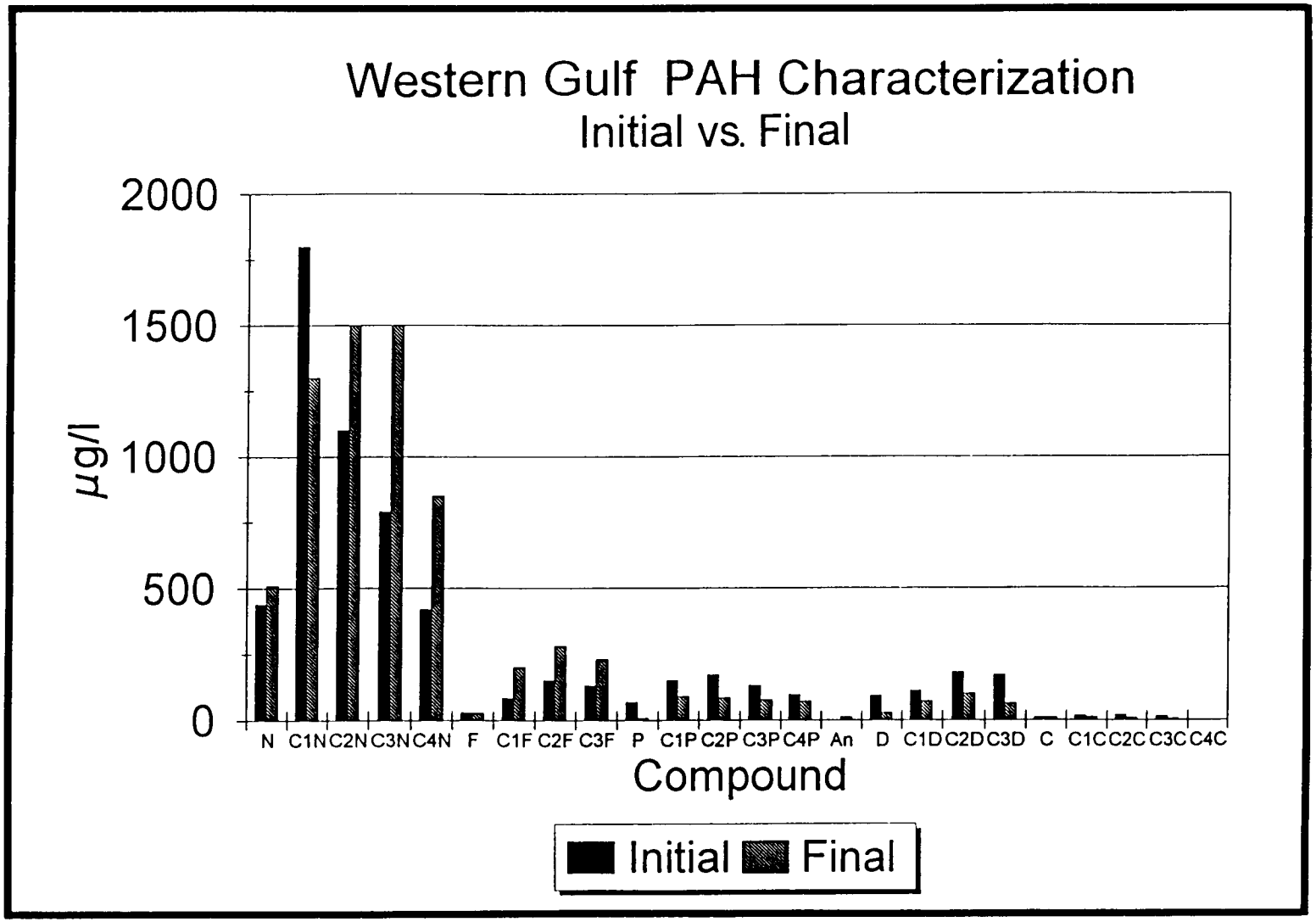

Figure 2. Polynuclear aromatic hydrocarbon (PAH) characteristics of the Western Gulf oil, as determined by the GC/MS-SIM technique.

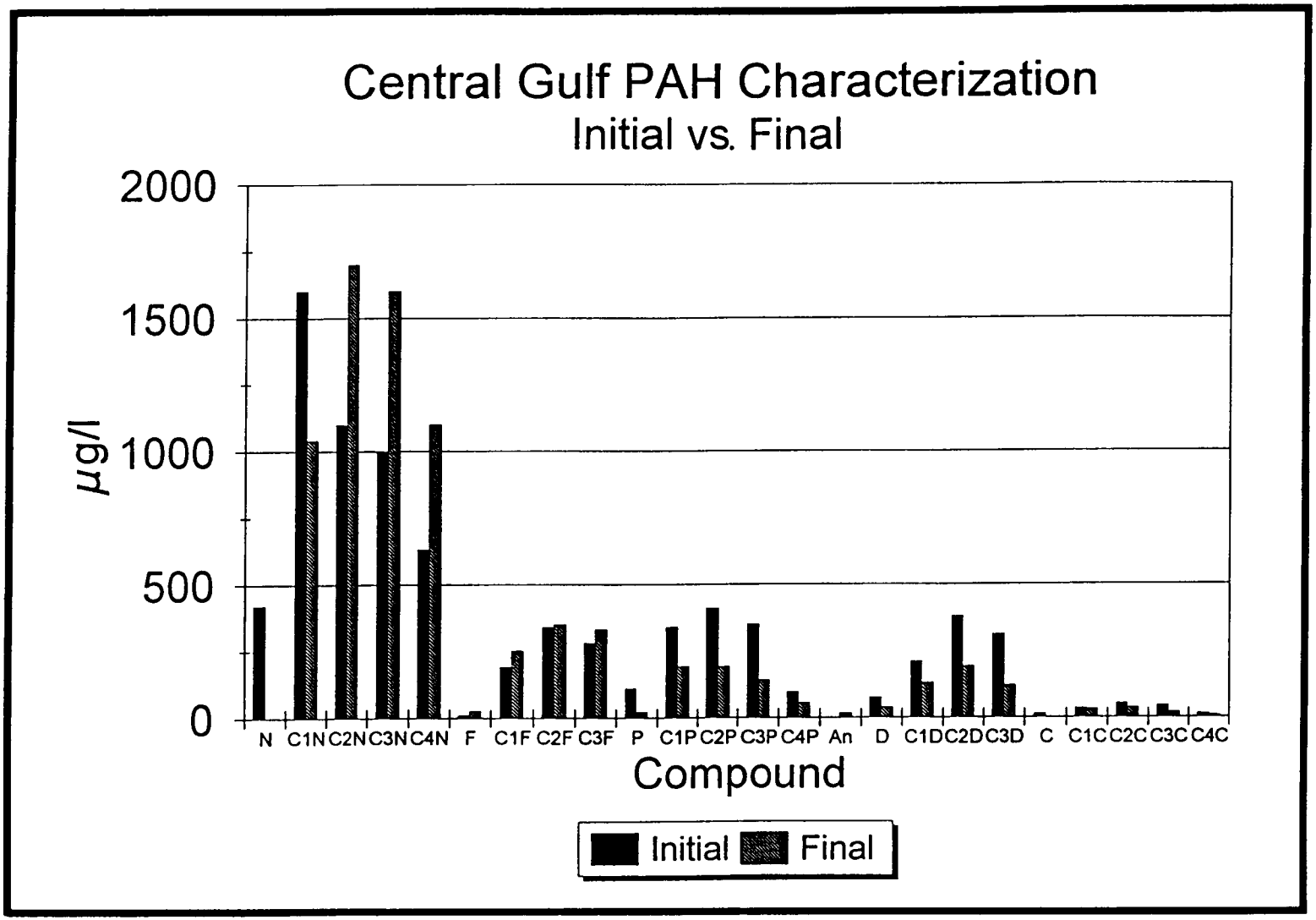

Figure 3. Polynuclear aromatic hydrocarbon (PAH) characteristics of the Central Gulf oil, as determined by the GC/MS-SIM technique.

Table 5. Average starting concentrations for each hydrocarbon type as measured in test exposures.

Treatment	Western Gulf Oil Water Accommodated Fraction	Western Gulf Oil and Dispersant	Central Gulf Oil Water Accommodated Fraction	Central Gulf Oil and Dispersant
Total Naphthalenes (µg/l)				
Static and Flowthrough	97 ± 16	318 ± 134	127 ± 18	327 ± 211
Total Petroleum Hydrocarbons (mg/l)				
Static	11 ± 7.5	49 ± 8.8	19 ± 8.7	50 ± 14.2
Flowthrough	14 ± 3	34 ± 26.1	9 ± 0.1	73 ± 34.3
Total BTEX (µg/l)				
Static	10,180 ± 1,340	588 ± 82	12,055 ± 35	439 ± 71
Flowthrough	9,300 ± 910	485 ± 71	7,985 ± 755	828 ± 84

Table 6. Comparison of average hydrocarbon concentrations integrated over 96-hr exposure periods.

Treatment	Western Gulf Oil Water Accommodated Fraction	Western Gulf Oil and Dispersant	Central Gulf Oil Water Accommodated Fraction	Central Gulf Oil and Dispersant
Total Naphthalenes (µg/l)				
Static and Flowthrough	3,290 ± 2,902	8,315 ± 5,057	1,513 ± 484	5,012 ± 4,048
Total Petroleum Hydrocarbons (mg/l)				
Static	544 ± 332	2,570 ± 619	572 ± 96	2,288 ± 849
Flowthrough	486 ± 37	422 ± 37	34 ± 17	1,062 ± 54
Total BTEX (µg/l)				
Static	690,462 ± 136,480	25,399 ± 9,719	684,480 ± 198,991	29,495 ± 1,270
Flowthrough	44,223 ± 2,263	2,717 ± 39	47,173 ± 10,814	4,914 ± 208

Overall, the initial concentrations show similar patterns as those for the integrated 96-hr concentrations. This would suggest that concentrations throughout the tests were consistent in the various exposures. **Figure 4** provides a comparison of the TPH concentrations in the WAF and dispersed oil exposures over time. The two oils showed similar patterns of degradation and decline over the 96-hr period. This was true for both the WAF and the dispersed oil. However, higher rates of decline were evident in the dispersed oil mixture. At the end of the 96-hr period, it was noteworthy that the final concentrations of TPH in the dispersed oil mixtures and in the WAF did not markedly differ.

Figures 5 and **6** compare the distribution of the Western and Central Gulf oils, respectively, with time in the flowthrough versus the static system. The initial concentrations were similar in both the static and flowthrough systems but the final concentrations tended to be slightly higher in the static system. With time, the greatest loss of hydrocarbons in the flowthrough system occurred within the first 24 hrs, particularly in the dispersed oil mixture.

4.2.2 Total BTEX Characteristics

The total number of samples analyzed for BTEX compounds are summarized in **Table 7**. Average total BTEX concentrations have been presented in **Table 6**. Average BTEX distributions are graphically presented in **Figure 7**.

Table 7. Numbers of BTEX samples analyzed for each type of test condition.

Test Condition	Flowthrough	Static
Central Gulf Oil		
Water Accommodated Fraction	2	2
Dispersed Oil	2	3
Western Gulf Oil		
Water Accommodated Fraction	2	3
Dispersed Oil	2	2

BTEX concentrations integrated over 96-hr exposures did not differ markedly between the two oils tested. BTEX concentrations in the static system were at least an order of magnitude higher than in the flowthrough system (**Figures 8** and **9**). Similarly, concentrations in the WAF were an order of magnitude greater than in the dispersed oil mixtures. The range of variation between samples was generally less than 30% (**Table 6**).

In spite of the order of magnitude differences in concentrations, the majority of the BTEX determinations noted for both the dispersed oil mixtures and the WAF in the flowthrough system were lost within the first 6 hrs, with concentrations close to detection limits within 24 hrs. These patterns are shown in **Figure 10**.

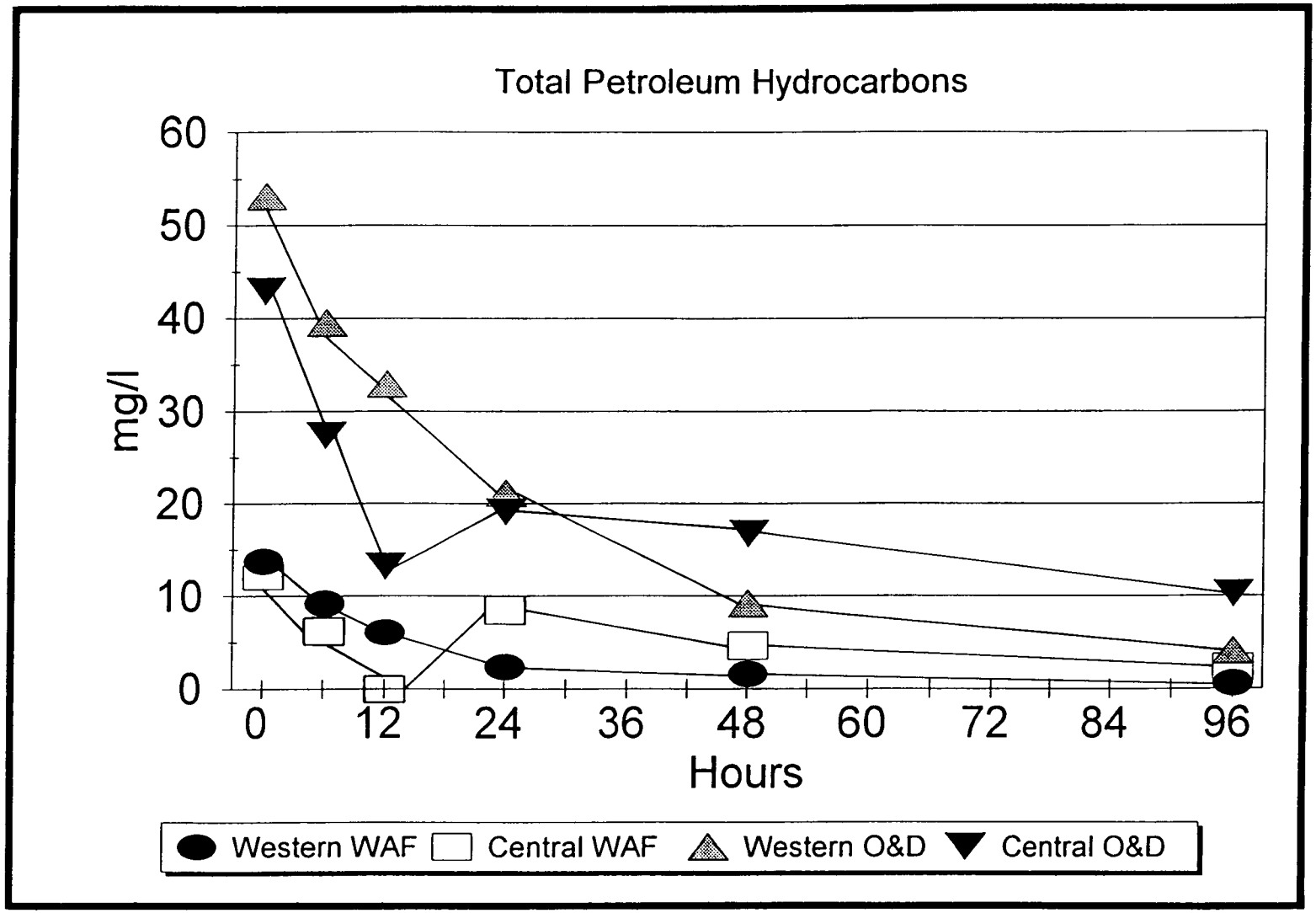

Figure 4. Comparison of the total petroleum hydrocarbon (TPH) concentrations over time in the water accommodated fractions (WAF) and dispersed oil mixtures (O&D), as measured from the flowthrough system.

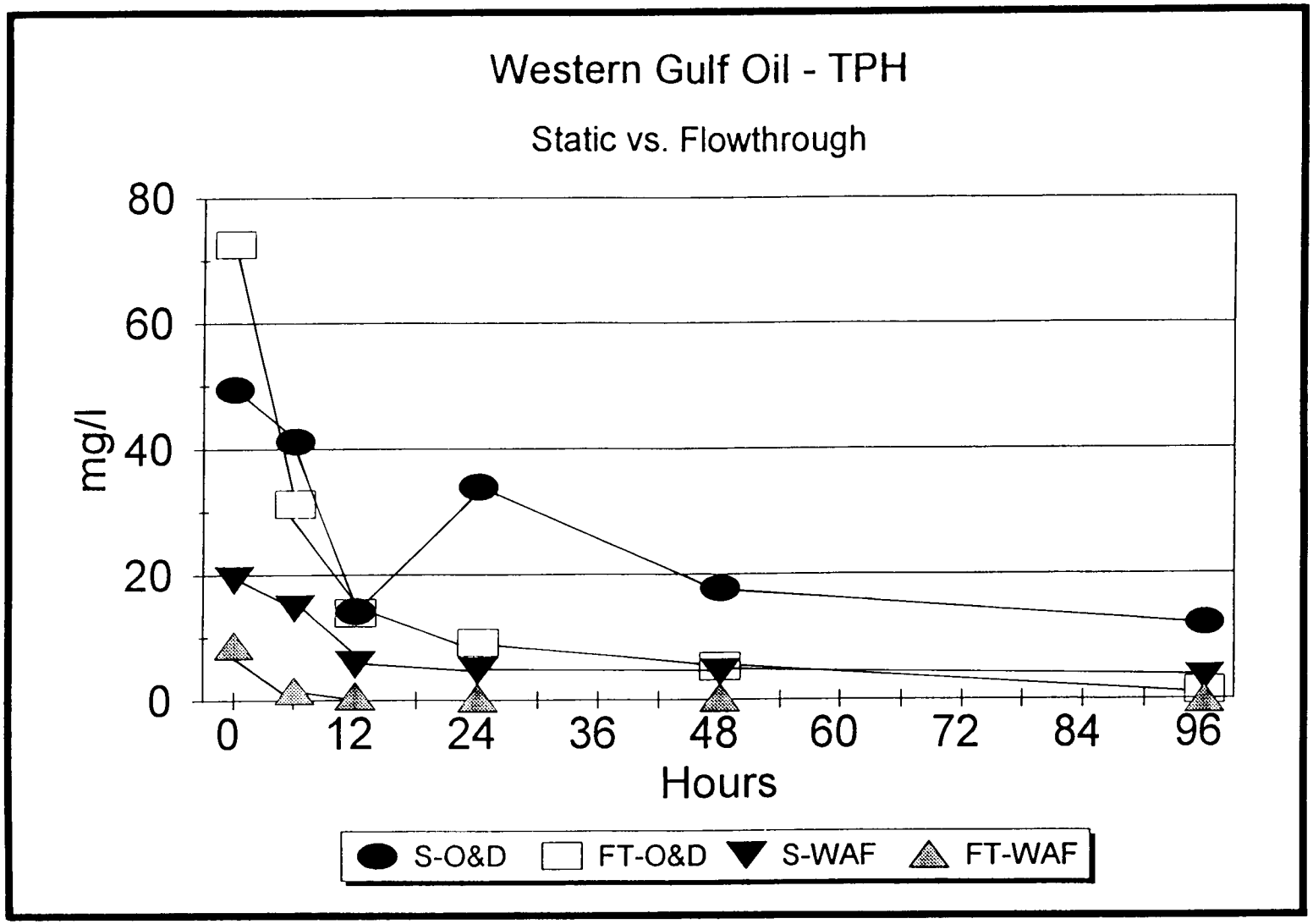

Figure 5. Comparison of Western Gulf oil total petroleum hydrocarbon concentrations in water accommodated fractions (WAF) and dispersed oil mixtures (O&D) for flowthrough (FT) versus static (S) tests.

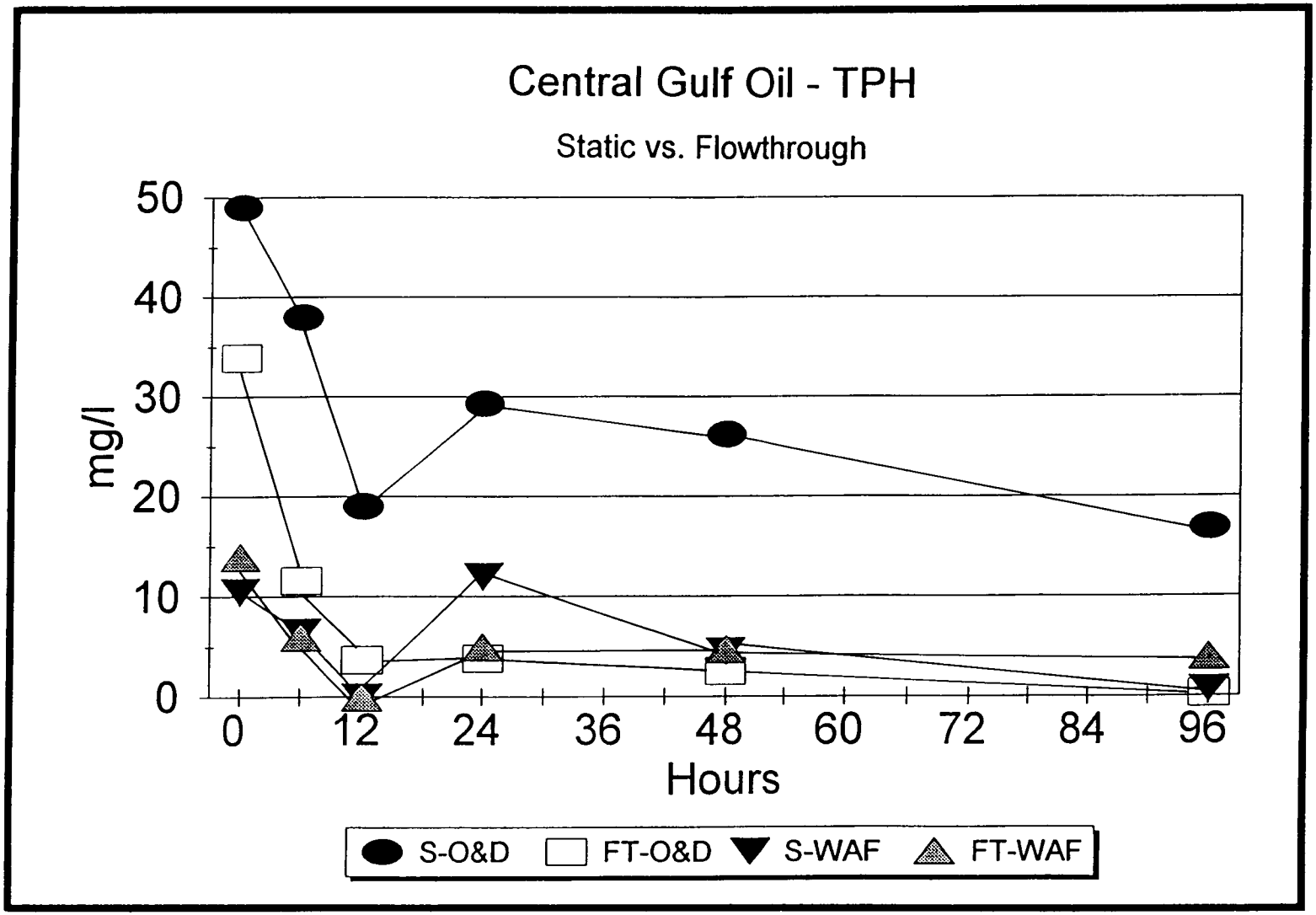

Figure 6. Comparison of Central Gulf oil total petroleum hydrocarbon concentrations in water accommodated fractions (WAF) and dispersed oil mixtures (O&D) for flowthrough (FT) versus static (S) tests.

