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TOXICITY OF VARIOUS OFF-SHORE CRUDE OILS AND DISPERSANTS TO MARINE AND ESTUARINE SHRIMP

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ABSTRACT

The acute effects of four crude oils and two oil spill removers on four species of marine shrimp (*Penaeus setiferus*, *P. aztecus*, *Palaemonetes vulgaris*, and *P. pugio*) were determined. Results of 48-hour bioassays showed that distinctive differences in toxicity existed between crude oils from different areas with all shrimp tested. The oil spill removers were much more toxic than the crude oils. Addition of the oil spill removers to all crude oils at recommended application ratios increased the toxicity of both the crude oils and the oil spill removers, indicating a synergistic effect. The *Palaemonetes* species appeared more tolerant to all toxicants.

Evidence indicates that the most serious effects of oil pollution would be noted in the shallower areas where high concentrations of toxic compounds may build up.

INTRODUCTION

Recent studies of oil pollution have mainly been concerned with the ecological effects of spilled petroleum products on the marine ecosystem (Diaz-Piferrer, 1962; Hawkes, 1961; McCauley, 1966; North, 1961; O'Sullivan and Richardson, 1967; Rutzler and Sterner, 1970). Due to lack of quantitative field data, it is impossible to predict the biological

effects of specific oil spills or subsequent treatment of slicks. Until quantitative and qualitative data on the volume of crude oil and/or emulsifiers on a known quantity of water in the environment are available, recommendations for setting water quality standards for crude oil and emulsifiers in the environment will have to be interpreted from laboratory experiments.

This study provides laboratory toxicity data on: (1) the acute toxicity of four different crude oils collected from off-shore wells and two oil spill removers to four species of marine shrimp; (2) the effects of the addition of the two oil spill removers on the toxicity of the four crude oils to the same four species of shrimp.

MATERIALS AND METHODS

The bioassays were conducted using four species of marine shrimp: *Penaeus setiferus*, *P. aztecus*, *Palaemonetes vulgaris*, *P. pugio*. Sizes ranged from 20 mm to 100 mm for *Penaeus* and 15 mm to 25 mm for *Palaemonetes*.

Bioassays were conducted with four crude oils and two oil spill removers. Only the *Palaemonetes* sp. were tested with crude oil/oil spill remover mixtures.

All test organisms were collected between June 1, 1969, and November 15, 1969, from the southern reaches of Barataria Bay, Louisiana. Collected shrimp were aerated with portable air pumps, taken to the laboratory within 3 hours of collection, separated by species, acclimated to ambient laboratory conditions, and transferred to aerated holding tanks covered with fiberglass screens to prevent escape.

Two holding medias were used: settled natural bay water, and aged, artificial sea water, "Instant Ocean". Salinity was adjusted in the holding tank to that encountered in the collection area, by adding either fresh water or Instant Ocean.

Test organisms were held for a minimum of 96 hours prior to testing. During this time the shrimp were fed a commercial tropical fish food TETRAMIN, manufactured by the TetraKarftWerk Company of West Germany. Feeding was discontinued 24 hours before testing. Any population showing an unusually high death rate during this holding period was discarded.

Bioassay Methods

The basic bioassay procedures suggested by the American Association of Public Health (1965) were used throughout the tests. The test containers were glass, rectangular 6-liter aquaria filled with 4 liters of sea water. A minimum of two replicates were run at each concentration. All tests were held within the recommended limits of 2 grams of living tissue per liter of aerated water.

Because of the excess handling required in identification and separation of the shrimp by species, preliminary bioassays were conducted to determine if it would be possible to run all tests by combining the two species of each genus. Because the responses of the two species of each genera were similar for each toxicant in the preliminary tests the species were combined and testing was conducted on a generic level.

Since the Federal Water Pollution Control Administration Interim Procedures (1969) recommend using an artificial sea water for testing, preliminary tests were also conducted with two of the crude oils using natural sea water and "Instant Ocean" to determine if similar results would be obtained. Because no differences in toxicity levels were noted, tests were conducted with the prepared sea water. The artificial sea water was aged at least 72 hours prior to testing. The test water was added to aquaria 6 hours before introduction of the shrimp to allow the water to reach ambient conditions. Shrimp were introduced into the aquaria and allowed to acclimate to their conditions for an hour before addition of the toxicants.

The oils from each of the four wells were tested separately. Pre-measured amounts of crude oil were slowly poured into the aquaria. Constant aeration was used to assure maximum oil/water contact and to insure adequate oxygen for the test shrimp. Tests were conducted for 48 hours. Mortality was recorded at 12-hour intervals and any dead shrimp removed.

