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STUDY TITLE: Oil & Oil Dispersants Toxicity Program

REPORT TITLE: Dispersed Oil Toxicity Tests with Species Indigenous to the Gulf of Mexico

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KEY WORDS: Straits of Florida; Eastern Gulf; Central Gulf; Western Gulf; acute toxicity testing; chemistry; invertebrates; fish; larvae; eggs; hydrocarbons; dispersant; Gulf of Mexico Region

BACKGROUND: Testing on the fate and effects of oil and dispersed oil on marine organisms can be traced back to large spills which occurred during the 1960's. Anderson *et al.* (1974) identified the low molecular weight aromatic hydrocarbons as the primary contributors to oil toxicity and described a method by which a water soluble fraction (WSF) of the oil could be prepared for testing the effects of oil. "Water accommodated fraction" (WAFL) has also been used to reflect those hydrocarbon fractions which are water soluble and the microscopic oil droplets found in suspension. Regulatory agencies are frequently faced with decisions on how best to respond to oil spills, including the potential use of dispersants. Decisions to use dispersants can result in tradeoffs that must weigh the amount of oil which will be dispersed into the water column, thus affecting pelagic organisms. In commercially-important fishery areas, such decisions can have environmental, economic, and political implications.

OBJECTIVES: 1) To expose the eggs and/or larvae of seven Gulf of Mexico fish and shellfish species and one Atlantic fish species to the WAF of two different Gulf of Mexico oils, dispersed oil mixtures, and a single dispersant in controlled, flowthrough or static 96-hr acute toxicity tests; 2) To characterize the chemical composition of the test oils and exposure media (i.e., WAF, dispersed oil dispersant) during various phases of acute toxicity testing; 3) To summarize the results of acute toxicity testing and parallel chemical analyses of exposure media on each of the species using gas chromatography (GC), GC/mass spectrometry (GC/MS), and GC/MS with selected ion monitoring (GC/MS-SIM).

DESCRIPTION: Static and flowthrough exposure methods were utilized on egg and larval stages of commercially-important species from the Gulf of Mexico including brown shrimp (*Penaeus aztecus*), white shrimp (*P. setiferus*), blue crab (*Callinectes sapidus*), eastern oysters (*Crassostrea virginica*), red drum (*Scianops ocellatus*), inland silverside (*Menidia beryllina*), and spot (*Leiostomus xanthurus*). As attempts to secure eggs and larvae of gulf menhaden (Brevoortia patronus) were unsuccessful, a congener *(Atlantic menhaden, Brevoortia tyrannus*) was evaluated using study-specific acute toxicity testing protocols. Mysids (*Mysidopsis bahia*) were also evaluated in a chronic toxicity assessment. Test organisms were exposed to the WAF of two different oils (i.e., Western and Central Gulf of Mexico OCS) and dispersed oil mixtures, as well as a single dispersant (Corexit 9527). Specially-designed flowthrough exposure chambers were developed, evaluated, and utilized during the study. LC_{50} values and Toxicity Index (TI) determinations were made for all species. The TI is a relative measure of toxicity calculated from the product of exposure concentration and time (e.g., ppm x hours = ppm-hrs). TI values were determined for total naphthalenes, total petroleum hydrocarbons (TPH), and BTEX compounds (i.e., benzene, toluene, ethylbenzene, xylenes).

SIGNIFICANT CONCLUSIONS: Replication between flowthrough exposure was good, particularly with regard to hydrocarbon exposures. Agreement in the static exposures was less evident, suggesting that greater variability is likely in toxicity results originating from this exposure method. Embryonic stages of the species tested exhibited lower sensitivity than early larval stages. The overall sensitivity of fish versus invertebrates appeared to be similar. Invertebrates performed better as test organisms, as overall control survival was better. The naturally high mortality of fish larvae compounded efforts to obtain acceptable test results. Significantly higher concentrations of total naphthalenes and TPH were measured in the dispersed oil mixtures compared to the WAF; however, the dispersed oil did not reflect a correspondingly greater toxicity. BTEX was measured at levels an order of magnitude higher in the WAF than in the dispersed oil mixtures. These results suggest that the BTEX compounds were a possible source of toxicity in the WAF exposures, whereas naphthalenes may have been the primary cause of toxicity in the dispersed oil exposures. In most exposures, a majority of the toxicity occurred within the first 24 hrs.