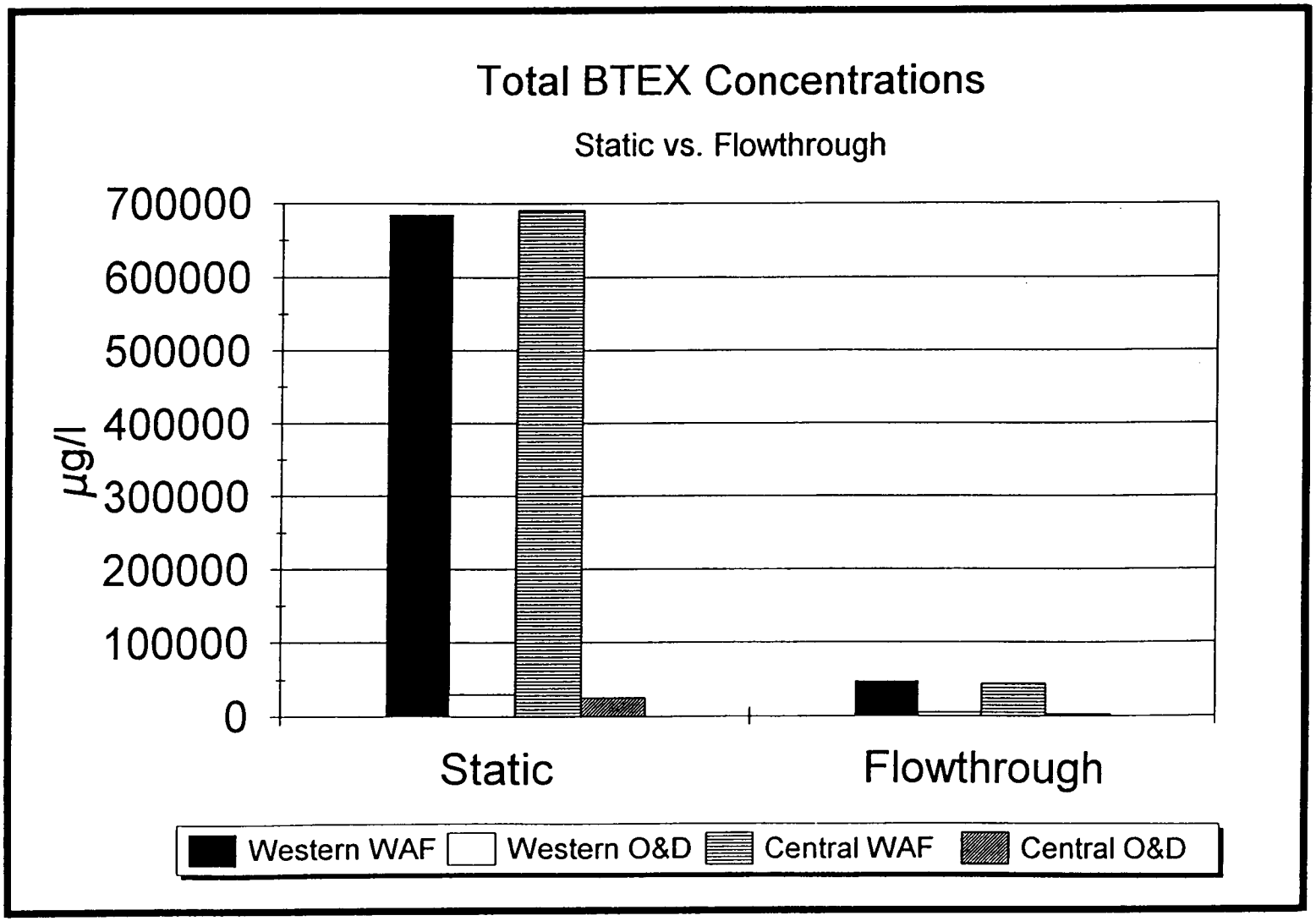

Figure 7. Comparison of total average BTEX concentrations in static versus flowthrough tests.

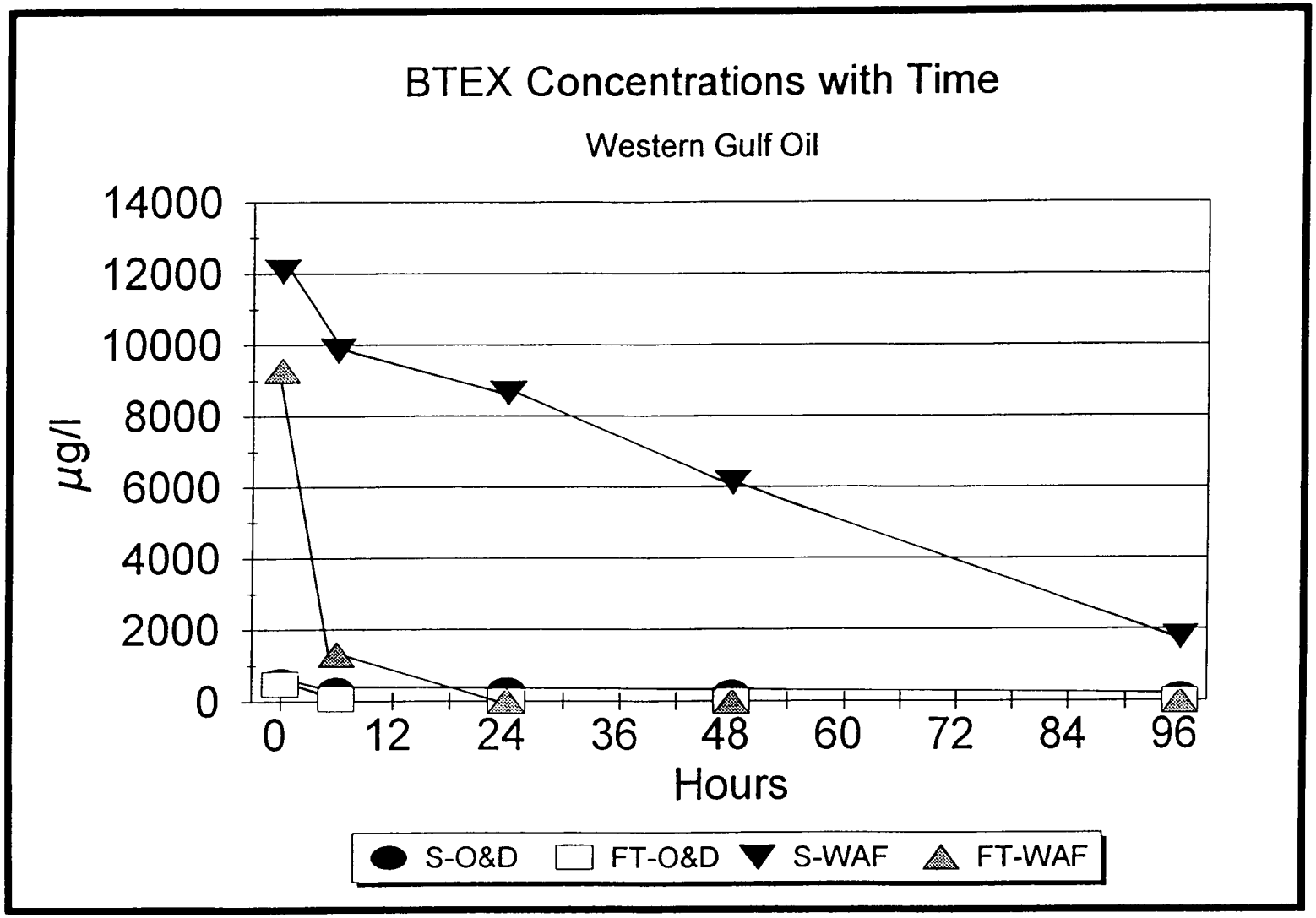

Figure 8. Measurements of BTEX concentrations over time for the Western Gulf oil comparing the water accommodated fractions (WAF) and dispersed oil mixtures (O&D) for flowthrough (FT) and static (S) systems.

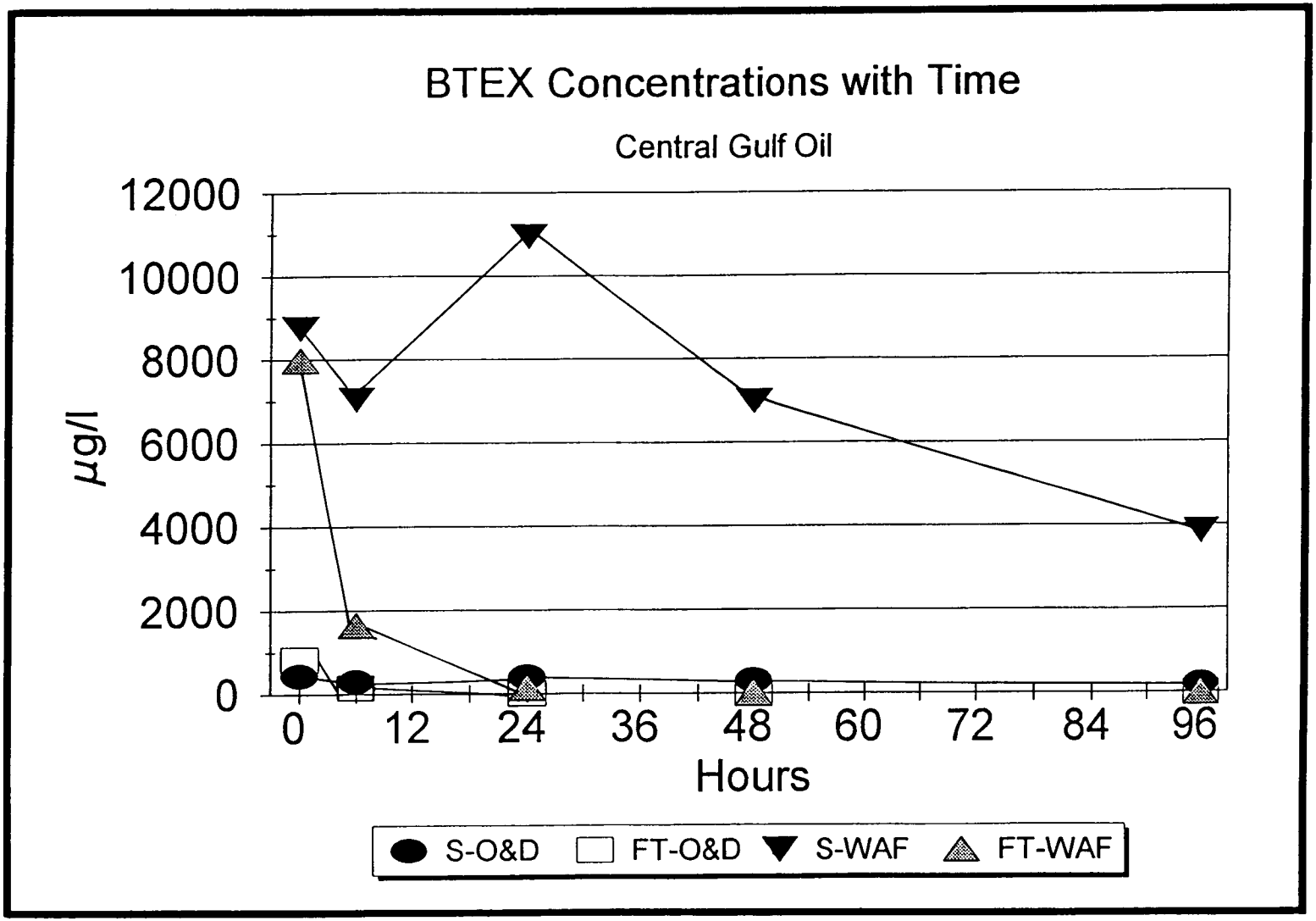

Figure 9. Measurements of BTEX concentrations over time for the Central Gulf oil comparing the water accommodated fractions (WAF) and dispersed oil mixtures (O&D) for flowthrough (FT) and static (S) systems.

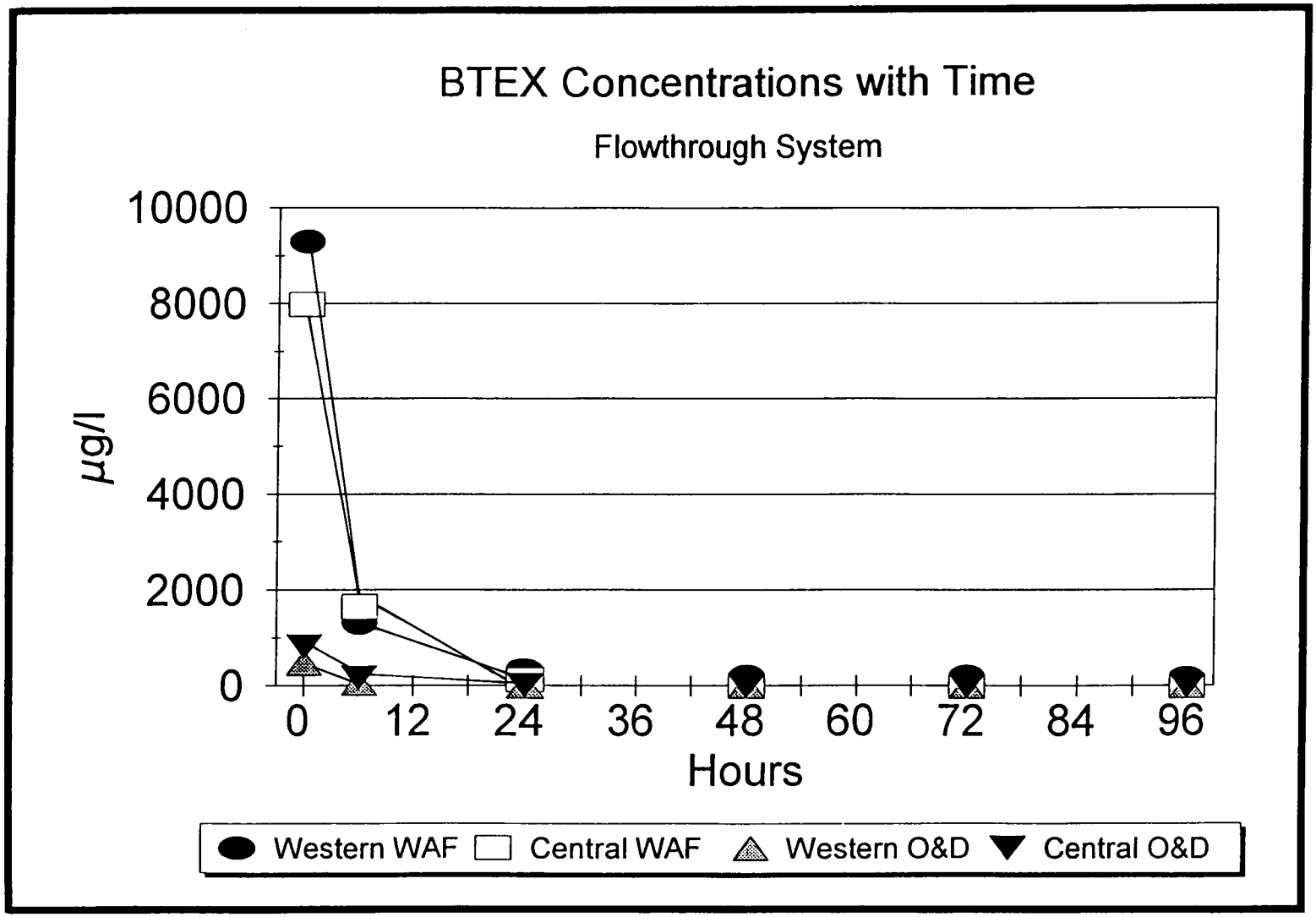

Figure 10. Measurements of total average BTEX concentrations over time in the flowthrough system comparing the water accommodated fractions (WAF) and dispersed oil mixtures (O&D) for both oils.

4.2.3 Polynuclear Aromatic Hydrocarbon Distributions

Total naphthalenes concentrations (in the highest exposure concentrations) are summarized in **Table 6**. The pattern for these hydrocarbons is representative of results for all of the measured PAHs. Fairly similar results were obtained for both Western and Central Gulf oils. Concentrations were two to three times higher in the dispersed oil when compared to the WAF. Variability between exposures was generally between 30 to 50%. Initial concentrations were higher in the dispersed oil compared to the WAF, however, after 96 hrs similar levels had been reached (**Figure 11**). A considerable amount of the PAHs had been lost within the first 24 hrs, particularly in the dispersed oil mixture. The rate of decrease could not all be accounted for by flow rates in the flowthrough system, suggesting that volatilization was a major contributor to losses during the exposure periods.

Figures 12 and **13** show the distribution of each of the individual PAHs with time. Data are presented for the Western Gulf oil as these are representative of the two oils tested. As noted previously, the dispersed oil had generally higher concentrations of the hydrocarbons than were evident in the WAF. Other than the naphthalenes, the hydrocarbons with the highest initial concentrations were dibenzothiophenes, phenanthrenes, and fluorenes, all of which were an order of magnitude less than was measured for the naphthalenes. These hydrocarbons were generally at or near detection limits within 24 hrs and most were absent from the exposures at 96 hrs.

4.2.4 Corexit 9527 Test Concentrations

Dispersant concentrations were measured by UV-spectrophotometric methods in a flowthrough test. The results of this analysis are presented in **Figure 14**. Beginning with an initial measured concentration of 82 mg/l (100 mg/l nominal), the concentration after the 96-hr exposure was approximately 32 mg/l. If flow rates were such that 50% of the exposure medium was replaced every 24 hrs, then the final concentration should have been approximately 5 mg/l after 96 hrs. The fact that the final concentration was higher than this suggests that flow rates were less than estimated or that mixing was not homogeneous. The latter hypothesis is supported by the fact that hydrocarbons in most tests were lost at rates greater than that which could be accounted for solely by dilution and flow rates.

4.3 ACUTE TOXICITY TESTING

The nine species-specific discussions which comprise **Section 4.3** outline the results of toxicity testing using the two Gulf of Mexico oils and the dispersant, as summarized in **Table 8**. Summary data from acute toxicity testing is presented in **Appendix B**. For ease of discussion, **Section 4.3** has been subdivided, allowing for separate discussions of acute toxicity testing results for invertebrates and fish. A brief summary of salient life and natural history information prefaces the presentation of acute toxicity testing results for each species.

4.3.1 Invertebrates

A summary of the LC_{50} determinations for all invertebrate tests is included in **Table 9**. **Tables 10** through **12** compare these results based on a toxicity index (TI) which uses ppb- or ppm-hrs as the measure of toxicity. For example, in **Table 10**, the TI used in the present set of tests was calculated using the product of total naphthalenes exposure and time of exposure. Total naphthalenes have been used for comparison because these have been shown

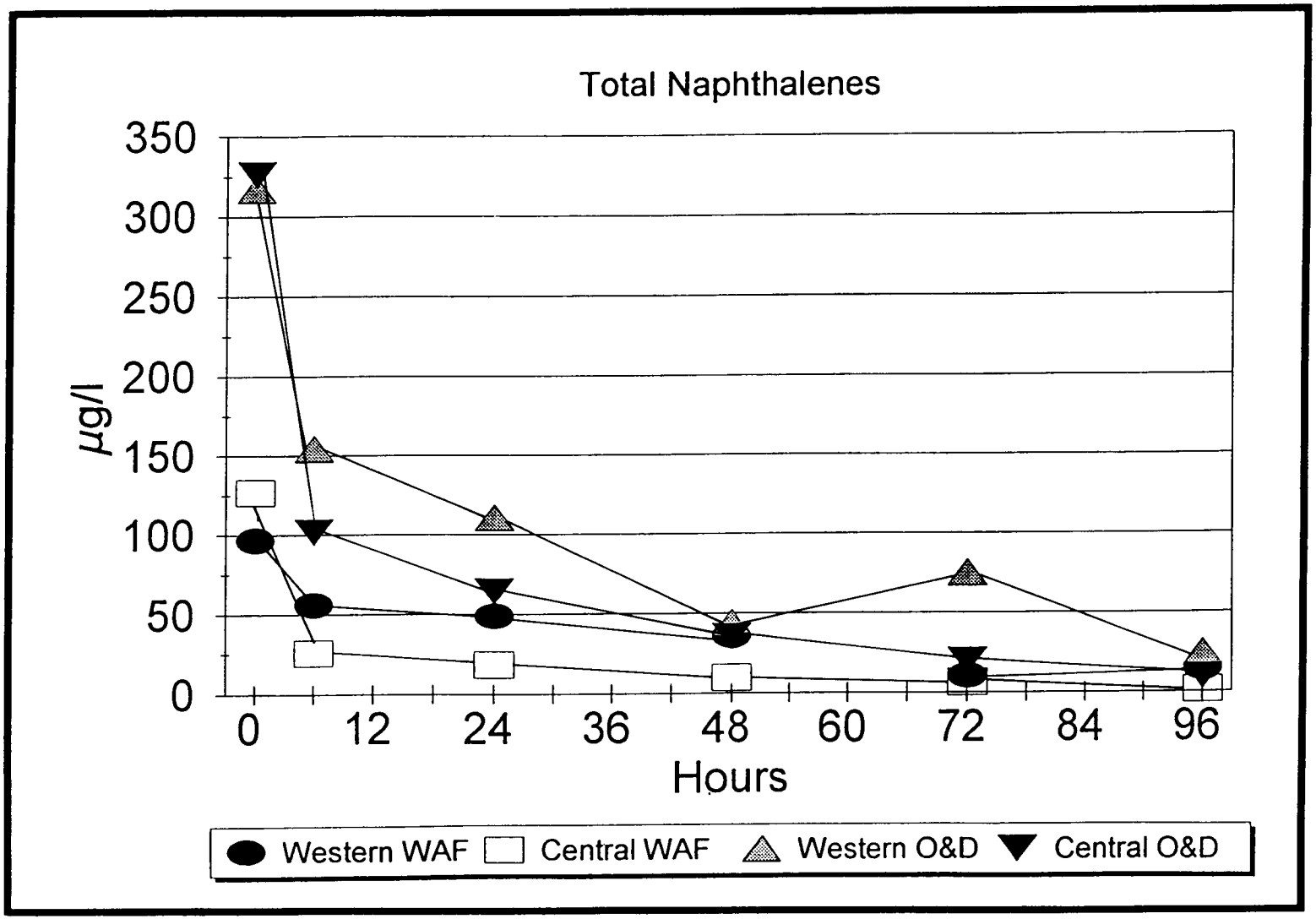

Figure 11. Measurements of naphthalenes in the water accommodated fractions (WAF) and dispersed oil mixtures (O&D) for both oils over time.

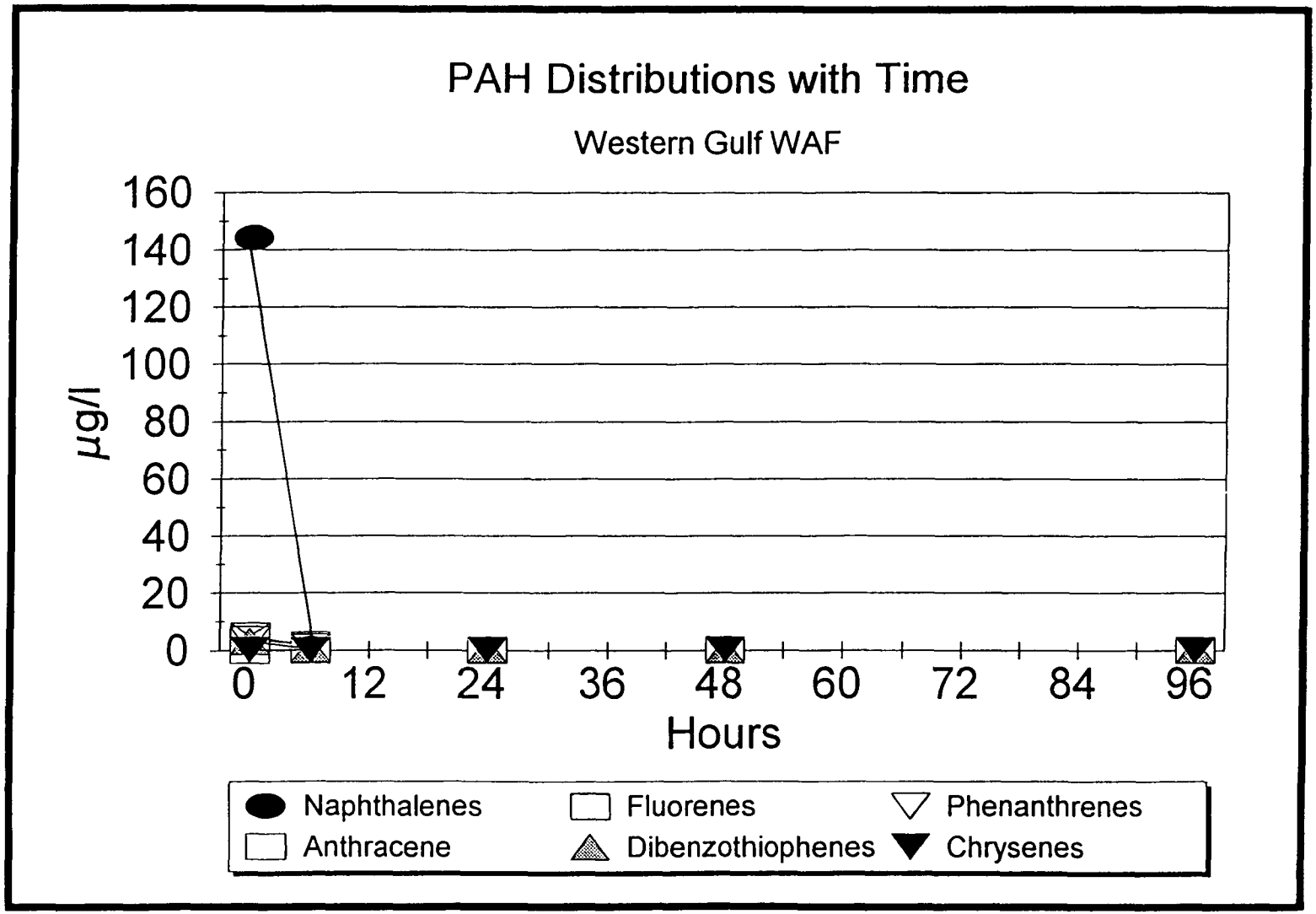

Figure 12. Polynuclear aromatic hydrocarbon (PAH) distributions over time for the Western Gulf water accommodated fraction (WAF).

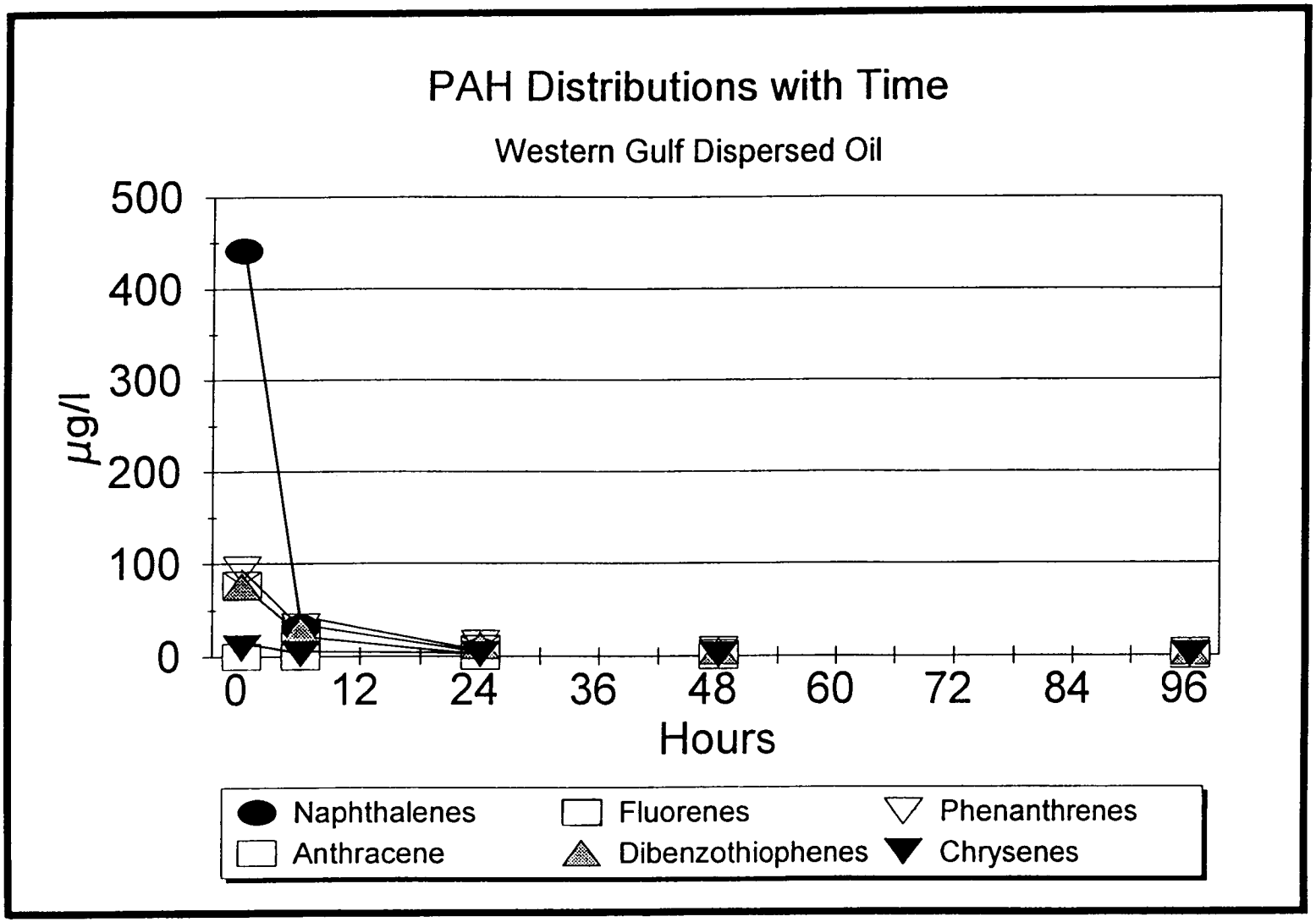

Figure 13. Polynuclear aromatic hydrocarbon (PAH) distributions over time for the Western Gulf dispersed oil mixture.

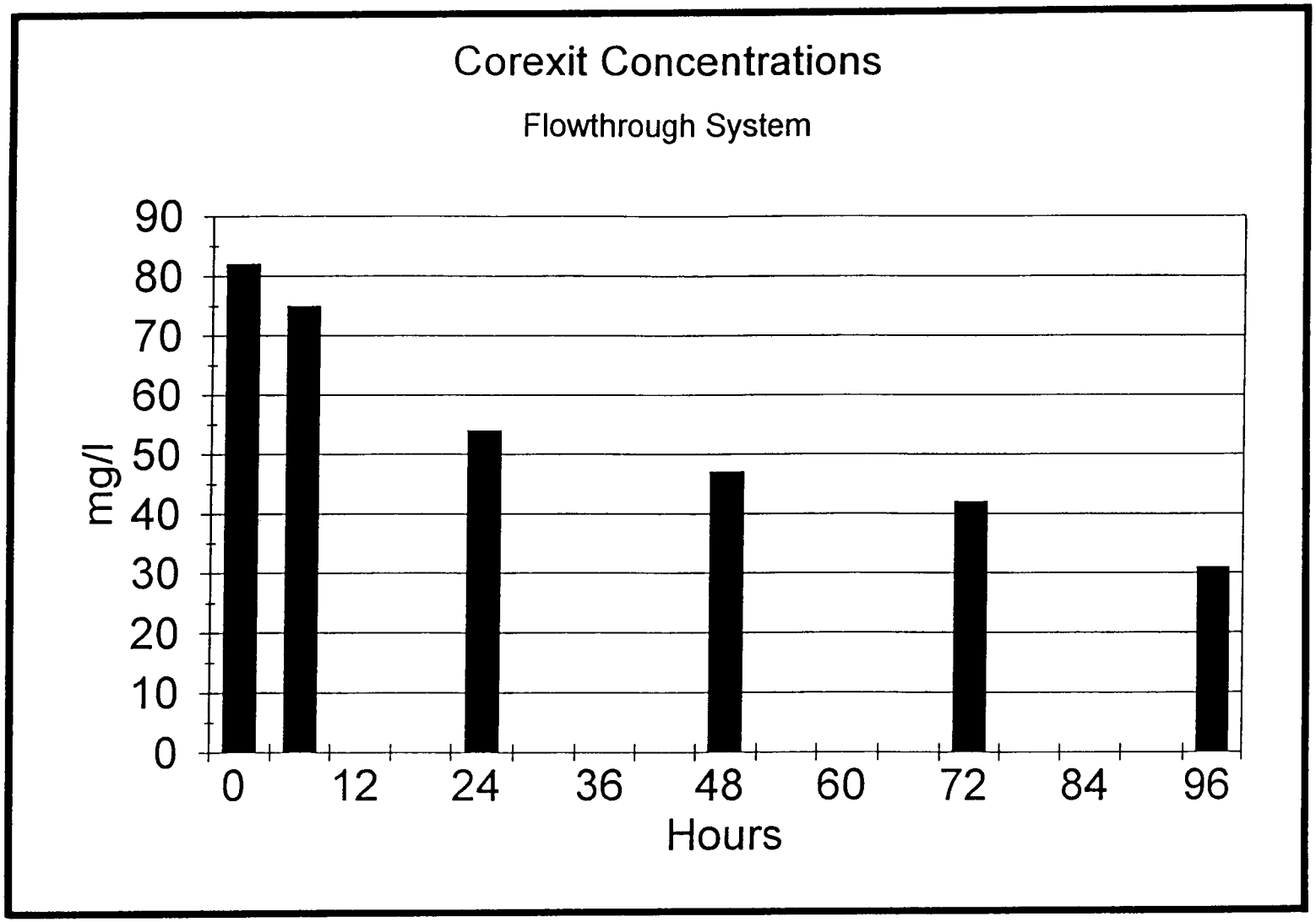

Figure 14. Results of ultraviolet spectrophotometric analysis of the dispersant Corexit 9527 in a flowthrough test.

Table 8. Summary of invertebrate and fish species tested with the water accommodated fractions (WAF) of Western and Central Gulf of Mexico oils, dispersed oils, and dispersant alone (Corexit 9527).

Species	Test[b]	Western Gulf of Mexico Oil[a]		Central Gulf of Mexico Oil[a]		Corexit 9527
		WAF[c] of Oil	Oil + Corexit 9527	WAF[c] of Oil	Oil + Corexit 9527	
Brown shrimp (*Penaeus aztecus*)	FT/A	post larvae tested 4/92	post larvae tested 4/92	Not tested[d]	Not tested[d]	Not tested[d]
White shrimp (*Penaeus setiferus*)	FT/A	PL22 tested 8&9/92; PAH[e] run 9/92	PL22 tested 8&9/92; PAH run 9/92; mysis stage tested 6/93; TPH run 6/93	PL15 tested 8/92; PAH run 8/92	PL15 tested 8/92; PAH run 8/92	PL15 tested 8/92; PAH run 8/92 PL22 tested 9/92; PAH run 9/92; mysis stage tested 6/93; TPH run 6/93
Blue crab (*Callinectes sapidus*)	FT/A	megalopae tested 9/93; TPH run 9/93	megalopae tested 9/93; TPH run 9/93	megalopae tested 9/92; BTEX run 9/92	megalopae tested 9/92; BTEX run 9/92	megalopae tested 9/92; BTEX run 9/92; megalopae tested 9/93; TPH run 9/93
Eastern oyster (*Crassostrea virginica*)	S/A	Not tested[d]	embryos tested 8/92; TPH run 8/92; embryos tested 9/93; TPH run 9/93	Not tested[d]	embryos tested 8/92; TPH run 8/92	embryos tested 8/92; TPH run 8/92; embryos tested 9/93; TPH run 9/93
Red drum (Redfish) (*Sciaenops ocellatus*)	S/A, FT/A	embryos tested 5/92; larvae tested 9/92; BTEX run 9/92	embryos tested 5/92; larvae tested 9/92; BTEX run 9/92	eggs/larvae[f] tested 6/92; PAH run 7/92; larvae tested 7&8/92; PAH run 8/92	eggs/larvae[f] tested 6/92; PAH run 7/92; larvae tested 7&8/92; PAH run 8/92	eggs/larvae[f] tested 5/92; PAH run 7/92; larvae tested 8&9/92

Table 8. Summary of invertebrate and fish species tested with the water accommodated fractions (WAF) of Western and Central Gulf of Mexico oils, dispersed oils, and dispersant alone (Corexit 9527) (continued).

Species	Test[b]	Western Gulf of Mexico Oil[a]		Central Gulf of Mexico Oil[a]		Corexit 9527
		WAF[c] of Oil	Oil + Corexit 9527	WAF[c] of Oil	Oil + Corexit 9527	
Inland silverside (Silverside minnow) (*Menidia beryllina*)	S/A, FT/A	eggs & larvae tested 4/92; PAH run 4/92; eggs & larvae tested 1/93; TPH and BTEX run 1/93	eggs & larvae tested 4/92; PAH run 4/92; eggs & larvae tested 1/93; TPH and BTEX run 1/93	eggs & larvae tested 12/92; TPH & BTEX run 12/92	eggs & larvae tested 12/92; TPH & BTEX run 12/92	eggs & larvae tested 4/92; PAH run 4/92; eggs & larvae tested 12/92; TPH & BTEX run 12/92
Spot (*Leiostomus xanthurus*)	S/A	eggs/larvae[f] tested 2/93; TPH and BTEX run 2/93	eggs/larvae[f] tested 2/93; TPH and BTEX run 2/93	eggs/larvae[f] tested 2/93; TPH and BTEX run 2/93	eggs/larvae[f] tested 2/93; TPH and BTEX run 2/93	eggs/larvae[f] tested 2/93; TPH and BTEX run 2/93
Mysid (*Mysidopsis bahia*)	S/C	larvae tested 10/93; no chemistry	Not tested[d]	Not tested[d]	Not tested[d]	Not tested[d]
Atlantic menhaden (*Brevoortia tyrannus*)	S/A	eggs/larvae[f] tested 3/93; PAH run 3/93	eggs/larvae[f] tested 3/93; PAH run 3/93	eggs/larvae[f] tested 3/93; PAH run 3/93	eggs/larvae[f] tested 3/93; PAH run 3/93	eggs/larvae[f] tested 3/93; PAH run 3/93

[a] oil characterization of both test oils conducted via GC/MS-SIM in November 1991 (initial) and June 1993 (19-month).
[b] FT/A = flowthrough, acute exposure; S/A = static and/or static renewal, acute exposures; S/C = static renewal, chronic exposure testing.
[c] water accommodated fraction, prepared per the protocol of Anderson *et al.* (1974).
[d] not tested due to a lack of available test specimens and/or an inability to induce spawning.
[e] analytical methodologies used included GC/MS-SIM for PAHs, GC-FID for TPH, and GC/MS for BTEX.
[f] indicates a species-specific egg stage of short duration where larval hatching occurs during the 96-hr acute toxicity test.

Table 9. LC_{50} determinations for invertebrate species exposed for 96 hours to nominal concentrations of the water accommodated fractions (WAF) of Western and Central Gulf oils, dispersed oil mixtures, and dispersant alone (Corexit 9527). Parenthetic entries represent either 24- or 48-hr LC_{50} values, as footnoted.

Species	WAF (%)	Dispersant (mg/l)	Oil & Dispersant (mg/l dispersant)
Western Gulf Oil			
Blue crab (*Callinectes sapidus*)	>100[a]	81.2 (94.6)[b]	90.8 (>100)[b]
White shrimp (*Penaeus setiferus*)	>100[c]	11.9[d]	18.6[c] (55.2)[b,c]
Brown shrimp (*P. aztecus*)	59.9 (59.9)[b]	--	52.7 (55.2)[b]
Central Gulf Oil			
Blue crab (*Callinectes sapidus*)	70.7 (73.7)[e]	77.9 (85.6)[e]	19.8 (56.7)[b]
White shrimp (*Penaeus setiferus*)	30.2[f] (85.6)[b,f]	11.9[d]	13.8[f] (24.8)[b,f]

[a] 64% survival in 100% concentration.
[b] 24-hr value.
[c] PL22 shrimp.
[d] PL15 and PL22 stages.
[e] 48-hr value.
[f] PL15 shrimp.

Table 10. Toxicity Index determinations (ppb-hrs) based on total naphthalenes concentrations for invertebrates exposed to the water accommodated fractions (WAF) and dispersed oil mixtures of Western and Central Gulf oils.

Species	WAF		Dispersed Oil	
	Western	Central	Western	Central
White shrimp (*Penaeus setiferus*)				
24 hr	>1,407[a]	737[b]	2,103[a]	691[b]
96 hr	>3,290[a]	457[b]	1,547[a]	692[b]
Brown shrimp (*Penaeus aztecus*)				
24 hr	843	--	2,103	--
96 hr	1,971	--	4,382	--
Blue crab (*Callinectes sapidus*)				
24 hr	>1,407	635	>3,809	1,579
96 hr	>3,290	1,070	7,550	992
Eastern oyster (*Crassostrea virginica*)	--	--	930	200

[a] PL22 shrimp.
[b] PL15 shrimp.

Table 11. Toxicity Index determinations (ppm-hrs) based on TPH concentrations for invertebrates exposed to the water accommodated fractions (WAF) and dispersed oil mixtures of Western and Central Gulf oils.

Species	WAF		Dispersed Oil	
	Western	Central	Western	Central
White shrimp (*Penaeus setiferus*)				
24 hr	>486[a]	21[b]	152[a]	148[b]
96 hr	>486[a]	10[b]	78[a]	147[b]
Brown shrimp (*Penaeus aztecus*)				
24 hr	95	--	153	--
96 hr	291	--	222	--
Blue crab (*Callinectes sapidus*)				
24 hr	>486	18	>277	337
96 hr	>486	24	383	210
Eastern oyster (*Crassostrea virginica*)				
96 hr	--	--	288	92

[a] PL22 shrimp.
[b] PL15 shrimp.

Table 12. Toxicity Index determinations (ppb-hrs) based on total BTEX concentrations in flowthrough tests for invertebrates exposed to the water accommodated fractions (WAF) and dispersed oil mixtures of Western and Central Gulf oils.

Species	WAF		Dispersed Oil	
	Western	Central	Western	Central
White shrimp (*Penaeus setiferus*)				
24 hr	>43,896[a]	38,571[b]	1,431[a]	1,137[b]
96 hr	>44,222[a]	14,246[b]	505[a]	678[b]
Brown shrimp (*Penaeus aztecus*)				
24 hr	29,294	--	1,431	--
96 hr	26,489	--	1,432	--
Blue crab (*Callinectes sapidus*)				
24 hr	>43,896	33,209	>2,592	2,599
96 hr	>44,222	33,351	2,467	973
Eastern oyster (*Crassostrea virginica*)				
96 hr	--	--	304	197

[a] PL22 shrimp.
[b] PL15 shrimp.

to be the most toxic components of oil (Anderson *et al.* 1974). In addition, because previous investigators have based TI calculations on total petroleum hydrocarbons, this index was calculated for comparative purposes (**Table 11**). Finally, TI determinations for BTEX were calculated (**Table 12**) because of the high concentrations measured in the exposures; BTEX compounds may account for some of the toxicity evident in testing results.

The TI approach, as described by Anderson *et al.* (1984), is based on the premise that toxicity is a function of not only concentration but also duration of exposure. As an example, if toxicity is a function of time and concentration, then it can be expected that an exposure to 5 ppm of a contaminant for 4 hrs is equivalent to 10 ppm for 2 hrs (i.e., 20 ppm-hrs).

The application of this approach is particularly appropriate for the present study. In the flowthrough tests, hydrocarbon concentrations were rapidly changing during the course of the exposures. Therefore, it is not valid to compare toxicity to various species solely on the basis of initial hydrocarbon exposures. At the same time, some tests were done under static conditions where contaminant levels were more constant. These tests are not then comparable to flowthrough exposure conditions. The TI approach allows a comparison of exposure results under different conditions.

4.3.1.1 Brown Shrimp (*Penaeus aztecus*)

Life History

Brown shrimp, *Penaeus aztecus*, ranges from Massachusetts through the Gulf of Mexico to the Yucatan Peninsula, with the exception of the Gulf coast between Sanibel and Apalachicola Bay (Florida) where it is absent (Lassuy 1983). According to Christmas *et al.* (1966) and Kutkuhn (1966), spawning of brown shrimp is reported to occur primarily offshore in waters deeper than 18 m. Spawning extends from September through May, however reports of year-round spawning are not uncommon. Eggs are externally fertilized and released into the water column, where they hatch within 24 hrs into the first naupliar stage (Lassuy 1983). As with other penaeid species, brown shrimp larvae pass through five naupliar, three protozoeal, and three mysis stages over a 10- to 25-day period before becoming postlarvae (Perez-Farfante 1969; Lassuy 1983).

Toxicity Testing Results

Brown shrimp were obtained on only one occasion (April 1992) during the course of the study and only in sufficient numbers to allow testing with the Western Gulf WAF and dispersed oil. The TI values for this species are presented in **Tables 10** through **12**. All of the toxicity in the exposures occurred within the first 24 hrs with little to no toxicity observed in subsequent days. This is consistent with hydrocarbon concentration patterns where the majority of the materials were lost within the first 24 hrs of exposure.

4.3.1.2 White Shrimp (*Penaeus setiferus*)

Life History

White shrimp, *Penaeus setiferus*, are found in U.S. Atlantic waters from Fire Island, New York, to St. Lucie Inlet, Florida; within the Gulf of Mexico, this species is found from

Apalachee Bay, Florida, to Ciudad, Mexico (Muncy 1984). Preferred water depths for this species range from 8 to 55 m. Spawning is directly correlated with water temperature (i.e., spawning begins in spring with increases in water temperature, then decreases in fall with decreasing temperatures). Fertilized eggs are discharged directly into the water column and sink to the bottom (Muncy 1984). Eggs hatch into planktonic nauplii within 10 to 12 hrs after fertilization (Klima *et al.* 1982). Nonfeeding nauplii undergo five molts over a 24- to 36-hr period to become free-feeding protozoea (Anderson 1966). As with other penaeid shrimp species, five naupliar, three protozoeal, and three mysis stages are realized over a 10- to 12-day period, after which the initial postlarval stage is reached (Muncy 1984).

Toxicity Testing Results

White shrimp tests were conducted in August and September 1992 with the Western and Central Gulf oils using postlarvae at ages of 15 and 22 days, respectively (i.e., PL15, PL22). As with the oil exposures, the majority of the toxicity occurred within the first 24 hrs. **Tables 10** through **12** present TI values for this species.

In tests with the Western Gulf oil, a higher level of mortality was seen in control and low-concentration exposures than was observed with the Central Gulf oil. It is unknown whether this diminished vitality was due to the fact that these shrimp may have been less healthy (e.g., due to their longer stay in the laboratory) or to timing associated with molting. It should be noted, however, that survivors from the acute toxicity testing were maintained for 30 days after completion of the test; a few shrimp were kept in the laboratory for a year after completion of the tests.

In the WAF, survival remained fairly constant in all exposure concentrations until Days 3 and 4, at which time mortality increased in all concentrations including controls. Nevertheless, at the end of the test, equal survival was measured in all concentrations (i.e., 36 to 44%). This would suggest that these animals were, on the whole, less sensitive to the dispersed oil exposure than were the PL15 shrimp used with the Central Gulf oil.

It is noteworthy that this same pattern was evident in the dispersant only test where mortalities in the control and low concentrations suddenly increased on Days 3 and 4. However, unlike the lower concentrations (where survival was greater than 50% in concentrations less than 25 ppm after 48 hrs), only 20 and 35% of the animals were alive in the 100- and 50-ppm concentrations, respectively. By the end of the test, similar levels of survival were measured in all concentrations (i.e., 28 to 52%), with the exception of the 100-ppm level where no shrimp survived. These results indicate that the slightly older shrimp were less sensitive to the dispersant and hydrocarbon exposures overall but may have experienced a period of sensitivity during a molt.