STUDY RESULTS: 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 polynuclear aromatic hydrocarbon compounds analyzed. Hydrocarbon concentrations at the end of the study (i.e., in Month 19) revealed several basic differences from the initial analyses. Naphthalenes and fluorenes were generally higher, while the phenanthrenes, dibenzothiophenes, and chrysenes exhibited lower or similar concentrations when compared to initial measurements. Differences in concentrations may suggest variability within the testing method rather than being indicative of any real change in the oils over time. Dispersant characterization indicated minimal naphthalene and phenanthrene concentrations of 0.16 and 0.012 µg/g, respectively. Static tests tended to have the highest overall TPH concentrations with the dispersed oil levels being 4-5 times greater than that measured in the WAF. Flowthrough concentrations tended to be more variable without the clear distinctions seen in the static tests. 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 note 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. Brown shrimp were tested with the Western Gulf WAF and dispersed oil. All of the toxicity in the two exposures occurred within the first 24 hrs with little to no toxicity observed in subsequent days; consistent with hydrocarbon patterns were the majority of the materials were lost within the first 24 hrs of exposure. White shrimp tests were conducted with both the Western ad Central Gulf oils using postlarvae at ages of 15 days (Central Gulf oil) and 22 days (Western Gulf oil). The dispersant itself had a measured LC_{50} of 11.9 mg.l. 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 grown for an additional 30 days in clean seawater and weighed. 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 concentrations 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, 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. These results also closely paralleled toxicity testing results. Blue crab exposures were conducted with both Central and Western Gulf oils. Based on results of two dispersant only tests, similar sensitivities to the dispersant were obtained. Similarly, the results from WAF exposure did not differ greatly. 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, suggesting a greater sensitivity to the dispersed oil. However, it was also suggested that molting may have occurred at a particularly critical period with regard to the severity of effects and may explain the

differences observed between the two oils. Oysters were exposed in static tests to the dispersant and to dispersed oil mixtures; no tests were conducted with WAFs due to difficulties encountered during spawning. In addition to the determination of an EC_{50} value for oysters, lowest observed effect concentrations (LOEC) and no observed effects concentrations (NOEC) were calculated using the Kruskai-Wallis method. The dispersant was particularly toxic to the embryo/larval stages, resulting in an EC_{50} <6.25 ppm. Similarly, the dispersed oil mixtures had statistically significant effects at the lowest concentrations tested. In exposures to the Central Gulf dispersed oil, the EC_{50} was, 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 pp.

Tests with embryos and larvae of inland silverside were conducted under both static and flowthrough exposures. Numbers of surviving embryos and larvae were similar after 96 hrs in the three different exposure media. 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 controls, the 50% and 50 mg/l concentrations showed a similar rate of mortality in the three exposures, suggesting a delayed response. A subsequent 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 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. Atlantic menhaden were subjected to static exposure conditions because of sensitivity to flowthrough testing protocols. 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 due to significant control mortalities. Hatching success and fry survival were evaluated in various exposures to Central and Western 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. Tests with red drum were limited due to difficulties encountered in maintaining controls. In those tests were acceptable results were obtained for the two oils, this species proved to be relatively insensitive. 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 concentrations of the dispersed oil and in the higher concentrations of the dispersant. The WAF did not appear to significantly affect the hatching success. In summary, significantly higher concentrations of total naphthalenes and TPH were measured in the dispersed oil mixtures compared to the WAF; however, the dispersed oil did not reflect a correspondingly greater toxicity. BTEX was measured at levels an order of magnitude higher in the WAF than in the dispersed oil mixtures. Results suggest that the BTEX compounds were a possible source of toxicity in the WAF exposures, whereas naphthalenes may have been the primary cause of toxicity in the dispersed oil exposures.

STUDY PRODUCTS: Fucik, K.W., K.A. Carr, and B.J. Balcom. 1994. Dispersed Oil Toxicity Tests with Biological Species Indigenous to the Gulf of Mexico. OCS Study/MMS 94-0021. A final reports prepared by Continental Shelf Associates, Inc. for the U.S. Department of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA. MMS Contract No. 14-35-0001-30617. 97 pp. + appendices.

*P.I.'s affiliation may be different than that listed for Program Manager.