After the 96-hr exposures were completed, surviving shrimp were transferred to clean artificial seawater and grown for an additional 30 days. Weights of the animals at the end of this period are shown in **Table 13**. Control weights in the two sets of test were consistent but slightly higher in the Central Gulf oil exposures. This may suggest age-related stress (i.e., shrimp in the Western Gulf exposures were about a week older at the time of their weighing). Effects on growth from the exposures themselves were noticeable in the surviving shrimp at the highest concentration in the Western Gulf dispersed oil and to a lesser extent in WAF. No effects were seen on growth in the 50-ppm concentration of the dispersant. These results are consistent with the findings from the acute toxicity testing.

Table 13. Summary of survival and growth data for white shrimp (*Penaeus setiferus*) following 96-hour exposures and 30-day recovery periods.

Test Concentration	% Survival after 96 Hours		Average Dry Weight (grams) after 30 Days Recovery	
	Western	Central	Western	Central
Corexit 9527				
Control	36	68	0.0204 ± 0.0074[a]	0.0224 ± 0.0076[a]
6.25 ppm	12	88	--	--
12.5 ppm	52	40	--	--
25 ppm	36	16	--	0.0140 ± 0.0010
50 ppm	28	0	0.0229 ± 0.0117	--
100 ppm	0	0	--	--
Water Accommodated Fraction				
Control	44	84	0.0204 ± 0.0074[a]	0.0224 ± 0.0076[a]
6.25%	40	76	--	--
12.5%	36	72	--	--
25%	40	72	--	--
50%	36	40	--	--
100%	44	12	0.0161 ± 0.0063	0.0156 ± 0.0054
Dispersed Oil				
Control	80	76	0.0204 ± 0.0074[a]	0.0224 ± 0.0076[a]
6.3 ppm	76	72	--	--
12.5 ppm	72	68	--	--
25 ppm	24	32	--	0.0202 ± 0.0060
50 ppm	36	0	--	--
100 ppm	8	0	0.0079 ± 0.0027	--

[a] represents the average of three pooled control samples.

In the Central Gulf exposures, reduced growth was noted in the 25-ppm dispersant concentration and in the 100% WAF. Slightly lower (though not significant) weights were observed in the 25-ppm concentration of the dispersed oil. Again, these results closely paralleled the results of the toxicity tests.

4.3.1.3 Blue Crab (*Callinectes sapidus*)

Life History

The blue crab, *Callinectes sapidus*, ranges from Nova Scotia to northern Argentina, including waters of the Gulf of Mexico, with introduced specimens also noted in waters off Europe and within the Mediterranean Sea (Oesterling 1976). Characterized as a coastal inhabitant, this species ranges from the shoreline to water depths of 90 m. This species also utilizes the extensive bays and estuaries of the Gulf coast during portions of its life cycle. According to Steele (1979), the eggs of blue crab change color from bright orange to brown through absorption of the yolk sac by the embryo and development of dark pigmentation in the eyes. The number of eggs shed during a spawn (i.e., as an egg "sponge") by a single female range from 700,000 to two million, with only one ten-thousandth of one percent (i.e., 0.000001) surviving to reach adulthood (Van Engel 1958). The remainder succumb to predators, fungal infection, or from excessively high or low salinities or temperatures. Eggs are typically carried from 7 to 14 days, after which they hatch into a planktonic zoea larval stage. Pre-zoeal stage hatching must occur between salinities of 23 to 33 ppt; similarly, ambient water temperatures must be between 19 and 29°C to ensure survival (Steele 1979). According to Steele (1979), larvae reared by Costlow and Bookhout (1959) exhibited seven zoeal stages, together lasting 31 to 49 days, and one megalopal stage lasting 6 to 20 days. Zoeal stage duration is highly dependant upon water temperature and salinity (Oesterling 1976).

Toxicity Testing Results

Blue crab exposures were conducted with Central Gulf oil in September 1992 and with Western Gulf oil in September 1993. Based on results of the dispersant only tests, similar sensitivities to the Corexit 9527 were obtained in the two tests (**Table 9**). Similarly, the results of the exposures to the WAF did not differ greatly. In both tests, survival in the 50% concentrations was 80%. The 100% concentrations had survival of 0% in the Central Gulf oil and 64% with the Western Gulf oil. In both sets of tests, the megalopa larvae were metamorphosing to the crab stage while the test was ongoing. While most of the toxicity in the dispersed oil tests appeared to occur within the first 24 hrs, mortalities in the WAF and dispersant mixtures were most evident after the first 24 hrs. This suggests a greater sensitivity to the dispersed oil based on test results. However, it may also be that the timing of molts was a particularly critical period with regard to the severity of effects and may explain the differences observed between the two oils.

4.3.1.4 Eastern Oyster (*Crassostrea virginica*)

Life History

The eastern or American oyster, *Crassostrea virginica*, inhabits estuaries and waters inshore of barrier islands of the Gulf of Mexico, ranging from Florida to Texas. This species is also found along the eastern U.S. coast from the Gulf of St. Lawrence (Canada) to the Florida Keys, and from the Yucatan Peninsula to Venezuela. This species is oviparous, where gametes are released directly into the water. The morphology of larval stages has been described by

Kilgen and Dugas (1984). Considerable variability has been noted by researchers regarding preferred spawning periods; however, spawning appears to be correlated to water temperature. The timing and intensity of spawning is directly related to the presence of male sperm and an associated pheromone in the water (Stanley and Sellers 1986). Female egg production ranges from 23 million to 86 million eggs, however egg counts as high as 115 million have been recorded. The eggs hatch 6 hrs after fertilization at water temperatures near 24°C (Loosanoff 1965). Oyster larvae are meroplanktonic, remaining in the water column for two to three weeks after hatching. During this period, the larvae pass through several developmental stages. According to Parrish (1969), after the blastula (3.2 hrs), gastrula (4.5 hrs), and trochophore (10 hrs) stages, larvae develop a shell and locomotory cilia (velum) and are termed prodissoconch I. A subsequent prodissoconch II stage follows. Two to three weeks after hatching, oyster larvae seek a solid surface for attachment.

Toxicity Testing Results

The oyster embryos were exposed in static tests to Corexit 9527 and to the dispersed oil mixtures during August 1992 and September 1993. No tests were conducted with the WAF due to difficulties in getting animals to spawn.

Table 14 summarizes results for the oyster tests based on an EC_{50} (for numbers of surviving larvae), lowest observed effects concentrations (LOEC), and no observed effects concentrations (NOEC). These latter two statistics were calculated using the Kruskal-Wallis method. The dispersant was particularly toxic to the embryo/larval stages, resulting in an EC_{50} of less than 6.25 ppm. **Table 14** reflects an EC_{50} of 4.9 mg/l, which represents an extrapolated value because the lowest exposure concentration was 6.25 ppm. Similarly, the dispersed oil mixtures had statistically significant effects at the lowest concentrations tested (i.e., 6.25 ppm). In exposures to the Central Gulf dispersed oil, the EC_{50} was less than 6.25 ppm dispersed oil. The Western Gulf dispersed oil had a lower toxicity than the Central Gulf dispersed oil with an NOEC of 6.25 ppm and an LOEC of 12.5 ppm.

4.3.2 Fish

Results of acute toxicity testing conducted on the early life stages of four fish species were qualified, to a limited extent, by difficulties realized during testing. The early larval stages were sensitive to any type of handling, a characteristic which was exacerbated by exposure in a flowthrough system. The fish tested all characteristically exhibited a high natural mortality which contributed to reduced survival in controls. Because of difficulties with survival encountered in the flowthrough tests, the majority of the most recent fish tests completed (i.e., in 1993) were conducted under static conditions. While this minimized handling, it was not possible to overcome the inherently high mortality of the larval organisms. Acceptable test data could generally be obtained over 48 hrs, but 96-hr exposures were only minimally successful.

Given these caveats, a summary of LC_{50} determinations for the fish is presented in **Table 15**. **Tables 16** through **18** compare these results based on a TI value, using either ppb- or ppm-hrs as the measure of toxicity. **Table 16** considers total naphthalenes, whereas **Tables 17** and **18** present results for total TPH and BTEX, respectively. Overall, the menhaden appeared to be the most sensitive of any of the animals tested. This may be somewhat misleading, however, because of the difficulty in obtaining acceptable test data for the red drum which were especially sensitive to handling. The dispersed oil also seemed to be less toxic to

Table 14. Test results for eastern oyster (*Crassostrea virginica*) exposed to Corexit 9527 and dispersed oils.

Test Statistic	Dispersant	Western Gulf Dispersed Oil	Central Gulf Dispersed Oil
EC_{50} (mg/l)	4.9[a] (3.1-8.9)	11.2 (7.9-13.9)	4.0 (3.3-5.8)
NOEC (mg/l)	<6.25	6.25	6.25
LOEC (mg/l)	6.25	12.5	12.5

[a] represents an extropolated EC_{50} value; mg/l = ppm.

Table 15. LC_{50} determinations (based on nominal concentrations) for vertebrate species exposed to the water accommodated fractions (WAF) of Western and Central Gulf oil, dispersed oil mixtures, and dispersant alone (Corexit 9527).

Species	WAF (%)	Dispersant (mg/l)	Oil & Dispersant (mg/l dispersant)
Western Gulf Oil			
Inland silverside (*Menidia beryllina*) larvae[a]			
24 and 96 hr	66.4[b]	42.5[b]	59.4[b]
embryos			
96 hr	>100	>100	>100
Atlantic menhaden (*Brevoortia tyrannus*)			
24 hr	>100	--	>100
48 hr	64.1	42.4	22.2
Red drum (*Sciaenops ocellatus*)			
24 hr	>100	--	>100
48 hr	>100	52.6	>100
Spot (*Leiostomus xanthurus*)			
24 hr	>100	--	>100
48 hr	>100	27.4	68.2
Central Gulf Oil			
Inland silverside (*Menidia beryllina*) larvae			
48 hr	81.1	--	>100
96 hr	59.1	46.7	>100
embryos			
96 hr	>100	>100	>100
Atlantic menhaden (*Brevoortia tyrannus*)			
24 hr	66.2	--	75.7
48 hr	42.1	42.4	64.6
Red drum (*Sciaenops ocellatus*)			
24 hr	>100	--	>100
48 hr	74.0	>100	>100
Spot (*Leiostomus xanthurus*)			
24 hr	>100	--	>100
48 hr	70.7	27.4	50.3

[a] indicates replicate analyses (Western Gulf WAF only).

[b] tablular entries represent average values; WAF ranged from 60-72.8%; dispersant ranged from 28.3-56.7 mg/l; dispersed oil ranged from 52.8-66.0 mg/l.

Table 16. Toxicity Index determinations (ppb-hrs) based on total naphthalenes concentrations for fish exposed to the water accommodated fractions (WAF) and dispersed oil mixtures of Western and Central Gulf oils.

Species	WAF		Oil & Dispersant	
	Western	Central	Western	Central
Inland silverside (*Menidia beryllina*)				
larvae				
24 hr	844 (1,024)[a]	--	2,285 (22,793)[a]	--
48 hr	--	977	--	>2,785
96 hr	1,974 (2,395)[a]	895	4,689 (6,053)[a]	>5,012
embryos				
96 hr	>3,290	>1,514	>8,315	>5,012
Atlantic menhaden (*Brevoortia tyrannus*)				
24 hr	>1,407	570	>3,809	2,108
48 hr	1,557	507	1,260	2,575
Red drum (*Sciaenops ocellatus*)				
24 hr	>1,407	>861	>3,809	>2,785
48 hr	>2,429	892	>2,429	>3,986
Spot (*Leiostomus xanthurus*)				
24 hr	>1,407	>861	>3,809	>2,785
48 hr	>2,429	852	3,870	2,005

[a] denotes results of duplicate analyses.

Table 17. Toxicity Index determinations (ppm-hrs) based on TPH concentrations for fish exposed to the water accommodated fractions (WAF) and dispersed oil mixtures of Western and Central Gulf oils.

Species	WAF		Oil & Dispersant	
	Western	Central	Western	Central
Inland silverside (*Menidia beryllina*)				
larvae				
24 hr	131 (458)[a]	--	159 (396)[a]	--
48 hr	--	338	--	>949
96 hr	326 (1,357)[a]	322	572 (1,696)[a]	>2,288
embryos				
96 hr	>544	>572	>2,570	>2,288
Atlantic menhaden (*Brevoortia tyrannus*)				
24 hr	>218	183	>867	718
48 hr	267	163	341	1,014
Red drum (*Sciaenops ocellatus*)				
24 hr	>218	>277	>867	>949
48 hr	>417	286	>1,534	>1,570
Spot (*Leiostomus xanthurus*)				
24 hr	>218	>277	>867	>949
48 hr	>417	273	1,046	790

[a] denotes results of duplicate analyses.

Table 18. Toxicity Index determinations (ppb-hrs) based on total BTEX concentrations for fish exposed to the water accommodated fractions (WAF) and dispersed oil mixtures of Western and Central Gulf oils.

Species	WAF		Oil & Dispersant	
	Western	Central	Western	Central
Inland silverside (*Menidia beryllina*)				
larvae				
24 hr	143,760 (174,429)[a]	--	4,572 (5,715)[a]	--
48 hr	--	358,108	--	>8,556
96 hr	414,277 (502,656)[a]	404,527	13,411 (16,763)[a]	29,495
embryos				
96 hr	>690,462	>684,479	>25,399	>29,495
Atlantic menhaden (*Brevoortia tyrannus*)				
24 hr	>239,600	138,577	>8,659	6,477
48 hr	283,043	178,751	2,639	11,101
Red drum (*Sciaenops ocellatus*)				
24 hr	>239,600	>209,331	>8,659	>8,556
48 hr	>441,564	314,194	>15,523	>17,184
Spot (*Leiostomus xanthurus*)				
24 hr	>239,600	>209,331	>8,659	>8,556
48 hr	>441,564	300,183	10,587	8,644

[a] denotes results of duplicate analyses.

the fish than did the WAF. In general, the ranges of the TI values for fish and invertebrates were similar.

4.3.2.1 Inland Silverside (*Menidia beryllina*)

Life History

Inland (or tidewater) silverside, *Menidia beryllina*, occur in estuaries along the Gulf coast. Spawning occurs in the upper reaches of estuarines during daytime high tides, with preference shown for the oligohaline and tidal freshwater portions of estuarines. Eggs are deposited on various substrates, all of which provide protection from thermal stress and desiccation. The egg stage lasts for 4 to 14 days (depending on temperature), whereas the *Menidia* larval stages last for 14 to 28 days (K. Fucik, personal communication 1994).

Toxicity Testing Results

Tests with inland silverside were conducted under both static and flowthrough exposures in April and December 1992 and January 1993. Animals were provided by a commercial supplier as laboratory-cultured specimens. Test data were acquired from both embryo and larval exposures. **Table 19** provides results of 96-hr embryo/larval exposures to the Western Gulf oil followed by recovery to Day 9. The numbers of surviving embryos and larvae were similar after 96 hrs in the three different exposure media (i.e., WAF, dispersed oil, and dispersant). By Day 9, the fewest survivors overall were found in the WAF and the dispersant exposures. When compared to the controls, the 50% and 50 mg/l concentrations showed a similar rate of mortality in the three exposures, suggesting a delayed response to the exposures.

A subsequent flowthrough test confirmed the relative insensitivity of the fish when the exposure was begun in a late embryonic stage (**Table 20**). In these tests, equally high hatching rates were measured in all of the exposures. No effects on fry survival were measured in the three exposures at the end of the 96-hr tests. No attempt was made to extend these tests beyond this period. By comparison, a static test exposure had effects in reducing hatching rates in both a 75% and 50% WAF (**Table 21**), possibly due to the effects of higher exposure concentrations than those observed in flowthrough tests.

Tests which had begun with the larvae produced similar results during exposures to Western and Central Gulf oils. An exposure to the Central Gulf dispersed oil gave somewhat anomalous results as no toxicity was measured. Low dissolved oxygen occurred in some of the 100% mixtures which could have accounted for some of the variability in the tests. These were static exposures and it appeared that the dissolved oxygen reductions were triggered by the decomposition of the animals since other static exposures did not experience oxygen problems.

4.3.2.2 Atlantic Menhaden (*Brevoortia tyrannus*)

Life History

According to Powell (1993), Atlantic menhaden (*Brevoortia tyrannus*) and gulf menhaden (*B. patronus*) exhibit significant differences in early life history traits, as determined under laboratory conditions. For example, Powell (1993) found that the eggs of the Atlantic species were larger and contained more yolk when compared to the gulf form; Atlantic larvae were larger at hatching and also contained more yolk. In general, developmental events (e.g., yolk depletion, age at first feeding, age at metamorphosis) were reached first by *B. tyrannus*. These differences between species have been attributed, in part, to the diverse nature of the

Table 19. Exposure and long-term recovery of inland silverside (*Menidia beryllina*) larvae exposed to the dispersant and the water accommodated fraction and dispersed oil mixture of Western Gulf oil.[a]

Concentration	Survival (%)					
	Water Accommodated Fraction		Dispersed Oil		Dispersant	
	96 hours	9 days	96 hours	9 days	96 hours	9 days
Control	98	40	96	74	94	50
3	100	70	98	82	96	58
6	96	34	92	68	98	52
12	96	30	98	84	94	52
25	96	20	96	74	94	62
50	98	28	98	40	96	30

[a] fish were exposed as embryos but had hatched by Day 9.

Table 20. Sensitivity of late embryonic stages (fry) of inland silverside (*Menidia beryllina*) from exposure to Western Gulf oil (i.e., water accommodated fraction, dispersed oil mixture) and dispersant in a flowthrough exposure.

Concentration	% Hatch	% Fry Survival[a]
Water Accommodated Fraction (%)		
Control	82	82
6.25	92	91
12.5	88	98
25	78	74
50	92	85
100	88	77
Dispersed Oil (mg/l)		
Control	56	68
6.25	90	82
12.5	92	89
25	98	84
50	96	67
100	90	69
Dispersant (mg/l)		
Control	96	88
6.25	74	89
12.5	66	82
25	52	90
50	92	91
100	92	87

[a] fry survival is based on the percent of embryos hatched, not the total number of embryos exposed.

Table 21. Hatching success of inland silverside (*Menidia beryllina*) exposed to the water accommodated fraction of the Western Gulf oil in a static exposure.

Concentration (%)	% Hatch
Control	100
6.25	98
12.5	98
25	96
50	78
75	38

coastal environment (e.g., temperature extremes) frequented by the Atlantic congener. Some general similarities, however, do exist relative to the early life histories of each species. Both species are morphologically-similar filter-feeding clupeids that utilize estuaries during their first year of life.

According to Trudel *et al.* (1988b), gulf menhaden (*Brevoortia patronus*) are found within the estuarine and nearshore waters of the Gulf of Mexico, ranging from Cape Sable, Florida to Vera Cruz, Mexico. Gulf menhaden spawn from October to March, with peak spawning evident in December. Considerable variability in spawning has been noted. Spawning appears to be most intense in the northern Gulf of Mexico, from the Florida-Alabama border to 90°30' W and from 91°30' W to 93°00' W, in water depths of 8 to 80 m. Menhaden eggs float near the surface, hatching in 2 to 3 days following their release. Larvae spend three to five weeks in gulf waters before moving into estuaries. Timing of estuarine immigration is variable; northern gulf studies indicate that larvae are present in gulf waters from October to April, with peak abundance evident from December to March. Larvae are found in northern gulf estuaries from September to May, with peak numbers variable from year to year; postlarvae are distributed throughout estuaries until June when they move to shallow, low salinity waters. The development time from hatching to juveniles is not known, but all young of the year appear to be juveniles by May or June (Trudel *et al.* 1988a).

Toxicity Testing Results

Atlantic menhaden were obtained on only one occasion during the course of the study (i.e., March 1993). Toxicity testing was conducted under static exposure conditions because of the sensitivity of the animals. Test specimens were received as embryos within approximately 36 hrs after their release from females. Tests were initiated with embryos and hatching occurred within the first 24 hrs of the test. Results were obtained in only the 48-hr exposures because of the significant mortalities which occurred in the controls. However, the test results provided clear patterns of toxicity during these exposures.

Two oils were tested and produced similar results in a comparison of the WAF and dispersed oil. Only one acceptable test was achieved in the dispersant exposures. The sensitivity of this species was comparable to that observed for spot (*Leiostomus xanthurus*).

4.3.2.3 Spot (*Leiostomus xanthurus*)

Life History

Spot, *Leiostomus xanthurus*, spawn during late fall and winter in the coastal waters of the Gulf of Mexico. Spot eggs are relatively small and develop rapidly (Powell *et al.* 1990). Young, pelagic larvae are typically transported shoreward towards nearby estuarine nursery areas to undergo transformation into juveniles (Govoni *et al.* 1983).

Toxicity Testing Results

Toxicity testing of spot, completed in February 1993, was conducted under similar conditions as those realized for Atlantic menhaden. Spot appeared to be more sensitive to the Central Gulf oil compared to the Western Gulf oil. As with Atlantic menhaden, test results were obtained for 48-hr exposures due to significant control mortality. An 80% mortality rate during a spawn is considered normal for this species (W. Hettler, NMFS, personal communication 1993).

Tables 22 and **23** summarize hatching success and fry survival in various exposures to Western and Central Gulf oils. In both sets of tests, similar results were obtained, indicating

Table 22. Hatching success and fry survival for spot (*Leiostomus xanthurus*) exposed to the water accommodated fraction of Central Gulf oil, dispersed oil mixture, and dispersant alone (Corexit 9527).

Concentration	% Hatch	48-hour Fry Survival	96-hour Fry Survival
Water Accommodated Fraction (%)			
Control	54	65	30
6.25	38	77	52
12.5	28	75	43
25	26	55	0
50	4	10	0
100	0	0	0
Dispersed Oil (mg/l)			
Control	50	90	20
6.25	30	80	3.3
12.5	38	89	0
25	28	75	0
50	26	72	0
100	16	40	0
Dispersant (mg/l)			
Control	38	85	42
6.25	48	100	47
12.5	42	97	10
25	44	90	0
50	32	50	0
100	8	0	0

Table 23. Hatching success and fry survival for spot (*Leiostomus xanthurus*) exposed to the water accommodated fraction of Western Gulf oil, dispersed oil mixture, and dispersant alone (Corexit 9527).

Concentration	% Hatch	48-hour Fry Survival	96-hour Fry Survival
Water Accommodated Fraction (%)			
Control	48	95	38
6.25	54	83	33
12.5	40	79	29
25	26	53	33
50	32	87	0
100	16	30	0
Dispersed Oil (mg/l)			
Control	58	97	0
6.25	40	50	0
12.5	46	50	0
25	36	48	0
50	36	73	0
100	28	29	0
Dispersant (mg/l)			
Control	38	85	42
6.25	48	100	47
12.5	42	97	10
25	44	90	0
50	32	50	0
100	8	0	0

that hatching success was similar between WAF and dispersed oil exposures and that effects were most evident in only the highest exposure concentrations after 48 hrs. In both cases, 96-hr survival was lowest in the dispersed oil mixtures.

4.3.2.4 Red Drum (*Sciaenops ocellatus*)

Life History

Red drum, *Sciaenops ocellatus*, also known as redfish or channel bass, range along the Atlantic coast from the Gulf of Maine to the Florida Keys; in the Gulf of Mexico, this species is found from extreme southwest Florida continuously along the Gulf coast into northern Mexico (Trudel *et al.* 1988b). Red drum spawn from late August to early October in the western Gulf of Mexico (Holt *et al.* 1989), however spawning is quite variable throughout the Gulf (Trudel *et al.* 1988b). Young red drum move into bays and estuaries as larvae (i.e., 1 to 7 mm) and come to rest in shallow vegetated areas where they quickly (i.e., within one month) mature into juveniles (>15 mm). Following their release, eggs hatch in approximately 1 day; larvae measurements range from 4 to 6 mm, whereas postlarvae range from 7 to 15 mm (Reagan 1985).

Toxicity Testing Results

Tests with red drum were attempted on numerous occasions during the course of the study, however this species proved to be the most difficult in attempts to obtain acceptable test results. In those tests where acceptable results were obtained for the two oils, this species proved to be relatively insensitive to the exposures (i.e., high TI values). **Table 24** indicates the hatching success realized by test organisms exposed to the Western Gulf oil in a flowthrough system. Hatching success was affected only at the highest concentration of the dispersed oil and in the higher concentrations of the dispersant. The WAF did not appear to significantly affect hatching success.

4.4 CHRONIC TEST RESULTS

4.4.1 Mysid (*Mysidopsis bahia*)

4.4.1.1 Life History

This species is found in salinities primarily above 15 ppt. In culture, the animals reach sexual maturity in 12 to 20 days. Females will begin having eggs in the ovary by Day 12. These eggs will develop after fertilization which generally occurs at night. By Day 15, brood pouches are generally fully formed with young being released between Days 17 and 20. Mature females can produce as many as 25 Stage I larvae (i.e., egg-shaped embryos) per brood but average about 11 Stage III larvae, the final stage before larvae are released. Broods are produced every 4 to 7 days. Juvenile mysids are planktonic for the first 24 to 48 hrs and then settle to the bottom. The juvenile molt occurs 24 to 48 hrs after release from the brood pouch (Weber 1991).

4.4.1.2 Toxicity Testing Results

The WAF at the highest concentration (i.e., 20%) was not toxic to the animals at 48 hrs, however, after seven days an LC_{50} of 17.9% WAF was measured. The NOEC for survival after seven days was 5% WAF. However, growth showed an NOEC of <1.25% WAF. The fecundity NOEC was 1.25% WAF.

Table 24. Hatching success of red drum (*Sciaenops ocellatus*) exposed to the water accommodated fraction (WAF) of Western Gulf oil, dispersed oil mixture, and dispersant alone (Corexit 9527).

Concentration	WAF (%)	Dispersed Oil (mg/l)	Dispersant (mg/l)
Control	58	82	76
6.25	59	87	74
12.5	100	93	82
25	94	87	59
50	78	82	44
100	61	39	0

CHAPTER 5 - DISCUSSION

5.1 OIL CHARACTERIZATION

Table 25 provides a comparison of the ratios obtained between the various kinds of test exposures and demonstrates several findings with regard to the hydrocarbon exposures realized during this study. First, this tabular comparison indicates that replication between tests was good, particularly with regard to hydrocarbon exposures. This finding is particularly noteworthy in comparisons between various flowthrough tests, where total naphthalenes for both the Western and Central Gulf oils were approximately three times that of the WAF. This finding is quite important as it allows one to extrapolate between tests (i.e., a complete characterization of hydrocarbons in test media could not be accomplished for every toxicity test). Further, if it is assumed that PAHs represent the toxic components of the oils tested, these data can also be used to support the hypothesis that the toxicity attributed to dispersed oil should have been three times greater than that attributed to the WAF. Agreement in these ratios was also evident for TPH and BTEX concentrations. This is further evidence for the consistency of flowthrough test exposures and comparability of associated toxicity test data.

Agreement in the static exposures was less evident, suggesting that greater variability is likely in toxicity results originating from this procedure. This variability could be a function of the way in which tests were conducted and the difficulty in reproducing an oyster embryo/larval test in such a way as to be comparable to a fish exposure (i.e., where different types of test containers may be used). Similarly, variability between test results may also reflect differences in the solubility and rates of volatilization of the TPH fraction as opposed to the BTEX compounds, the latter of which tend to be more uniformly soluble as well as volatile.

With only minor exception, it was possible to obtain fairly constant exposures between the flowthrough and static treatments, regardless of whether the test medium was dispersed oil or the WAF. Anomalous results were obtained for the TPHs in 1) the Western Gulf dispersed oil; and 2) the WAF of the Central Gulf oil. The ratios of the dispersed oil were approximately half that of the WAF when the flowthrough and static systems were compared. This may suggest that the overall volatility of the dispersed oil is greater than that of the WAF. This could also be suggested by the fact that the BTEX were found at much higher concentrations in the WAF than in the dispersed oil mixtures.

5.2 ACUTE TOXICITY CHARACTERIZATIONS

The chemical characterizations discussed in **Section 5.1** may explain many of the anomalies observed in the toxicity data. Much of the variability that was seen in the fish tests may have been attributed to the fact that most of these tests were run under static conditions and where some of the greatest variability in TPH concentrations was obtained. By comparison, the TPH, naphthalenes, and BTEX concentrations were relatively uniform in flowthrough tests where most of the invertebrate exposures were completed.

Another source of variability in the tests may arise from the organisms themselves. One of the biggest problems encountered during this study was the difficulty in achieving good control and animal survival during exposures. Although the objective of most toxicity testing is to achieve at least an 80% control survival, such rates were almost impossible to obtain with the larval fish. This was not isolated to this program as others have experienced similar results with

Table 25. Comparative ratios of hydrocarbon levels in the dispersed oil versus the water accommodated fraction (WAF) in flowthrough and static acute toxicity testing systems and in flowthrough versus static exposures.

Type of Exposure	Western Gulf Oil Dispersed	Western Gulf Oil WAF	Central Gulf Oil Dispersed	Central Gulf Oil WAF
Total Naphthalenes				
Flowthrough	2.7[a]		3.0	
Flowthrough: Static	--[b]	--[c]	--	0.1
Static	--[d]		--	
Total Petroleum Hydrocarbons				
Flowthrough	4.7		4	
Flowthrough: Static	0.9	0.16	0.06	0.46
Static	0.87		31.2	
Total BTEX				
Flowthrough	0.04		0.05	
Flowthrough: Static	0.06	0.11	0.07	0.17
Static	0.04		0.05	

[a] ratio of the respective hydrocarbon concentrations for dispersed oil:water accommodated fraction in flowthrough exposures.
[b] ratio of the respective hydrocarbon concentrations for flowthrough:static for dispersed oil.
[c] ratio of the respective hydrocarbon concentrations for flowthrough:static for the water accommodated fraction.
[d] ratio of the respective hydrocarbon concentrations for dispersed oil:water accommodated fraction in static exposures.

these species (based on verbal discussions with Dr. William Hettler, Dr. Joan Holt, and Mr. David Maus). Much of this problem arises because of the naturally high mortality experienced by some of these species. The larval fish were particularly sensitive to handling which necessitated modifying test procedures as well as switching to static rather than flowthrough tests. Numerous attempts were made to complete a successful red drum test, yet only two of these many tests were able to provide data.

Because the goal of this study effort was to use the youngest animals possible, better results might have been obtained with slightly older organisms. For example, the EPA protocols for *Menidia*, a commonly used aquatic toxicity test species, recommend the use of nine-day-old fish for testing. Prior to this age, the fish are difficult to keep alive in their transition through feeding stages. Considerable difficulty was realized during the course of the study in maintaining viable newborn *Menidia* during toxicity testing although the eggs were generally insensitive. If such species are to be used in the future, test protocols should consider the use of older animals which have passed through those life stages where mortality is naturally high.

The invertebrates were less sensitive than the fish to such handling and generally provided acceptable control results. As an example of their durability, larvae of *P. monodon* from a source in Australia were successfully used in tests after 30 hrs in transit to the U.S. However, it was difficult to duplicate invertebrate results which may suggest a wide variation in population sensitivity. It was also difficult to obtain these animals. As a consequence, it was not possible to complete replicate tests, a step which could narrow statistical limits.

As discussed previously, levels of total naphthalenes were typically three times higher in the dispersed oil exposures than in the WAF. This would suggest that the dispersed oil should also be three times as toxic as the WAF, assuming that napthalenes are the primary cause of toxicity. However, when the TI ratios are compared for the dispersed oil versus the WAF, a much different picture emerges. **Table 26** indicates that, on the basis of the TI determinations, dispersed oil toxicity was approximately 20 to 70% that of the WAF. An obvious question arises: Do these results suggest that the dispersed oil is less toxic than the WAF? The answer is no, as in many of the tests, a higher toxicity was frequently measured in the dispersed oil exposures compared to the WAF. However, on the basis of the measured hydrocarbon exposures, it would appear that the dispersed oil is proportionally less toxic than the WAF.

One explanation for this apparent anomaly in the measured toxicities could be related to the actions of the oil following treatment with the dispersant. Throughout this report, the term "water accommodated fraction" or WAF has been utilized to account for the combination of small droplets and dissolved hydrocarbons that are found in the exposure medium. Similarly, when the oil is dispersed, a large proportion of this oil is in the form of small droplets and/or emulsions. Given the size of the animals used in the tests, it is likely that such particles would not be available for ingestion by the animals and would eventually be lost to the system. Similarly, in the closed and semi-enclosed systems used in these tests, solubility limits could have been reached so that the dissolved hydrocarbon concentrations would have been similar in both the WAF and dispersed oil exposures.

Another possible explanation for the differences observed may involve the rate at which the hydrocarbons were lost from the system. As seen with the dispersed oil mixtures, BTEX hydrocarbons were initially present in much lower concentrations than in the WAF. Anderson *et al.* (1981) has previously reported a similar effect in dispersed oil exposures. The loss of these hydrocarbons almost immediately from the system can probably be accounted for

Table 26. Ratios of Toxicity Index determinations (ppb-hrs) for total naphthalenes concentrations in the dispersed oil versus the water accommodated fraction (WAF).

Species	Western Gulf Oil Dispersed:WAF	Central Gulf Oil Dispersed:WAF
White shrimp (*Penaeus setiferus*)	1.5	0.9
Brown shrimp (*Penaeus aztecus*)	2.5	ND[a]
Blue crab (*Callinectes sapidus*)	<2.7	2.5
Eastern oyster (*Crassostrea virginica*)	ND	ND
Inland silverside (*Menidia beryllina*) larvae embryos	2.7 >[b]	<5.6 >[b]
Atlantic menhaden (*Brevoortia tyrannus*)	0.8	3.7
Red drum (*Sciaenops ocellatus*)	>[b]	>4.5
Spot (*Leiostomus xanthurus*)	<1.6	2.4

[a] not determined.
[b] no measured toxicity evident for this species or life stage.

by an "explosive-like" volatilization. This effect may arise due to the increased surface area that would result when oil droplets form following dispersion. However, that would assume that these droplets must come into contact with the atmosphere where BTEX compounds can escape. Given the rate at which this would need to occur to account for the rapid loss, it is likely that the methods used to mix the dispersed oil may have had an influence on the rates of volatilization of BTEX. Shaking, as was used in the present set of tests, would not likely occur in the environment.

The rate of loss of hydrocarbons from the dispersed oil exposures was greater than could be accounted for solely by flow rates. This also indicates volatilization was an important factor in the fate of the hydrocarbons. It was also apparent that most of the toxicity in the dispersed oil exposures occurred within the first 24 hrs. If the total period during which toxic levels are present in the dispersed oil medium is shorter than with the WAF, a proportionately lower toxicity could be expected.

Tables 27 and **28** show TI relationships (i.e., ratios of TI determinations, dispersed oil: WAF) for the TPH and BTEX compounds present, respectively. When TPH TI ratios were compared, considerable variability was evident (**Table 27**), suggesting that TPH ratios alone could not adequately explain the toxicity.

BTEX in the dispersed oil mixtures resulted in <1-8% of the toxicity evident in the WAF. By comparison, BTEX levels in the dispersed oil mixtures were 4-17% of their concentrations in the WAF (**Table 25**). This might suggest that the observed toxicity in WAF exposures was due largely to effects from the BTEX levels rather than from the naphthalenes or TPH.

Identification of BTEX as the primary contributor to toxicity in these tests is consistent with the rapid loss of these compounds from test media and the fact that most of the toxicity occurred within the first 24 hrs of the test. This conclusion has implications with regard to toxic effects following oil spills and the use of dispersants. The data suggest that toxicity to the larval animals may occur very rapidly. It also suggests that the WAF may be as toxic as the dispersed oil because of the difference in BTEX levels that are attained in the WAF versus the dispersed oil mixture. Whether the necessary conditions to produce toxicity in an actual spill condition can be attained (i.e., sufficiently high exposure for the needed duration) will probably vary with environmental conditions at the time of a spill.

An important and historically-consistent finding in these tests was the lower sensitivity of the embryonic stages compared to the early larval stages. The investigations of Sharp *et al.* (1979) indicated that the timing of exposure was significant in influencing toxicity. The most sensitive period seemed to coincide with a period of organogenesis within the embryo. Similarly, Fisher and Foss (1993) found a correlation between toxicity and the stage of egg development in the grass shrimp, *Palaemonetes pugio*. The embryos of spot, Atlantic menhaden, and red drum were equally insensitive to the hydrocarbon exposures. These fish had short incubation periods of between 24 and 48 hrs. These embryos generally reached the laboratory when they were approximately 24 to 30 hrs old. Therefore, exposures probably did not begin until after the most sensitive periods for the embryos had passed. Somewhat greater sensitivity may have been obtained if the embryos had been immediately exposed to the hydrocarbons upon release from the females.

Table 27. Ratios of Toxicity Index determinations (ppm-hrs) for TPH concentrations in the dispersed oil versus the water accommodated fraction (WAF).

Species	Western Gulf Oil	Central Gulf Oil
	Dispersed:WAF	Dispersed:WAF
White shrimp (*Penaeus setiferus*)	<0.3	7.0
Brown shrimp (*Penaeus aztecus*)	1.6	--
Blue crab (*Callinectes sapidus*)	<0.8	18.7
Eastern oyster (*Crassostrea virginica*)	--	--
Inland silverside (*Menidia beryllina*) larvae embryos	1.2 >[a]	>7.1 >[a]
Atlantic menhaden (*Brevoortia tyrannus*)	1.3	3.9
Red drum (*Sciaenops ocellatus*)	>[a]	>5.4
Spot (*Leiostomus xanthurus*)	<2.5	2.9

[a] no measured toxicity evident for this species or life stage.

Table 28. Ratios of Toxicity Index determinations (ppb-hrs) for total BTEX concentrations in the dispersed oil versus the water accommodated fraction (WAF).

Species	Western Gulf Oil	Central Gulf Oil
	Dispersed:WAF	Dispersed:WAF
White shrimp (*Penaeus setiferus*)	<0.03	0.03
Brown shrimp (*Penaeus aztecus*)	0.05	--
Blue crab (*Callinectes sapidus*)	<0.06	0.08
Eastern oyster (*Crassostrea virginica*)	--	--
Inland silverside (*Menidia beryllina*) larvae embryos	0.03 >[a]	>0.08 >[a]
Atlantic menhaden (*Brevoortia tyrannus*)	0.009	0.05
Red drum (*Sciaenops ocellatus*)	>[a]	>0.05
Spot (*Leiostomus xanthurus*)	<0.02	0.03

[a] no measured toxicity evident for this species or life stage.

The overall sensitivity of the fish versus invertebrates appears to be relatively similar. The invertebrates performed better as test organisms, as overall survival in controls was better. The naturally high mortality of the fish larvae confounded efforts to obtain acceptable test results and necessitated repeating several of the tests many times. The invertebrate tests, however, were generally accomplished with good control survival and results.

A question that has often arisen with regard to this type of testing is the applicability of test results between geographic regions and species. McAuliffe (1987) has compared the relative sensitivities of marine fish and invertebrates using ppb- or ppm-hrs (i.e., TI). Many of the species compared by McAuliffe (1987) are from colder environments than those used in the present study. Nevertheless, the results reported by McAuliffe (1987) for both the fish and invertebrates are variable but within the ranges found in this investigation.

In other acute toxicity tests conducted on a tropical Australian shrimp species (*Penaeus monodon*) and a penaeid shrimp cultured in south Texas (*P. vannemai*), LC_{50} determinations for exposures to Corexit 9527 ranged from 35 to 45 ppm for both species (The SeaCrest Group 1993). This is less toxic than the approximately 12 ppm determined for white shrimp (*P. setiferus*) in the present study but is probably within the ranges of variability observed in these types of tests.

Finally, it was an objective of this study to compare the results of the embryo-larval testing with those obtained by Shuba and Heikamp (1989) for juvenile and adult fish and invertebrates of the same species. **Table 29** compares the results of Shuba and Heikamp (1989) with those realized during the present study. As was evident in this study, toxicity occurred rapidly within the tests. Within 24 hrs, the lowest LC_{50} value had been reached and did not differ markedly from the 96-hr LC_{50} values. This was consistent with their chemical analyses in which the hydrocarbon concentrations had largely been lost from the system within the first 24 hrs. Overall, the trends appear to be consistent between the two studies and suggest that the juvenile and adult invertebrates were only slightly less sensitive than the larval organisms. Comparable fish data were available only for the red drum (redfish); in this case, no clear distinctions were possible although this species appeared to be more sensitive than the invertebrates tested. These results are largely consistent with the existing literature (e.g., see McAuliffe 1987 for a review).

There are some obvious difficulties in comparing the data of Shuba and Heikamp (1989) with the current study results. Chief among these are the different oil types used in the exposures, as well as different methodologies for measuring the hydrocarbon concentrations during exposures. Shuba and Heikamp (1989) note that the methodologies used to measure the PAHs caused problems in obtaining satisfactory results in their analyses. This was evident in their test results which showed inconsistency between exposures.

By comparison, the methodologies used in the present set of tests gave fairly consistent results in terms of initial exposure concentrations. The only exception to this trend was evident in several of the TPH determinations from static test exposures.

A considerable effort has been expended over the years in evaluating the effects of oil and dispersed oil on marine organisms. Yet, the question still arises as to the value of these tests in making decisions concerning the use of dispersants during actual spill conditions. In all cases, such decisions regarding the use or non-use of dispersants will come down to a tradeoff between the natural resource damages that occur by dispersing oil versus oil-associated impacts

Table 29. Comparison of 96-hour LC_{50} determinations (mg/l or ppm) for dispersed oil exposures from the present study with those of Shuba and Heikamp (1989) using Mayan crude and Saudi Arabian light crude.

Species	Test[a]	LC_{50} Determination by Dispersed Oil Type			
		Central Gulf of Mexico[b]	Western Gulf of Mexico[b]	Mayan Crude[c]	Saudi Arabian Crude[c]
Blue crab (*Callinectes sapidus*)	FT/A	19.8	90.8	>150	--
	S/A	--	--	43	--
Brown shrimp (*Penaeus aztecus*)	FT/A	--	52.7	36	23
	S/A	--	--	--	<3
White shrimp (*Penaeus setiferus*)	FT/A	13.8	18.6	44	>16
Eastern oyster (*Crassostrea virginica*)	FT/A	--	--	effect at 2,500	--
	S/A	<6.25[d]	--	--	--
Red drum (*Sciaenops ocellatus*)	S/A	>100	>100	387	166
Atlantic menhaden (*Brevoortia tyrannus*)	S/A	64.6	22.2	--	--
Spot (*Leiostomus xanthurus*)	S/A	50.3	68.2	--	--
Inland silverside[e] (*Menidia beryllina*)	FT/A	>100	59.4	--	--

[a] FT/A = flowthrough, acute exposure; S/A = static, acute exposure.
[b] eggs and/or larvae tested during the present study.
[c] juveniles and adults tested by Shuba and Heikamp (1989).
[d] this represents an extrapolated EC_{50} value.
[e] larvae results only; values represent average LC_{50} determinations.

to sensitive coastal and shoreline habitats. It can certainly be asserted that dispersed oil would have more significant impacts during periods of the year when higher concentrations of larval fish and invertebrate stages are present in the water column. However, the high natural mortalities experienced by many of these species may overshadow any effects from exposure to dispersed oil.

There can be little question that dispersed oil can have effects on larval organisms given significant exposure. However, attempts to measure actual effects on larval organisms in the field following oil spills and dispersant usage is difficult if not impossible to obtain in a scientifically valid manner. While one can project toxic levels of oil from laboratory data and chemical concentrations can be measured in the field, it is much more difficult to get accurate pictures of larval distributions. This is because of the patchy distributions that are generally present and the need for multiple samples to obtain statistically valid numbers.

The present study effort extends our quantification of the effects of oil and dispersed oil on marine organisms. Data acquired do not differ appreciably from those previously obtained in other dispersed oil effects studies. In essence, results from this effort support the contention that dispersed oil can have effects on larval marine organisms. Data indicate that the toxicity occurred rapidly and that complete, 96-hr exposures were not required to induce lethal effects. The possible contribution of BTEX to total toxicity was a surprise given the fact that it is rapidly lost from the system. However, the dispersed oil was not markedly more toxic than the undispersed oil. While this may be an unexpected result in some regards, it would appear that an explanation based on the fate of hydrocarbons in the exposure system can account for the observations.

The TI determinations were useful in explaining these effects and should be considered in future field programs as a means to provide comparative data with laboratory investigations. Singer *et al.* (1990) measured toxicity of Corexit 9527 to the early life stages of four marine species. Concentrations of Corexit producing LC_{50}s were similar to those reported in this study. However, these authors also concluded that the use of a Toxicity Index may not provide truly comparable values across species. They suggested that this may be due to the fact that the index may overlook complex physiological and biochemical processes. However, our data suggest that the index should be calculated on the basis of the concentrations of the causative agent in a product. For instance, BTEX may have been a major contributor to toxicity in the test animals. A TI based on naphthalene or TPH concentrations alone could not wholly account for the observed toxicity. Standardization of the TI approach (i.e., use of total naphthalenes, TPH, total BTEX, etc.) would allow more comparative evaluations between species and geographic regions where oil effects are measured.

It is noteworthy that the present study effort provided repeatable testing conditions from which credible toxicity data were produced. More importantly, this study provided toxicity information for previously untested early life stages and underscored the difficulties inherent in the use of eggs and larvae in acute toxicity testing.

CHAPTER 6 - SUMMARY AND CONCLUSIONS

The flowthrough exposure methods employed in the current study provided consistent hydrocarbon exposures, making it possible to extrapolate between tests. The GC/MS-SIM technique also proved to be an acceptable method for measuring PAH concentrations in the test media. By comparison, Shuba and Heikamp (1989) experienced significant difficulties in their attempts to measure PAHs.

Overall, the two oils were similar in their PAH composition. In both WAF and dispersed oil exposures, hydrocarbons were significantly reduced within the first 24 hrs at rates higher than could be accounted for solely by flow rates. Volatilization has been identified as a major mechanism for the significant loss of hydrocarbons realized during this period.

The invertebrates proved to be the better test organisms because of their relative insensitivity to handling and lower natural mortality rates, both factors which severely limited larval fish testing. Fish embryos were less sensitive to the WAF and dispersed oil than were the larvae, however this may be related, in part, to the particular life stage at which exposures were initiated. Most of the toxicity generally occurred during the first 24 hrs of exposure with minimal toxicity occurring between 48 and 96 hrs. This trend has been interpreted as a function of the rates at which hydrocarbons were lost during the first 24 hrs. This is particularly important when considering the use of dispersants to treat oil spills. While much criticism has been offered concerning the applicability of 96-hr tests to actual field conditions, the existing data would seem to indicate that the degree of toxic effects, whether with the WAF or dispersed oil, will be determined within the first few hours of exposure. This confirms the value of laboratory exposures, particularly under flowthrough conditions, as a means to provide data for estimating effects of dispersants applied in the field.

Because the hydrocarbons are being rapidly lost either through dilution or volatilization, the use of a TI based on ppm- or ppb-hrs provides a means of comparison in situations where hydrocarbon concentrations are rapidly decreasing. However, this also indicates that under actual field conditions, it is not possible to extrapolate from single measurements at time zero to estimate possible toxic effects. The tests indicated that fairly high concentrations (ppm-hrs) were required to produce toxicity in the test species. Given the high dilution rates in the marine environment, frequent chemical sampling will be required within the first few hours of a spill to determine whether toxic conditions are reached.

The use of a TI to compare the effects of WAF versus dispersed oil exposures provided interesting comparisons. These comparisons suggested that the toxicity of the dispersed oil may be proportionally less than the toxicity of the WAF when total hydrocarbon concentrations are compared. Potential explanations include the possibility that either volatilization is enhanced in the dispersed oil compared to the WAF or that a higher proportion of the dispersed oil is emulsified as opposed to dissolved and is, therefore, unavailable to the test animals.

The results of the present study were similar when compared to other studies investigating the effects of dispersed oil on marine fish and invertebrates. The ranges of sensitivities are similar for species from different geographic regions, however, all data show a large variability. The major difficulties encountered in conducting the present set of tests related to the availability of the test organisms and their subsequent sensitivity to handling and high natural mortalities. Under such circumstances, it is not possible to recommend such species for

routine use in toxicological studies. This is unfortunate because the early life stages tend to be the most sensitive. However, under such circumstances, it may also indicate that impacts from such events as oil spills may have proportionally smaller effects than that expected from natural mortality.

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APPENDICES

APPENDIX A

CHEMISTRY RESULTS

APPENDIX A
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PAH RESULTS

				Naphthalene					Fluorene				Phenanthrene				
Sample No.	Oil	Exposure	Time	N	C1N	C2N	C3N	C4N	F	C1F	C2F	C3F	P	C1P	C2P	C3P	C4P
000000-000	Western Gulf	WAF	0	46	45	7.9	6.5	0.018	0.41	0.17	0.007	0.007	1.0	0.099	0.108	0.022	ND*
000000-000	Western Gulf	WAF	6	39	34	6.5	3.9	0.053	0.21	0.072	ND	0.031	0.65	0.3	0.25	0.036	ND
000000-000	Western Gulf	WAF	24	40	36	6.5	3.6	1.2	0.36	2.04	0.70	0.50	0.40	0.97	1.1	0.44	0.028
000000-000	Western Gulf	WAF	48	32	31	5.8	3.7	0.92	0.41	1.1	0.49	0.20	0.30	0.52	0.21	0.031	ND
000000-000	Western Gulf	WAF	96	19	17	3.5	3.0	0.84	0.37	0.95	0.48	0.35	0.30	0.86	1.1	0.47	0.047
924929-001	Western Gulf	O&D	0	25	140	78.0	59.0	35	3.8	9.7	18	18.00	11.00	12	13	14	5.8
924929-003	Western Gulf	O&D	6	5.4	28	33.0	24.0	16	1.2	4.6	11	8.90	2.30	7.3	9.7	13	4.3
924929-005	Western Gulf	O&D	24	0.38	1.6	3.2	9.2	6.7	0.61	1.6	5.1	5.7	1.2	5.4	6.7	5.7	1.2
924929-007	Western Gulf	O&D	48	0.018	0.006	0.004	0.31	0.20	ND	2.0	0.16	0.09	0.12	0.76	1.2	1.1	0.48
924929-009	Western Gulf	O&D	96	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.15
924929-002	Western Gulf	WAF	0	43	50	8.7	4	0.48	0.43	2.5	0.98	0.14	0.76	1.5	0.77	0.60	0.54
924929-004	Western Gulf	WAF	6	7.9	7.7	1.3	0.91	0.076	0.21	3.3	0.068	0.007	0.23	0.56	0.35	0.34	0.44
924929-006	Western Gulf	WAF	24	0.075	ND	ND	ND	ND	0.11	0.006	ND	ND	0.021	0.005	ND	ND	ND
924929-008	Western Gulf	WAF	48	ND	ND	ND	ND	ND	0.004	ND	ND	ND	0.008	ND	ND	ND	ND
924929-010	Western Gulf	WAF	96	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
925214	Central Gulf	O&D	0	38	140	110	97	57	4.9	15	30	28	8.7	26	32	22	5.8
925214	Central Gulf	O&D	6	0.26	1.8	5.4	14.0	10	0.63	3.7	9.2	11	2.1	9	12	8.2	1
925214	Central Gulf	O&D	24	0.056	0.27	0.47	2.3	3.3	0.16	0.92	3.50	3.8	0.39	3.2	5.8	4.3	0.53
925214	Central Gulf	O&D	48	0.019	0.038	0.1	0.48	0.83	0.043	0.24	1.3	1.80	0.07	1.1	2.7	2.4	0.32
925214	Central Gulf	O&D	96	0.014	0.033	0.053	0.3	0.38	0.022	0.12	0.77	0.48	0.04	0.57	1.8	1.8	0.26
925214	Central Gulf	WAF	0	53	67	16.0	5.9	2.4	1	1.3	1.9	0.93	1.2	1.4	0.81	0.38	0.035
925214	Central Gulf	WAF	6	0.16	0.053	0.2	0.1	0.096	0.56	0.24	0.38	ND	1.6	0.24	0.081	0.036	ND
925214	Central Gulf	WAF	24	0.045	0.12	0.06	0.1	0.14	ND	ND	0.14	ND	0.25	ND	0.024	ND	ND
925214	Central Gulf	WAF	48	0.034	0.2	0.1	0.1	0.007	0.27	0.11	0.094	ND	0.30	ND	0.014	0.012	ND

PAH RESULTS (Continued)

				Anthracene		Dibenzothiophene			Chrysene				
Sample No.	Oil	Exposure	Time	An	D	C1D	C2D	C3D	C	C1C	C2C	C3C	C4C
000000-000	Western Gulf	WAF	0	ND	0.022	0.048	0.036	ND	0.067	ND	ND	ND	ND
000000-000	Western Gulf	WAF	6	ND	0.059	0.084	0.083	ND	0.086	ND	ND	ND	ND
000000-000	Western Gulf	WAF	24	ND	0.33	1.3	1.1	0.31	0.045	ND	ND	ND	ND
000000-000	Western Gulf	WAF	48	ND	0.097	1.4	0.59	0.072	0.031	ND	ND	ND	ND
000000-000	Western Gulf	WAF	96	ND	0.12	1.7	0.73	0.14	0.069	ND	ND	ND	ND
924929-001	Western Gulf	O&D	0	ND	7.3	8.8	17	11	0.94	0.63	0.063	0.001	0.011
924929-003	Western Gulf	O&D	6	ND	2.7	7.1	9.7	9	0.61	0.17	0.004	ND	ND
924929-005	Western Gulf	O&D	24	ND	3.4	3	5.1	3.5	0.093	ND	ND	ND	ND
924929-007	Western Gulf	O&D	48	ND	0.24	0.12	0.38	0.18	ND	ND	ND	ND	ND
924929-009	Western Gulf	O&D	96	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
924929-002	Western Gulf	WAF	0	ND	0.24	0.42	0.15	0.002	0.009	ND	ND	ND	ND
924929-004	Western Gulf	WAF	6	ND	0.084	0.095	0.021	ND	ND	ND	ND	ND	ND
924929-006	Western Gulf	WAF	24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
924929-008	Western Gulf	WAF	48	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
924929-010	Western Gulf	WAF	96	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
925214	Central Gulf	O&D	0	ND	5.8	17	29	24	0.97	2.3	3.1	2.5	0.76
925214	Central Gulf	O&D	6	ND	2	5.5	11	9	0.22	1.1	1.1	1	0.081
925214	Central Gulf	O&D	24	ND	0.74	1.8	5.1	4.7	0.3	0.6	0.66	0.62	0.055
925214	Central Gulf	O&D	48	ND	0.3	0.62	2.3	2.2	0.2	0.32	0.5	0.34	0.03
925214	Central Gulf	O&D	96	0.029	0.12	0.24	1.6	1.8	ND	0.22	0.38	0.23	ND
925214	Central Gulf	WAF	0	ND	0.16	1.1	1.1	0.84	0.14	0.035	0.072	0.042	ND
925214	Central Gulf	WAF	6	ND	0.007	0.17	0.18	0.068	0.12	0.016	0.005	0.014	ND
925214	Central Gulf	WAF	24	ND	ND	0.092	0.13	0.041	0.12	0.005	0.003	0.004	ND
925214	Central Gulf	WAF	48	ND	0.005	0.13	0.1	0.039	0.11	0.004	0.001	0.01	ND

PAH RESULTS (Continued)

				Naphthalene					Fluorene				Phenanthrene				
Sample No.	Oil	Exposure	Time	N	C1N	C2N	C3N	C4N	F	C1F	C2F	C3F	P	C1P	C2P	C3P	C4P
925214	Central Gulf	WAF	96	0.019	0.15	0.0	0.1	ND	ND	0.11	0.11	ND	0.34	ND	0.01	0.015	ND
925293-002	Central Gulf	O&D	0	28	57	110	73	84	4.8	17	28	28	8.8	27	31	20	7.8
925293-004	Central Gulf	O&D	6	7.4	23	46	46	53	2.6	10	17	21	5.4	17	20	14	5
925293-006	Central Gulf	O&D	24	0.72	2.70	11	20	32	0.92	4.70	10	14	2.3	9.2	13	9.5	3.4
925304-002	Central Gulf	O&D	48	0.16	0.37	2.3	4.6	12.3	0.26	1.7	4.9	7.8	0.82	4.3	6.5	5.8	2.1
925304-004	Central Gulf	O&D	72	0.07	0.06	0.59	3	6	0.12	0.78	2.3	4.2	0.32	2.2	4.1	3.9	1.6
925304-006	Central Gulf	O&D	96	0.03	ND	0.28	2.1	3.2	0.08	0.44	1.6	2.6	0.18	1.3	2.7	2.8	0.94
925293-001	Central Gulf	WAF	0	51	35	22	9.7	6.0	1.8	2.2	2.2	1.2	1.3	2.3	1.8	1.5	0.13
925293-003	Central Gulf	WAF	6	5.5	3.3	3.1	3.2	0.60	0.39	1.7	1.5	ND	0.59	0.78	0.52	0.51	ND
925293-005	Central Gulf	WAF	24	0.31	0.30	0.52	ND	0.38	ND	0.50	0.37	ND	0.27	0.4	0.24	0.29	ND
925304-001	Central Gulf	WAF	48	0.16	0.06	0.17	ND	0.22	ND	0.6	0.25	ND	ND	ND	0.14	0.21	ND
925304-003	Central Gulf	WAF	72	0.04	0.1	0.74	ND	0.16	ND	1.1	0.59	0.08	0.1	ND	0.11	0.15	ND
925304-005	Central Gulf	WAF	96	0.02	ND	0.2	0.64	0.2	ND	1.8	1.6	0.22	0.07	0.16	0.09	0.16	ND
925344-002	Western Gulf	O&D	0	5.9	32	38	31	12	0.79	2.2	6.9	ND	1.1	7.7	2.7	0.47	ND
925344-004	Western Gulf	O&D	6	0.65	4.3	13	14	4.4	0.21	0.67	2.5	ND	0.7	4.2	2.2	0.36	ND
925344-006	Western Gulf	O&D	24	ND	ND	ND	2	0.34	ND	ND	0.21	ND	0.15	1	1.4	0.4	ND
925344-008	Western Gulf	O&D	48	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.22	0.02	ND
925344-010	Western Gulf	O&D	72	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
925344-012	Western Gulf	O&D	96	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
925344-001	Western Gulf	WAF	0	42	43	20	3.1	ND	0.34	0.09	ND	ND	0.57	0.01	ND	ND	ND
925344-003	Western Gulf	WAF	6	4.5	2.9	1.3	ND	ND	0.02	ND	ND	ND	0.09	ND	ND	ND	ND
925344-005	Western Gulf	WAF	24	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.03	ND	ND	ND	ND
925344-007	Western Gulf	WAF	48	ND	ND	ND	ND	ND	0.01	ND	ND	ND	0.05	ND	ND	ND	ND
925344-009	Western Gulf	WAF	72	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

PAH RESULTS (Continued)

				Anthracene		Dibenzothiophene			Chrysene				
Sample No.	Oil	Exposure	Time	An	D	C1D	C2D	C3D	C	C1C	C2C	C3C	C4C
925214	Central Gulf	WAF	96	ND	ND	0.077	0.09	0.021	0.11	0.006	0.001	0.007	ND
925293-002	Central Gulf	O&D	0	ND	6.5	17	31	23	1	2.5	2.9	2.9	1.2
925293-004	Central Gulf	O&D	6	ND	4.2	9.1	20	17	0.67	1.8	2.5	2.1	0.87
925293-006	Central Gulf	O&D	24	ND	2.2	4.7	13	11	0.5	1.2	1.4	1.4	0.59
925304-002	Central Gulf	O&D	48	ND	0.96	2.6	6.8	6	0.33	0.81	0.88	0.78	0.37
925304-004	Central Gulf	O&D	72	ND	0.5	1.3	3.8	3.6	0.26	0.6	0.51	0.4	0.37
925304-006	Central Gulf	O&D	96	ND	0.33	0.81	2.1	2.3	0.22	0.37	0.28	0.29	0.23
925293-001	Central Gulf	WAF	0	ND	0.31	2.5	2.3	2.4	0.22	ND	ND	ND	0.20
925293-003	Central Gulf	WAF	6	ND	0.05	1.1	1.2	0.33	0.1	ND	ND	ND	0.29
925293-005	Central Gulf	WAF	24	ND	0.01	0.44	ND	ND	0.24	ND	ND	ND	0.15
925304-001	Central Gulf	WAF	48	ND	ND	0.61	0.7	ND	0.17	ND	ND	ND	0.13
925304-003	Central Gulf	WAF	72	ND	ND	0.6	0.84	ND	0.12	ND	ND	ND	0.19
925304-005	Central Gulf	WAF	96	ND	ND	0.54	0.84	0.34	0.12	ND	ND	ND	0.22
925344-002	Western Gulf	O&D	0	ND	1.2	1.5	2.5	0.71	ND	ND	ND	ND	ND
925344-004	Western Gulf	O&D	6	ND	0.68	0.87	1.8	0.61	ND	ND	ND	ND	ND
925344-006	Western Gulf	O&D	24	ND	0.18	0.21	0.92	0.53	ND	ND	ND	ND	ND
925344-008	Western Gulf	O&D	48	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
925344-010	Western Gulf	O&D	72	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
925344-012	Western Gulf	O&D	96	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
925344-001	Western Gulf	WAF	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
925344-003	Western Gulf	WAF	6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
925344-005	Western Gulf	WAF	24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
925344-007	Western Gulf	WAF	48	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
925344-009	Western Gulf	WAF	72	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

PAH RESULTS (Continued)

				Naphthalene					Fluorene				Phenanthrene				
Sample No.	Oil	Exposure	Time	N	C1N	C2N	C3N	C4N	F	C1F	C2F	C3F	P	C1P	C2P	C3P	C4P
925344-011	Western Gulf	WAF	96	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
925344-013		D Only		0.16	ND	ND	ND	ND	ND	ND	ND	ND	0.012	ND	ND	ND	ND
935950-001	Western Gulf	WAF	0	39	30	8.8	0.59	ND	0.1	ND	ND	0.02	0.1	ND	ND	ND	0.01
935950-002	Western Gulf	WAF	6	33	26	7.9	0.34	ND	0.1	ND	ND	0.02	0.1	0.01	ND	ND	0.02
935950-003	Western Gulf	WAF	24	29	23	7.9	0.26	ND	0.1	ND	ND	ND	0.11	ND	ND	ND	0.03
935950-004	Western Gulf	WAF	48	16	13	5.3	0.17	ND	0.07	ND	ND	ND	0.08	ND	ND	ND	0.02
935950-005	Western Gulf	WAF	72	4.2	4.1	2.3	ND	ND	0.02	ND	ND	ND	0.04	ND	ND	ND	0.01
935950-006	Western Gulf	WAF	96	ND	0.25	0.45	ND	ND	ND	ND	ND	0.01	0.01	ND	ND	ND	ND
935950-007	Western Gulf	O&D	0	27	73	94	76	28	1.5	2.3	8.3	9.2	2.5	8	11	8	2.9
935950-008	Western Gulf	O&D	6	18	49	66	52	18	1	1.3	5.3	6	1.7	5.6	6.2	4.1	1.1
935950-009	Western Gulf	O&D	24	12	38	62	63	26	1.4	3.8	6.8	10	2.6	8.7	12	9	3
935950-010	Western Gulf	O&D	48	3.9	18	32	26	8.4	0.53	0.01	2.7	3.6	1.3	3.4	3.7	2	0.28
935950-011	Western Gulf	O&D	72	0.15	4.5	33	28	9.6	0.59	0.03	3	4.3	1.3	3.7	4.3	2.6	0.61
935950-012	Western Gulf	O&D	96	0.21	0.66	24	19	6.5	0.42	ND	2.1	3.4	1	2.9	3.2	1.7	0.26
935950-013	Central Gulf	WAF	0	65	50	16	1.7	ND	0.32	ND	ND	ND	0.53	0.2	ND	ND	0.01
935950-014	Central Gulf	WAF	6	36	31	12	1.1	ND	0.26	ND	ND	ND	0.51	0.22	0.01	ND	0.02
935950-015	Central Gulf	WAF	24	31	29	12	0.81	ND	0.24	ND	ND	ND	ND	0.14	ND	ND	0.01
935950-016	Central Gulf	WAF	48	14	16	8.5	0.47	ND	0.2	ND	ND	ND	0.42	0.16	ND	ND	0.02
935950-017	Central Gulf	WAF	72	5.7	8.4	5.2	ND	ND	0.13	ND	ND	ND	0.3	0.06	ND	ND	ND
935950-018	Central Gulf	WAF	96	1	2.4	2.2	0.1	ND	0.05	ND	ND	ND	0.14	ND	ND	ND	ND
935950-019	Central Gulf	O&D	0	35	92	130	100	36	2.6	2.6	12	18	6.9	21	23	15	4.2
935950-020	Central Gulf	O&D	6	20	25	62	44	14	1.3	0.03	5.6	6	3.6	9.2	9.6	5.1	0.91
935950-021	Central Gulf	O&D	24	13	25	63	58	24	1.4	1.2	7.9	8.4	3.2	9.5	11	7.9	2.3
935950-022	Central Gulf	O&D	48	7.1	28	45	31	10	1.1	0.05	5.1	5.4	3.3	8.6	8.6	4.4	0.8

PAH RESULTS (Continued)

				Anthracene		Dibenzothiophene			Chrysene				
Sample No.	Oil	Exposure	Time	An	D	C1D	C2D	C3D	C	C1C	C2C	C3C	C4C
925344-011	Western Gulf	WAF	96	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
925344-013	Western Gulf	D Only		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
935950-001	Western Gulf	WAF	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
935950-002	Western Gulf	WAF	6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
935950-003	Western Gulf	WAF	24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
935950-004	Western Gulf	WAF	48	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
935950-005	Western Gulf	WAF	72	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
935950-006	Western Gulf	WAF	96	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
935950-007	Western Gulf	O&D	0	ND	5.1	5.4	9.4	5.9	0.16	0.07	ND	ND	ND
935950-008	Western Gulf	O&D	6	ND	3.4	3.4	5.7	4.7	ND	ND	ND	ND	ND
935950-009	Western Gulf	O&D	24	ND	4.9	5.9	10	8.3	0.19	0.07	ND	ND	ND
935950-010	Western Gulf	O&D	48	ND	1.5	2	3.3	2.8	0.02	ND	ND	ND	ND
935950-011	Western Gulf	O&D	72	ND	1.6	2.2	3.8	3.3	0.04	ND	ND	ND	ND
935950-012	Western Gulf	O&D	96	ND	1.2	1.7	2.7	2.9	0.02	ND	ND	ND	ND
935950-013	Central Gulf	WAF	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
935950-014	Central Gulf	WAF	6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
935950-015	Central Gulf	WAF	24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
935950-016	Central Gulf	WAF	48	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
935950-017	Central Gulf	WAF	72	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
935950-018	Central Gulf	WAF	96	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
935950-019	Central Gulf	O&D	0	ND	26	12	20	15	0.5	1	0.23	ND	ND
935950-020	Central Gulf	O&D	6	ND	2.4	5.1	8.2	4.1	0.13	0.05	ND	ND	ND
935950-021	Central Gulf	O&D	24	ND	4.8	5.8	10	6.1	0.16	0.04	ND	ND	ND
935950-022	Central Gulf	O&D	48	ND	2	4.8	7.4	4.8	0.11	0.04	ND	ND	ND

PAH RESULTS (Continued)

				Naphthalene					Fluorene				Phenanthrene				
Sample No.	Oil	Exposure	Time	N	C1N	C2N	C3N	C4N	F	C1F	C2F	C3F	P	C1P	C2P	C3P	C4P
935950-023	Central Gulf	O&D	72	1.2	12	30	21	6.1	0.69	ND	2.6	3.4	2.4	5.8	5.3	2.5	0.31
935950-024	Central Gulf	O&D	96	ND	1.4	19	17	5.7	0.48	ND	2.7	2.7	2.1	5.4	5.1	2.3	0.31
End Oil	Western Gulf			510	1300	1500	1500	850	27	200	280	230	6	89	84	74	69
End Oil	Central Gulf			0	1040	1700	1600	1100	26	250	350	330	22	190	190	140	54
Initial Oil	Western Gulf			440	1800	1100	790	420	29	83	150	130	68	150	170	130	96
Initial Oil	Central Gulf			420	1600	1100	1000	630	9.9	190	340	280	110	340	410	350	97

PAH RESULTS (Continued)

				Anthracene		Dibenzothiophene			Chrysene				
Sample No.	Oil	Exposure	Time	An	D	C1D	C2D	C3D	C	C1C	C2C	C3C	C4C
935950-023	Central Gulf	O&D	72	ND	1	3.2	4.5	4	0.05	0.01	ND	ND	ND
935950-024	Central Gulf	O&D	96	ND	1	2.9	4.2	2.9	0.05	ND	ND	ND	ND
End Oil	Western Gulf			11	27	70	99	63	8	11	6	4	ND
End Oil	Central Gulf			16	38	130	190	120	0	32	36	19	7
Initial Oil	Western Gulf			ND	91	110	180	170	8.7	16	18	14	ND
Initial Oil	Central Gulf			ND	75	210	380	310	13	34	52	44	14

* ND = concentration less than detection limit of 0.01 μg/l.

BTEX RESULTS

SAMPLE NO.	OIL TYPE	EXPOSURE	TIME	BENZENE	TOLUENE	ETHYLBENZENE	XYLENE
925379-002	Central Gulf	O&D	0	61	280	70	500
925379-004	Central Gulf	O&D	6	6.4	41	9.5	72
925379-006	Central Gulf	O&D	24	<*	3.2	0.75	9.6
925379-008	Central Gulf	O&D	48	<	<	<	4.2
925379-010	Central Gulf	O&D	72	<	<	<	1.7
925379-012	Central Gulf	O&D	96	<	<	<	<
925379-001	Central Gulf	WAF	0	3300	2800	170	960
925379-003	Central Gulf	WAF	6	1200	1000	70	380
925379-005	Central Gulf	WAF	24	84	76	6.2	30
925379-007	Central Gulf	WAF	48	4.2	3.5	<	2.1
925379-009	Central Gulf	WAF	72	2.0	1.4	<	1.4
925379-011	Central Gulf	WAF	96	1.1	1.1	<	<
925418-002	Western Gulf	O&D	0	61	220	45	230
925418-004	Western Gulf	O&D	6	5.9	19	5.1	39
925418-006	Western Gulf	O&D	24	<	<	<	2.3
925418-008	Western Gulf	O&D	48	<	<	<	1.4
925418-010	Western Gulf	O&D	72	0.56	3.2	0.57	3.1
925418-012	Western Gulf	O&D	96	<	<	<	<
925418-001	Western Gulf	WAF	0	3800	2900	160	1100
925418-003	Western Gulf	WAF	6	680	560	31	220
925418-005	Western Gulf	WAF	24	2.8	3.9	<	1.2
925418-007	Western Gulf	WAF	48	<	<	<	<
925418-009	Western Gulf	WAF	72	<	<	<	<
925418-011	Western Gulf	WAF	96	<	0.75	<	1.1
925674-001	Central Gulf	WAF	0	3900	3300	240	1300
925674-002	Central Gulf	WAF	6	290	260	16	100
925674-003	Central Gulf	WAF	24	31	29	1.6	11
925674-004	Central Gulf	WAF	48	8.9	9.9	<	1.9
925674-005	Central Gulf	WAF	72	2.5	1.4	<	<
925674-006	Central Gulf	WAF	96	1.7	1.0	<	<
925674-007	Central Gulf	O&D	0	81	250	53	360
925674-008	Central Gulf	O&D	6	20	62	15	100
925674-009	Central Gulf	O&D	24	2.0	5.8	1.2	9.7
925674-010	Central Gulf	O&D	48	<	2.0	<	2.1

BTEX RESULTS (Continued)

SAMPLE NO.	OIL TYPE	EXPOSURE	TIME	BENZENE	TOLUENE	ETHYLBENZENE	XYLENE
925674-011	Central Gulf	O&D	72	<	1.0	<	<
925674-012	Central Gulf	O&D	96	<	0.92	<	<
925818-001	Western Gulf	WAF	0	6100	4400	220	1600
925818-002	Western Gulf	WAF	6	4000	2900	140	1200
925818-003	Western Gulf	WAF	24	4300	2900	120	770
925818-004	Western Gulf	WAF	48	2900	2000	92	640
925818-005	Western Gulf	WAF	96	870	560	25	190
925818-006	Western Gulf	O&D	0	55	160	37	310
925818-007	Western Gulf	O&D	6	23	64	20	130
925818-008	Western Gulf	O&D	24	19	55	15	110
925818-009	Western Gulf	O&D	48	17	41	12	79
925818-010	Western Gulf	O&D	96	5.7	5.8	1.4	13
935723-002	Central Gulf	O&D	0	30	110	28	200
935723-004	Central Gulf	O&D	6	23	89	24	160
935723-006	Central Gulf	O&D	24	38	130	31	220
935723-008	Central Gulf	O&D	48	46	120	23	160
935723-010	Central Gulf	O&D	96	44	27	2.9	18
935723-001	Central Gulf	WAF	0	4400	3700	100	520
935723-003	Central Gulf	WAF	6	2800	2400	86	560
935723-005	Central Gulf	WAF	24	5300	4400	160	1100
935723-007	Central Gulf	WAF	48	2100	1600	66	430
935723-009	Central Gulf	WAF	96	140	110	7.7	74
935756-001	Western Gulf	WAF	0	6000	4300	190	1300
935756-002	Western Gulf	WAF	6	6000	4200	150	1100
935756-003	Western Gulf	WAF	24	5800	3400	<	<
935756-004	Western Gulf	WAF	48	3900	2100	79	520
935756-005	Western Gulf	WAF	96	1300	550	5.2	38
935756-006	Western Gulf	O&D	0	49	160	34	260
935756-007	Western Gulf	O&D	6	27	82	17	120
935756-008	Western Gulf	O&D	24	37	120	25	200
935756-009	Western Gulf	O&D	48	29	73	13	100
935756-010	Western Gulf	O&D	96	17	43	7.6	60
935782-001	Western Gulf	WAF	0	6100	4100	<	440
935782-002	Western Gulf	WAF	6	580	420	18	130

BTEX RESULTS (Continued)

SAMPLE NO.	OIL TYPE	EXPOSURE	TIME	BENZENE	TOLUENE	ETHYLBENZENE	XYLENE
935782-003	Western Gulf	WAF	24	11	17	<	<
935782-004	Western Gulf	WAF	48	0.88	4.7	<	<
935782-005	Western Gulf	WAF	96	<	<	<	<
935782-006	Western Gulf	O&D	0	53	130	31	200
935782-007	Western Gulf	O&D	6	3.9	20	5.6	28
935782-008	Western Gulf	O&D	24	<	1.0	<	<
935782-009	Western Gulf	O&D	48	<	<	<	<
935782-010	Western Gulf	O&D	96	<	<	<	<
935858-001	Central Gulf	WAF	0	4000	3300	190	1300
935858-002	Central Gulf	WAF	6	3900	3200	150	990
935858-003	Central Gulf	WAF	24	5000	4200	240	1500
935858-004	Central Gulf	WAF	48	4700	3700	180	1200
935858-005	Central Gulf	WAF	96	3700	2800	110	740
935858-006	Central Gulf	O&D	0	81	210	39	180
935858-007	Central Gulf	O&D	6	43	110	27	120
935858-008	Central Gulf	O&D	24	49	150	36	160
935858-009	Central Gulf	O&D	48	43	100	22	110
935858-010	Central Gulf	O&D	96	44	110	26	130
935858-011	Western Gulf	WAF	0	5200	3700	180	1100
935858-012	Western Gulf	WAF	6	5400	3800	140	1200
935858-013	Western Gulf	WAF	24	5600	3900	140	1200
935858-014	Western Gulf	WAF	48	5400	3600	130	1000
935858-015	Western Gulf	WAF	96	2300	2300	53	560
935858-016	Western Gulf	O&D	0	77	220	52	350
935858-017	Western Gulf	O&D	6	62	170	35	250
935858-018	Western Gulf	O&D	24	47	120	27	190
935858-019	Western Gulf	O&D	48	48	120	29	190
935858-020	Western Gulf	O&D	96	38	110	22	160

* < = concentration less than respective reporting (detection) limit; limits of detection for benzene, toluene, and ethylbenzene are 0.5 μg/l; the limit for xylene is 1.0 μg/l.

TPH RESULTS

Sample No.	Oil Type	Exposure	Time	TPH	Limit	Units
924971-002	Western Gulf	O&D	0	40	0.05	mg/l
924971-004	Western Gulf	O&D	4	40	0.05	mg/l
924971-006	Western Gulf	O&D	12	30	0.05	mg/l
924971-008	Western Gulf	O&D	24	16	0.05	mg/l
924971-010	Western Gulf	O&D	48	11	0.05	mg/l
924971-012	Western Gulf	O&D	96	3.5	0.05	mg/l
924971-001	Western Gulf	WAF	0	8.8	0.05	mg/l
924971-003	Western Gulf	WAF	4	3.2	0.05	mg/l
924971-005	Western Gulf	WAF	12	1.4	0.05	mg/l
924971-007	Western Gulf	WAF	24	0.89	0.05	mg/l
924971-009	Western Gulf	WAF	48	0.72	0.05	mg/l
924971-011	Western Gulf	WAF	96	<*	0.05	mg/l
924971-013	Western Gulf	WAF	96	<	0.05	mg/l
925241-001	Central Gulf	O&D	0	34	0.05	mg/l
925241-002	Central Gulf	O&D	12	33	0.05	mg/l
925241-003	Central Gulf	O&D	48	23	0.05	mg/l
925285-001	Western Gulf	O&D	0	73	0.05	mg/l
925285-002	Western Gulf	O&D	6	65	0.05	mg/l
925285-003	Western Gulf	O&D	24	57	0.05	mg/l
925285-004	Western Gulf	O&D	48	46	0.05	mg/l
925285-006	Central Gulf	O&D	0	58	0.05	mg/l
925285-008	Central Gulf	O&D	4	34	0.05	mg/l
925285-010	Central Gulf	O&D	24	19	0.05	mg/l
925285-012	Central Gulf	O&D	48	12	0.05	mg/l
925285-014	Central Gulf	O&D	72	7.7	0.05	mg/l
925285-016	Central Gulf	O&D	96	3.8	0.05	mg/l
925285-005	Central Gulf	WAF	0	3.0	0.05	mg/l
925285-007	Central Gulf	WAF	6	<	0.05	mg/l
925285-009	Central Gulf	WAF	24	<	0.05	mg/l
925285-013	Central Gulf	WAF	72	<	0.05	mg/l
925285-015	Central Gulf	WAF	96	<	0.05	mg/l
925674-001	Central Gulf	WAF	0	17	0.05	mg/l
925674-002	Central Gulf	WAF	6	1	0.05	mg/l
925674-003	Central Gulf	WAF	24	<	0.05	mg/l
925674-004	Central Gulf	WAF	48	<	0.05	mg/l

TPH RESULTS (Continued)

Sample No.	Oil Type	Exposure	Time	TPH	Limit	Units
925674-005	Central Gulf	WAF	72	<	0.05	mg/l
925674-006	Central Gulf	WAF	96	<	0.05	mg/l
925674-007	Central Gulf	O&D	0	60	0.05	mg/l
925674-008	Central Gulf	O&D	6	20	0.05	mg/l
925674-009	Central Gulf	O&D	24	7.5	0.05	mg/l
925674-010	Central Gulf	O&D	48	4.5	0.05	mg/l
925674-011	Central Gulf	O&D	72	0.84	0.05	mg/l
925674-012	Central Gulf	O&D	96	0.58	0.05	mg/l
925818-001	Western Gulf	WAF	0	14	0.05	mg/l
925818-002	Western Gulf	WAF	6	10	0.05	mg/l
925818-003	Western Gulf	WAF	24	8.7	0.05	mg/l
925818-004	Western Gulf	WAF	48	5.7	0.05	mg/l
925818-005	Western Gulf	WAF	96	2.4	0.05	mg/l
925818-006	Western Gulf	O&D	0	37	0.05	mg/l
925818-007	Western Gulf	O&D	6	28	0.05	mg/l
925818-008	Western Gulf	O&D	24	20	0.05	mg/l
925818-009	Western Gulf	O&D	48	13	0.05	mg/l
925818-010	Western Gulf	O&D	96	5.5	0.05	mg/l
935723-002	Central Gulf	O&D	0	37	0.05	mg/l
935723-004	Central Gulf	O&D	6	39	0.05	mg/l
935723-006	Central Gulf	O&D	24	40	0.05	mg/l
935723-008	Central Gulf	O&D	48	36	0.05	mg/l
935723-010	Central Gulf	O&D	96	13	0.05	mg/l
935723-001	Central Gulf	WAF	0	18	0.05	mg/l
935723-003	Central Gulf	WAF	6	13	0.05	mg/l
935723-005	Central Gulf	WAF	24	24	0.05	mg/l
935723-007	Central Gulf	WAF	48	9.2	0.05	mg/l
935723-009	Central Gulf	WAF	96	1.4	0.05	mg/l
935756-001	Western Gulf	WAF	0	15	0.05	mg/l
935756-002	Western Gulf	WAF	6	16	0.05	mg/l
935756-003	Western Gulf	WAF	24	14	0.05	mg/l
935756-004	Western Gulf	WAF	48	8.4	0.05	mg/l
935756-005	Western Gulf	WAF	96	4	0.05	mg/l
935756-006	Western Gulf	O&D	0	40	0.05	mg/l

TPH RESULTS (Continued)

Sample No.	Oil Type	Exposure	Time	TPH	Limit	Units
935756-007	Western Gulf	O&D	6	34	0.05	mg/l
935756-008	Western Gulf	O&D	24	33	0.05	mg/l
935756-009	Western Gulf	O&D	48	25	0.05	mg/l
935756-010	Western Gulf	O&D	96	11	0.05	mg/l
935782-001	Western Gulf	WAF	0	17	0.05	mg/l
935782-002	Western Gulf	WAF	6	1.9	0.05	mg/l
935782-003	Western Gulf	WAF	24	<	0.05	mg/l
935782-004	Western Gulf	WAF	48	<	0.05	mg/l
935782-005	Western Gulf	WAF	96	<	0.05	mg/l
935782-006	Western Gulf	O&D	0	120	0.05	mg/l
935782-007	Western Gulf	O&D	6	23	0.05	mg/l
935782-008	Western Gulf	O&D	24	6.8	0.05	mg/l
935782-009	Western Gulf	O&D	48	4.0	0.05	mg/l
935782-010	Western Gulf	O&D	96	0.86	0.05	mg/l
935858-001	Central Gulf	WAF	0	11	0.05	mg/l
935858-002	Central Gulf	WAF	6	11	0.05	mg/l
935858-003	Central Gulf	WAF	24	10	0.05	mg/l
935858-004	Central Gulf	WAF	48	9.5	0.05	mg/l
935858-005	Central Gulf	WAF	96	8	0.05	mg/l
935858-006	Central Gulf	O&D	0	52	0.05	mg/l
935858-007	Central Gulf	O&D	6	41	0.05	mg/l
935858-008	Central Gulf	O&D	24	36	0.05	mg/l
935858-009	Central Gulf	O&D	48	35	0.05	mg/l
935858-010	Central Gulf	O&D	96	34	0.05	mg/l
935858-011	Western Gulf	WAF	0	12	0.05	mg/l
935858-012	Western Gulf	WAF	6	14	0.05	mg/l
935858-013	Western Gulf	WAF	24	10	0.05	mg/l
935858-014	Western Gulf	WAF	48	12	0.05	mg/l
935858-015	Western Gulf	WAF	96	11	0.05	mg/l
935858-016	Western Gulf	O&D	0	48	0.05	mg/l
935858-017	Western Gulf	O&D	6	38	0.05	mg/l
935858-018	Western Gulf	O&D	24	37	0.05	mg/l
935858-019	Western Gulf	O&D	48	33	0.05	mg/l
935858-020	Western Gulf	O&D	96	32	0.05	mg/l

TPH RESULTS (Continued)

Sample No.	Oil Type	Exposure	Time	TPH	Limit	Units
936234-001	Central Gulf	O&D	0	7.9	0.05	mg/l
936234-002	Central Gulf	O&D	6	3.3	0.05	mg/l
936234-003	Central Gulf	O&D	24	3.2	0.05	mg/l
936234-004	Central Gulf	O&D	48	4.3	0.05	mg/l
936234-005	Central Gulf	O&D	96	NA	0.05	mg/l
936462-001	Western Gulf	O&D	0	46	0.05	mg/l
936462-002	Western Gulf	O&D	24	23	0.05	mg/l
936462-003	Western Gulf	O&D	48	16	0.05	mg/l
936490-001	Dispersant	D	0	1	0.05	mg/l
936490-002	Dispersant	D	6	1.4	0.05	mg/l
936490-003	Dispersant	D	24	<	0.05	mg/l
936490-004	Dispersant	D	48	<	0.05	mg/l
936490-005	Dispersant	D	72	<	0.05	mg/l
936490-006	Dispersant	D	96	<	0.05	mg/l
936490-007	Western Gulf	O&D	0	58	0.05	mg/l
936490-008	Western Gulf	O&D	6	31	0.05	mg/l
936490-009	Western Gulf	O&D	24	12	0.05	mg/l
936490-010	Western Gulf	O&D	48	5	0.05	mg/l
936490-011	Western Gulf	O&D	72	1.1	0.05	mg/l
936490-012	Western Gulf	O&D	96	<	0.05	mg/l
936490-013	Western Gulf	WAF	0	9	0.05	mg/l
936490-014	Western Gulf	WAF	6	<	0.05	mg/l
936490-015	Western Gulf	WAF	24	<	0.05	mg/l
936490-016	Western Gulf	WAF	48	<	0.05	mg/l
936490-017	Western Gulf	WAF	72	<	0.05	mg/l
936490-018	Western Gulf	WAF	96	<	0.05	mg/l

* < = concentration less than detection limit of 0.05 mg/l.

APPENDIX B

SUMMARY DATA FOR ACUTE TOXICITY TESTING

APPENDIX B
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MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulfoil & Corexit 9527

SPECIES Menidia beryllina - embryos to fry

STATIC/FLOWTHROUGH Flowthrough

TEST CONTAINER SIZE 250 ml beakers

SALINITY 27 °/oo

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	~~100~~ 50	~~50~~ 25	~~25~~ 12.5	~~12.5~~ 6.25	~~6.25~~ 3.13
24	98	100	98	100	96	100
48	98	100	98	100	94	100
72	98	98	96	98	92	98
96	96	98	96	98	92	98

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 21-23°C one reading Day 0 and Day 4

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 4.2 mg/L last day

COMMENTS Ran 3/31/92 - 4/4/92. A preliminary test.

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Menidia beryllina - embryos to fry

STATIC/FLOWTHROUGH Flow through

TEST CONTAINER SIZE 250 ml beakers

SALINITY 26‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	~~100~~ 50	~~50~~ 25	~~25~~ 12.5	~~12.5~~ 6.25	~~6.25~~ 3.13
24	96	98	100	100	100	96
48	96	96	96	98	100	96
72	94	96	96	94	98	96
96	94	96	94	94	98	96

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 19.8 – 21.5 °C (DAYS 1+4)

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.2 – 6.9 mg/L (DAYS 1 4+4)

COMMENTS Ran 4/1/92 – 4/5/92. A preliminary test.

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Wkestern Gulf oil WSF

SPECIES Menidia beryllina - embryos to fry

STATIC/FLOWTHROUGH Flowthrough

TEST CONTAINER SIZE 250 ml beakers

SALINITY 27‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	~~**12.5**~~ 6.25	~~**6.25**~~ 3.13
24	100		98	98	96	100
48	98		98	98	96	100
72	98	AC	98	96	96	100
96	98		98	96	96	100

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 20.6 - 20.9 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.5 - 6.3 mg/L

COMMENTS Ran 4/1/92 - 4/5/92

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Water Soluble Fraction Western Gulf oil.

SPECIES Menidia beryllina - embryos to fry

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 100 ml

SALINITY 22‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	CONTROL	~~100~~ 75	50	25	12.5	6.25
24	100%	98%	94%	100%	96%	96%
48	88	96%	88	84	84	88
72	48	84	64	90	44	38
96	38	78	52	86	44	KMC 1/25/94 ~~38~~ 36

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 21.0 - 23.5 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 4.0 - 4.4 mg/L

COMMENTS Run 4/30/92 - 5/4/92 - ~~ite~~ dose response except for control survival

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil Water soluble fraction

SPECIES Menidia beryllina - embryos to fry

STATIC/FLOWTHROUGH Flow through

TEST CONTAINER SIZE 250 ml beakers

SALINITY 27 ‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS Percent %

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	96	98	100	100	98	98
48	92	92	100	100	96	96
72	78	78	94	98	90	92
96	60	68	80	94	86	84

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 23.6 - 25.1 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.1 - 6.1 mg/L

COMMENTS Run 5/12/92 (92) KRC 1/25/94 - 5/16/92 (92) KRC 1/25/94 - acute lack of effects compared to static system tests on 4/30/92 - 5/4/92

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil + Corexit 9527

SPECIES Menidia beryllina - embryos to fry

STATIC/FLOWTHROUGH Flowthrough

TEST CONTAINER SIZE 250 ml beakers

SALINITY 25 ‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg / L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	98	100	100	100	100	100
48	96	90	100	100	100	100
72	90	84	96	100	96	100
96	52	62	64	82	84	76

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 22.3 - 24.8°C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.0 - 5.3 mg/L

COMMENTS Run 5/12/92 - 5/16/92 - no effect

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527 (Run with Western Gulf oil tests.)

SPECIES Menidia beryllina - embryos to fry

STATIC/FLOWTHROUGH Flowthrough

TEST CONTAINER SIZE 250 ml beakers

SALINITY 25 %

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	98	100	100	~~100~~ 96 RMC 1/25/94	100	100
48	96	98	96	88	98	98
72	96	92	94	82	92	94
96	84	80	84	40	64	70

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 23.0 - 24.2 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.4 - 6.0 mg/L

COMMENTS Run 5/12/92 - 5/16/92 720 effect

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil WSF

SPECIES Menidia beryllina - embryos to fry

STATIC/FLOWTHROUGH Flow-through

TEST CONTAINER SIZE 250 mL beakers

SALINITY 20‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	100	100	96	100	94
48	100	100	100	96	100	94
72	100	98	100	96	100	94
96	98	96	100	94	100	94

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.9 - 25.5 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.8 - 6.2 mg/L

COMMENTS Ran 12/9/92 - 12/13/92 no effects

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil + Corexit 9527

SPECIES Menidia beryllina - embryos to fry

STATIC/FLOWTHROUGH Flow-Through

TEST CONTAINER SIZE 250 ml beakers

SALINITY 20‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	98	~~100~~ 98 (AKC 11/25/94)	100	100	100
48	100	98	98	100	100	100
72	100	98	98	100	100	100
96	98	98	96	100	100	100

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.4 - 25.3 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.5 - 6.2 mg/L

COMMENTS Ran 12/9/92 - 12/13/92 no effects

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Menidia beryllina - embryos to fry

STATIC/FLOWTHROUGH Flow-through

TEST CONTAINER SIZE 250 mL beakers

SALINITY 20‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	100	100	100	100	100
48	100	100	100	100	100	100
72	100	100	100	100	100	100
96	98	96	98	100	100	100

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.1 – 25.6 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.4 – 6.2 mg/L

COMMENTS Ran 12/9/92 – 12/13/92 no effects

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil WSF

SPECIES Menidia beryllina – 7 day old larvae

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 30 ml plastic cups

SALINITY 20 ‰

NO. EXPOSED/CONCENTRATION 50 5 per cup of 25 mL

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	80	100	100	100	100
48	98	24	98	98	98	100
72	96	0	88	92	96	98
96	92	0	88	88	94	98

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.2 – 25.6 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 4.8 – 5.7 mg/L

COMMENTS Can 12/29/92 – 1/2/93 good dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil + Corexit 9527

SPECIES Menidia beryllina - 7 day old larvae

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 30 mL plastic cup

SALINITY 20 ‰

NO. EXPOSED/CONCENTRATION 50 5 per cup of 25 mL

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	100	100	100	100	100
48	100	100	100	100	100	98 MC 1/25/94
72	100	92	100	100	98	96
96	94	90	100	100	98	96

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.3 - 26.0 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 4.0 - 10.0 mg/L

COMMENTS Ran 12/29/92 - 1/2/93 no effects

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Menidia beryllina - 7 day old larvae

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 30 ml plastic cup

SALINITY 20 ‰

NO. EXPOSED/CONCENTRATION 50 5 per cup of 25 ml

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	0	56	98	98	100
48	98	0	54	96	98	100
72	96	0	52	96	98	98
96	94	0	52	96	98	96

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 25.3 - 25.5 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION (43%) 2.9 - 11.5 mg/L

COMMENTS Ran 12/29/92 - 1/2/93 good dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil WSF

SPECIES Menidia beryllina - 9 day old larvae

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 260 ml plastic cup

SALINITY 20 ‰

NO. EXPOSED/CONCENTRATION 50 10 per cup of 100 mL

EXPOSURE UNITS ~~Setting lbs/gal~~ ~~percent~~ Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	2	98	98	100	98
48	98	2	94	98	100	98
72	98	0	94	98	98	98
96	98	0	94	96	98	98

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.3 - 25.8 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 3.0 - 6.0 mg/L

COMMENTS Ran 1/5/93 - 1/9/93 good dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil + Corexit 9527

SPECIES Menidia beryllina — 9 day old larvae

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 240 ml plastic cup

SALINITY 20 ‰

NO. EXPOSED/CONCENTRATION 50 10 per cup of 100 mL

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	98	10	88	98	100	100
48	98	4	66	92	100	100
72	98	4	64	92	100	100
96	98	4	64	92	100	100

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.6 - 25.8 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 3.0 (45%) - 6.1 mg/L

COMMENTS Ran 1/5/93 - 1/9/93 good dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Menidia beryllina - 9 day old larvae

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 260 ml plastic cup

SALINITY 20 ‰

NO. EXPOSED/CONCENTRATION 50 10 per cup of 100 ml

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	0	82	100	98	100
48	98	0	82	100	98	100
72	98	0	78	98	98	100
96	98	0	78	98	98	100

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.8 - 26.1 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION Day 1 6.4 - 6.8 mg/L

COMMENTS Ran 1/5/93 - 1/9/93 good dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil WSF

SPECIES Menidia beryllina - embryos only

STATIC/FLOWTHROUGH Flow-through

TEST CONTAINER SIZE 250 ml beakers

SALINITY 21 %

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	98	100	100	100	100
48	100	96	100	98	100	96
72	100	96	100	98	100	96
96	100	96	100	98 (1 hatched)	100 (4 hatched)	96 (1 hatched)

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.7 - 25.2 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 6.0 - 6.2 mg/L

COMMENTS Ran 1/13/93 - 1/17/93 no effects

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil + Corexit 9527

SPECIES Menidia beryllina embryos only

STATIC/FLOWTHROUGH Flow-Through

TEST CONTAINER SIZE 250 mL beakers

SALINITY 21 ~~20~~ ‰ KAE 1/25/94

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	96	100	100	100	100
48	100	96	100	100	100	100
72	100	96	100	100	100	100
96	100	96	1 hatched 100	100	1 hatched 98	1 hatched 100

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.4 - 24.9 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.9 - 6.3 mg/L

COMMENTS Ran 1/13/93 - 1/17/93 no effects

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Menidia beryllina, embryos only

STATIC/FLOWTHROUGH Flow-through

TEST CONTAINER SIZE 250 mL beakers

SALINITY 21 ‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	CONTROL	100	50	25	12.5	6.25
24	98	96	100	100	100	98
48	98	96	100	100	100	98
72	98	96	100	100	100	98
96	98	96	100	100	100	98 (2 hatched)

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 25.0 - 25.5 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 6.0 - 6.3 mg/L

COMMENTS Ran 1/13/93 - 1/17/93 no effects

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil WSF

SPECIES Menidia beryllina 7 days old

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 260 ml

SALINITY 20 °/oo

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	CONTROL	100	50	25	12.5	6.25
24	98	10	100	96	100	96
48	94	6	98	96	100	96
72	90	6	98	96	100	94
96	86	6	98	96	100	94

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.7 - 24.9 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 4.4 - 5.6 mg/L

COMMENTS Ran 1/26/93 - 1/30/93 good dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil + Corexit 9527

SPECIES Menidia beryllina 7 days old

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 200 ml plastic cups

SALINITY 20‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	98	18	90	100	98	100
48	98	MAC 1/26/94 to 14	82	92	98	100
72	98	14	80	86	96	100
96	96	14	80	86	96	98

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.9 - 25.7 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 4.2 - 6.4 mg/L

COMMENTS Ran 1/26/93 - 1/30/93 good dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Menidia beryllina 7 days old

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 260 ml plastic cups

SALINITY 20 ‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	78	0	4	66	100	100
48	96	0	4	64	100	98
72	96	0	4	64	100	98
96	96	0	4	64	100	98

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 25.2 °C (2 readings)

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.0 - 6.4 mg/L

COMMENTS Ran 1/26/93 - 1/30/93 good dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil WSF

SPECIES Leiostomus xanthurus embryos to fry

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 30 ml plastic cups

SALINITY 30 ‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	98	82	100	94	100	98
48	68	0	74	68	78	64
72	32	0	0	10	18	26
96	18	0	0	0	16	20

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 19.2 - 20.2 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.7 - 6.0 mg/L

COMMENTS Ran 2/11/93 - 2/15/93 good 48 hr. results; note 96 hr. dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil + Corexit 9527

SPECIES Leiostomus xanthurus embryos to fry

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 30 mL plastic cups

SALINITY 31 ‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	96	60	92	90	98	96
48	76	22	40	24	68	54
72	28	0	6	12	4	16
96	10	0	0	0	0	4

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 19.9 - 20.9 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.7 - 6.0 mg/L

COMMENTS Ran 2/11/93 - 2/15/93 use 48hr. results, note 72 + 96 hr. survival only in control + low concentrations

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Leiostomus xanthurus embryos to fry

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 30 mL plastic cups

SALINITY 30‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	90	84	94	96	98	96
48	72	0	28	74	70	80
72	28	0	0	24	28	38
96	14	0	0	0	2	22

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 19.4 - 21.0 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.1 - 6.1 mg/L

COMMENTS Ran 2/11/93 - 2/15/93 good 48 hr. results, note 72 + 96 hr. survival patterns

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil WSF

SPECIES Leiostomus xanthurus - embryos to fry

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 30 mL plastic cups

SALINITY 30‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	92	100	96	100	100	100
48	~~36~~ 56	102	84	86	80	78
72	36	0	10	22	26	36
96	18	0	0	10	16	22

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 19.8 - 20.0 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.4 - 6.4 mg/L

COMMENTS Ran 2/11/93 - 2/15/93 use 48 hr. results (no effect)
but note 72 hr. effects, 96 hr. survival pattern

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil + Corexit 9527

SPECIES Leiostomus xanthurus - embryos to fry

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 30 ml. plastic cups

SALINITY 30 ‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	96	86	98	92	96	94
48	80	30	68	70	66	42
72	12	0	0	6	2	2
96	0	0	0	0	0	0

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 19.9 - 20.6 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.9 - 6.2 mg/L

COMMENTS Ran 2/11/93 - 2/15/93 good 48 hr. results, not 72 hr. survival

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil WSF

SPECIES Brevoortia embryos → fry

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 260 mL plastic cups

SALINITY 30‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	96	0	88	96	96	96
48	78	0	28	88	98	84
72	20	0	0	64	76	52
96	2	0	0	4	26	20

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 19.9 - 20.9 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.2 - 6.3 mg/L

COMMENTS Ran 3/11/93 - 3/15/93 use 48 hr. results but compare 72 hr.

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil + Corexit 9527

SPECIES Brevoortia embryos → fry

STATIC/FLOWTHROUGH ~~Static~~

TEST CONTAINER SIZE 260 ml plastic cups

SALINITY 30‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	84	16	80	94	100	98
48	20	0	2	8	34	40
72	12	0	0	0	0	8
96	0	0	0	0	0	0

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 19.0 - 20.0 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.5 - 6.1 mg/L

COMMENTS Ran 3/11/93 - 3/15/93 used 48 hr. based on dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Crevoortia embryos → fry

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 260 mL plastic cups

SALINITY 30 ‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	72	0	24	72	84	74
48	0	0	4	0	14	18
72	0	0	0	0	0	4
96	0	0	0	0	0	4

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 20.3 - 20.4 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.0 - 6.2 mg/L

COMMENTS Ran 2/11/93 - 2/15/93 used 24 hr. pattern

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil WSF

SPECIES Brevoortia embryos → fry

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 260 ml plastic cups

SALINITY 30‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	96	70	96	94	98	94
48	62	4	48	52	62	46
72	38	0	36	44	56	24
96	24	0	2	16	24	12

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 19.9 - 21.2 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.0 - 6.8 mg/L

COMMENTS Ran 3/11/93 - 3/15/93 use 48 hr. results, note 72 + 96 hr. results

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil + Corexit 9527

SPECIES Brevoortia embryos → fry

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 260 mL plastic cups

SALINITY 30‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	82	54	86	88	90	92
48	28	0	4	18	20	24
72	14	0	0	6	12	22
96	8	0	0	0	0	0

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 19.9 - 20.0 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.4 - 5.9 mg/L

COMMENTS Ran 3/11/93 - 3/15/93 use 48 hr results, note 72 hr. dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil WSF

SPECIES Penaeus aztecus Post larvae (? age)

STATIC/FLOWTHROUGH Flow Through

TEST CONTAINER SIZE 250 ml beaker

SALINITY 27 ‰

NO. EXPOSED/CONCENTRATION 25

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	0	88	92	92	
48	100	0	88	88	88	
72	100	0	84	88	80	
96	100	0	88 (1 found in tank)	92 (1 found in tank)	76	

Not used

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 23.1 °C Initial

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.8 Initial 4.4 Final

COMMENTS Ran 4/15/92 - 4/19/92

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil + Corexit 9527

SPECIES Penaeus azteeus Post larvae (? age)

STATIC/FLOWTHROUGH Flow Through

TEST CONTAINER SIZE 250 ml beakers

SALINITY 27‰

NO. EXPOSED/CONCENTRATION 25

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	84	12	56	96	88	
48	92 (2 found in tank)	8	60 (1 found in beaker)	92	88	
72	88	8	60	92	92 (1 found in tank)	NC
96	88	8	60	88	88 (1 not found)	

Not used

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 23.8 °C Initial

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.7 Initial

COMMENTS Ran 4/15/92 - 4/19/92

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil + Corexit 9527

SPECIES Penaeus setiferus mysis stage

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 260 ml plastic cup

SALINITY 35 ‰

NO. EXPOSED/CONCENTRATION 20

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	85	0	0	0	5	60
48	70	0	0	0	0	20
72	55	0	0	0	0	15
96	45	0	0	0	0	10

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 20.0 - 20.5 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 3.3 - 6.8 mg/L

COMMENTS Ran 6/25/93 - 6/29/93

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Penaeus setiferus mysis stage

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 260 ml plastic cups

SALINITY 35 ‰

NO. EXPOSED/CONCENTRATION 20

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	90	0	20	not enough animals	65	85
48	70	0	0		55	70
72	45	0	0		55	KME 1/21/99 ~~40~~ 60
96	40	0	0	KP	55	45

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 20.1 - 21.0 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 3.4 - 6.7 mg/L

COMMENTS Exp. 6/25/93 - 6/29/93 note dose responses

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil WSF

SPECIES Callinectes sapidus - megalope

STATIC/FLOWTHROUGH Flow Through

TEST CONTAINER SIZE Individually capped 30 mL plastic cups with 16 holes in each for water flow!

SALINITY 30 ‰

NO. EXPOSED/CONCENTRATION 25

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	96	100	100	100	100
48	100	92	88	92	84	84
72	72*	0	80	80	76	76
96	104	0	80	76	72	68

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 25.0 - 25.6 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 2.1 - 4.7 mg/L

COMMENTS Ran 9/10/92 - 9/14/92 good dose response

*7 dead megalopae

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil + Corexit 9527

SPECIES Callinectes sapidus - megalopae

STATIC/FLOWTHROUGH Flow-Through

TEST CONTAINER SIZE Individually capped 30 mL plastic cups with 16 holes in each for water flow.

SALINITY 30 ‰

NO. EXPOSED/CONCENTRATION 25

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	0	100	100	80	96
48	100	0	96	92	80	96
72	100	0	28	32	68	96
96	100	0	28	24	68	96

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 25.8 - 26.0 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION *1.9 (33%) - 3.6 mg/L

COMMENTS Ran 9/10/92 - 9/14/92 good dose response!

* 25 mg/L conc. had 24 crabs alive with DO readings of 2.0 mg/L

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Callinectes sapidus - megalope

STATIC/FLOWTHROUGH Flow-through

TEST CONTAINER SIZE ~~Individually~~ capped 30 mL plastic cups with 16 holes in each for water flow.

SALINITY 30 ‰

NO. EXPOSED/CONCENTRATION ~~25 for Control, 50, 100 ppm~~ ~~23 for 6.25, 12.5~~ 25 ppm

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	92	100	100	100	96
48	100	36	96	91	100	91
72	96	20	96	87	83	78
96	92	20	96	87	83	78

TEMPERATURE RANGE IN HIGHEST CONCENTRATION ~~25.2 - 25.9~~ 25.2 - 25.9 °C
KLC 1/25/94

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 1.7 (28%) - 5.2 mg/L

COMMENTS Ran 9/10/92 - 9/15/92 good dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil WSF

SPECIES Callinectes sapidus megalopae

STATIC/FLOWTHROUGH Flow-through

TEST CONTAINER SIZE 250 ml beakers

SALINITY 24 ‰

NO. EXPOSED/CONCENTRATION 25

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	96	80	92	100	100	100
48	96	80	88	100	100	100
72	88	68	80	96	84	100
96	88	64	80	96	84	96

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.9 - 25.5 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 4.3 - 5.5 mg/L

COMMENTS Ran 9/22/93 - 9/26/93 dose response OK

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil + Corexit 9527

SPECIES Callinectes sapidus megalopae

STATIC/FLOWTHROUGH Flow-through

TEST CONTAINER SIZE 250 ml beaker

SALINITY 24‰

NO. EXPOSED/CONCENTRATION 25

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	CONTROL	100	50	25	12.5	6.25
24	100	52	88	96	96	96
48	100	52	84	96	96	96
72	100	48	84	96	84	92
96	100	44	84	96	84	92

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.6 - 25.3 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 4.4 - 8.1 mg/L

COMMENTS Ran 9/22/93 - 9/26/93 good dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Callinectes sapidus megalopae

STATIC/FLOWTHROUGH Flow-through

TEST CONTAINER SIZE 250 ml beakers

SALINITY 24 ‰

NO. EXPOSED/CONCENTRATION 25

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	96	52	96	96	96	96
48	92	40	88	96	96	96
72	68	40	88	96	92	88
96	68	32	88	96	92	88

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 23.4 - 24.3 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.0 - 8.1 mg/L

COMMENTS Ran 9/22/93 - 9/26/93 good dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil and Corexit 9527

SPECIES Crassostrea virginica embryos

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 100 ml crystallizing dish

SALINITY 30 ‰

NO. EXPOSED/CONCENTRATION ~30 embryos/ml 4 reps Control
3 reps test concentrations

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	—	—	—	—	—	—
48	$\bar{x}$ = 117 embryos/rep 9.0 embryos/ml	0	0	0	0	$\bar{x}$ = 21 embryos/rep 1.6 embryos/ml
72	—	—	—	—	—	—
96	—	—	—	—	—	—

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 19.0 - 22.9 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 6.1 - 7.0 mg/L

COMMENTS Ran 8/5/92 - 8/7/92 statistically significant differences in all concentrations

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western oil + Corexit 9527

SPECIES Crassostrea virginica embryos

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 100 mL

SALINITY 26‰

NO. EXPOSED/CONCENTRATION 30 embryos/mL

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	—	—	—	—	—	—
48	$\bar{x}$ = 43.3 embryos/rep 1.1 embryos/mL	0	0	$\bar{x}$ = 1.7 embryos/rep 0.04 embryos/mL	$\bar{x}$ = 18.7 embryos/rep 0.5 embryos/mL	$\bar{x}$ = 40 embryos/rep 1.0 embryos/mL
72	—	—	—	—	—	—
96	—	—	—	—	—	—

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.0 - 25.1 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 16.3 - 16.8 mg/L

COMMENTS Ran 8/31/93 - 9/2/93 statistically significant effects @ 25 + 12.5 mg/L

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Crassostrea virginica embryos

STATIC/FLOWTHROUGH Static

TEST CONTAINER SIZE 100 mL crystallizing dish

SALINITY 27‰

NO. EXPOSED/CONCENTRATION ~30 embryos/mL 4 reps Control 3 reps test concentration

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	—	—	—	—	—	—
48	$\bar{X}$ = 27.5 embryos/rep 0.16 embryos/ml	0	0	0	0	$\bar{X}$ = 7.7 embryos/rep 0.15 embryos/ml
72	—	—	—	—	—	—
96	—	—	—	—	—	—

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.1 - 24.8 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 6.5 - 6.7 mg/L

COMMENTS San 9/8/93 - 9/10/93 statistical difference from controls at all concentration

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf WAF

SPECIES Redfish - Sciaenops ocellatus

STATIC/FLOWTHROUGH flowthrough

TEST CONTAINER SIZE 250 ml beaker

SALINITY 30 °/oo

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS %

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	80	48	34	100	36	80
48	10	10	12	4	0	4
72	2	0	6	4	0	0
96	2	0	4	2	0	0

TEMPERATURE RANGE IN HIGHEST CONCENTRATION ± 24.7-25.6 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.3-5.9 mg/l

COMMENTS only WAF run from 7/28/92 - 8/1/92, no apparent difference due to high mortalities

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil WSF

SPECIES Sciaenops ocellatus larvae

STATIC/FLOWTHROUGH Flow Through

TEST CONTAINER SIZE 250 ml beakers

SALINITY 32‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	70	54	82	94	74	70
48	44	4	64	52	42	34
72	16	0	50	14	18	10
96	2	0	0	0	0	0

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.6 - 25.5 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.2 - 6.2 mg/L

COMMENTS Run 8/18/92 - 8/22/92 note 48 hour effects in 100%

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil + Corexit 9527

SPECIES Sciaenops ocellatus larvae

STATIC/FLOWTHROUGH Flowthrough

TEST CONTAINER SIZE 250 ml beaker

SALINITY 30 ‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	30	88	98	94	78	70
48	8	52	94	72	54	50
72	0	6	26	38	34	26
96	0	0	0	6	4	0

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.8 – 25.2 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.5 – 6.1 mg/L

COMMENTS Run 8/18/92 - 8/22/92 no effects after 48 hrs

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Sciaenops ocellatus larvae

STATIC/FLOWTHROUGH Flowthrough

TEST CONTAINER SIZE 250 ml beakers

SALINITY 31 ‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	82	74				
48	64	70				
72	36	38		XC		
96	0	0				

Not enough fish to set up all concentrations.

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 25.0 - 25.6 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.2 - 5.8 mg/L

COMMENTS Ran 8/18/92 - 8/22/92

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf + Corexit 9527

SPECIES Redfish - Sciaenops ocellatus

STATIC/FLOWTHROUGH flowthrough

TEST CONTAINER SIZE 250 ml

SALINITY 31 °/oo

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/l

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	34	88	92	84	54	28
48	10	78	70	40	12	4
72	2	8	12	4	8	0
96	0	0	0	0	0	0

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 25.3-26.0 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.3-5.6 mg/l

COMMENTS test run 9/22/92 - 9/26/92, Lack of effects in higher concentrations

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Redfish - Sebastes marinus

STATIC/FLOWTHROUGH flowthrough

TEST CONTAINER SIZE 250 ml

SALINITY 31 °/oo

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS mg/l

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	28	82	78	46	94	64
48	22	2	14	22	28	14
72	6	0	0	2	0	0
96	0	0	0	0	0	0

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 25.1-25.4 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 4.5-5.3 mg/l

COMMENTS Test run 9/22/92 - 9/26/92; no apparent effects @ 24 hrs; a dose response effect @ 48 hrs, poor overall survival

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf - WAF

SPECIES Redfish - Sciaenops ocellatus

STATIC/FLOWTHROUGH flowthrough

TEST CONTAINER SIZE 250 ml beakers

SALINITY 32 ‰

NO. EXPOSED/CONCENTRATION 50

EXPOSURE UNITS %

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	20	64	44	12	48	74
48	20	28	16	2	6	12
72	0	6	2	0	2	0
96	0	0	0	0	0	0

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 25.0-25.4°C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.2-5.7 ml/l

COMMENTS test run 9/22/92-9/26/92 overall test results poor

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil + Corexit 9527

SPECIES Redfish Embryos Sciaenops ocellatus

STATIC/FLOWTHROUGH Flow through

TEST CONTAINER SIZE 250 ml beakers

SALINITY 30‰

NO. EXPOSED/CONCENTRATION 100

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	32	52	52	64	73	68
48	13	13	42	46	30	30
72	7	9	32	32	22	14
96	3	7	22	14	10	7

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 23.6 - 23.8 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.6 - 6.6 mg/L

COMMENTS Run 5/24/92 - 5/28/92 ~~data not used~~ 4F

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil - Water Soluble Fraction

SPECIES Redfish embryos Sciaenops ocellatus

STATIC/FLOWTHROUGH Flow through

TEST CONTAINER SIZE 250 ml beakers

SALINITY 30‰

NO. EXPOSED/CONCENTRATION 100

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	KMC 1/25/1993 93 97	55	46	51	69	67
48	17	17	47	27	48	24
72	9	7	37	14	17	4
96	2	4	15	5	2	0

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 25.6 - 27.4 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.2 - 5.9 mg/L

COMMENTS Run 5/24/92 - 5/28/92

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527 (Run with Western Gulf oil tests)

SPECIES Redfish embryos Sciaenops ocellatus

STATIC/FLOWTHROUGH Flowthrough

TEST CONTAINER SIZE 250 ml beakers

SALINITY 30‰

NO. EXPOSED/CONCENTRATION 100

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	20	0	54	59	45	46
48	9	0	11	21	35	21
72	0	0	4	13	11	14
96	0	0	3	9	6	1

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 25.8 - 26.8 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 5.2 - 6.4 mg/L

COMMENTS Run 5/24/92 - 5/28/92 data not used

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil WSF

SPECIES Penaeus setiferus Post larvae (PL) 15

STATIC/FLOWTHROUGH Flow through

TEST CONTAINER SIZE 250 ml beakers

SALINITY 30 ‰

NO. EXPOSED/CONCENTRATION 25

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	36	76	100	84	92
48	88	24	56	84	80	88
72	88	16	44	80	72	84
96	84	12	40	72	72	76

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.1 - 25.3 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 4.2 - 6.6 mg/L

COMMENTS Ran 8/25/92 - 8/29/92 note dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Central Gulf oil and Corexit 9527

SPECIES Penaeus setiferus Post larvae (PL)

STATIC/FLOWTHROUGH Flow Through

TEST CONTAINER SIZE 250 ml beakers

SALINITY 30‰

NO. EXPOSED/CONCENTRATION 25

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	96	0	4	40	88	76
48	92	0	0	48	84	72
72	80	0	0	36	68	72
96	76	0	0	32	68	72

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.8 - 25.8 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 4.4 - 7.3 mg/L

COMMENTS Ran 8/25/92 - 8/29/92 good dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Penaeus setiferus Post larvae (PL) 15

STATIC/FLOWTHROUGH Flow through.

TEST CONTAINER SIZE 250 ml beakers

SALINITY 30‰

NO. EXPOSED/CONCENTRATION

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	88	0	0	32	56	96 INF
48	76	0	0	24	44	100
72	68	0	0	24	40	92
96	68	0	0	16	40	88

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 25.3 - 25.8

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 4.7 - 7.2 mg/L

COMMENTS Ran 8/25/92 - 8/29/92 good dose response

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil WSF

SPECIES Penaeus setiferus Post larvae (PL) 22

STATIC/FLOWTHROUGH Flowthrough

TEST CONTAINER SIZE 250 ml beakers

SALINITY 28 ‰

NO. EXPOSED/CONCENTRATION 25

EXPOSURE UNITS Percent (%)

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	88	76	84	92	84	96
48	68	56	72	68	68	68
72	44	44	36	40	44	40
96	44	44	36	40	36	40

TEMPERATURE RANGE IN HIGHEST CONCENTRATION * 24.3 – 27.6 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 4.2 – 5.4 mg/L

COMMENTS Ran 9/1/92 – 9/5/92 no effects

*believe this temp is questionable. Taken last day by a different person and/or probe. All temp. readings on last day much higher than throughout rest of test.

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Corexit 9527

SPECIES Penaeus setiferus Post larvae (PL) 22

STATIC/FLOWTHROUGH Flow through

TEST CONTAINER SIZE 250 ml beaker

SALINITY 28 ‰

NO. EXPOSED/CONCENTRATION 25

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	CONTROL	100	50	25	12.5	6.25
24	100	44	68	96	88	96
48	60	16	28	56	68	24
72	36	0	28	36	56	16
96	36	0	28	36	52	12

TEMPERATURE RANGE IN HIGHEST CONCENTRATION * 25.4 - 27.7 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 4.1 - 5.9 mg/L

COMMENTS Ran 9/1/92 - 9/5/92 note dose response

* Believe this temp is questionable. All temp readings taken on the last day were done by a different technician, perhaps using a different probe. All readings that day were much higher than at any other day of the test.

MMS DISPERSANTS STUDY
SUMMARY DATA SHEET

TEST MATERIAL Western Gulf oil + Corexit 9527

SPECIES Penaeus setiferus Post larvae (PL) 22

STATIC/FLOWTHROUGH Flowthrough

TEST CONTAINER SIZE 250 ml beakers

SALINITY 28‰

NO. EXPOSED/CONCENTRATION 25

EXPOSURE UNITS mg/L

% SURVIVAL

	CONCENTRATION					
TIME (HRS)	**CONTROL**	**100**	**50**	**25**	**12.5**	**6.25**
24	100	36	48	72	88	92
48	100	16	40	76 1 found in tank	76	92
72	80	12	36	24	72	80
96	80	8	36	24	72	76

TEMPERATURE RANGE IN HIGHEST CONCENTRATION 24.5 - 25.9 °C

DISSOLVED OXYGEN RANGE IN HIGHEST CONCENTRATION 3.8 - 5.6 mg/L

COMMENTS Ran 9/1/92 - 9/5/92 note good dose response

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Royalty Management Program** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.