In testing the combined crude oil/oil spill remover, the oil was poured into the aquaria first. The oil spill remover was sprayed into the oil at the concentration recommended by the manufacturer of one part remover to ten parts crude oil. Mixing during the test period was accomplished by constant aeration.

At least four concentrations of each toxicant were tested. Each test included control aquaria containing no toxicants. If any of the aquaria showed low dissolved oxygen levels the test results were discarded. Dissolved oxygen concentrations were maintained between 75-100% saturation. Water temperatures were maintained between 19-21 C. Salinity was maintained at 25 ± 2 ppt.

Description of Toxicants

The crude oils were provided by the Humble Oil and Refining Company from four of their offshore oil wells. Each oil came from different depths and strata. The oils were collected directly from production lines on the platforms and were not exposed to the open air for any length of time. The oils were stored in tightly covered containers to prevent evaporation of any component. Table 6 shows the oil analyses run by the E. W. Saybolt Company, Houston, Texas.

The two oil spill removers tested were obtained from local distributors. Corexit 7664 is a product of the Enjay Chemical Company, a subsidiary of Humble Oil and Refining Company. Corexit is described by its manufacturer as a water based, amber colored liquid that is a proprietary surface active compound (Anonymous, 1969). They state that the product acts as an emulsifier and is reported to be "non-toxic" to aquatic life.

The ameroid brand oil spill remover is manufactured by the Drew Chemical Company of Ajax, Ontario. This product appears as a viscose, amber-colored water-soluble liquid having a strong kerosene odor. The flash point of this product is reported to be 75 C on the label. The product also has a strong foaming action. From the odor, flash point and foaming action of this product it appeared to be a kerosene-synthetic detergent mixture. This product is described on the label as being relatively harmless when mixed with oil.

RESULTS

Crude Oil Tests

Table 1 indicates that the ranges in 48-hour LC₅₀ values for the four oils on *Penaeus* shrimp were from 1.0 to 40.0 ppt. The maximum concentrations of oil that produced no mortality varied from slightly less than 1 ppt to 10 ppt. Concentrations causing 100% mortality ranged from 7.5 ppt to 75 ppt. Crude oil from well Q-4-D was the most toxic, followed by Q-30 and W-30 (which gave similar responses) and the

TABLE 1. Approximate 48-hour LC₀, LC₅₀ and LC₁₀₀ values for *Penaeus* shrimp and crude oil (ppt).

Oil Number	LC ₀	LC ₅₀	LC ₁₀₀
Q-30	<1.0	7.5	15.0
W-30	2.5	5.0-7.5	>10.0
W-4-D	10.0	40.0	50.0-75.0
Q-4-D	<1.0	1.0-2.5	7.5

W-4-D oil was least toxic (symbols represent field, block, and well number).

Table 2 indicates that the LC₅₀ values for *Palaemonetes* were similar to the LC₅₀ values for *Penaeus* (Table 1) for the different crude oils. The ranges were slightly lower with the *Palaemonetes* shrimp, 1.0 to 25.0 ppt (Table 2). Again, oil Q-4-D appeared to be the most toxic at all LC levels, followed by Q-30 and W-30 (similar), and W-4-D. A higher concentration of the oils was necessary to kill all *Palaemonetes* shrimp as compared to the *Penaeus*. It took from 1.25 to 2.0X more oil to kill 100% of the *Palaemonetes* sp. The oil had to be increased 8 to 20X in going from LC₀ to LC₁₀₀ values with *Palaemonetes* shrimp (Table 2). With *Penaeus* shrimp the increase in oil concentration was from 4 to 15X over the LC₀ to LC₁₀₀ spread (Table 1). The data indicates that the *Palaemonetes* species may be more tolerant to crude oils than the *Penaeus*.

TABLE 2. Approximate 48-hour LC₀, LC₅₀ and LC₁₀₀ values for *Palaemonetes* shrimp and crude oil (ppt).

Oil Number	LC ₀	LC ₅₀	LC ₁₀₀
Q-30	<2.5	5.0-10.0	>20.0
W-30	1.0	7.5	20.0
W-4-D	<5.0	10.0-25.0	100.0
Q-4-D	<1.0	1.0-5.0	10.0

Oil Spill Removers

Table 3 illustrates the LC values noted with the *Penaeus* shrimp tested with the two oil spill removers. The Ameroid remover was up to 2000X more toxic than the Corexit brand. LC₅₀ values of 2.5 ppm with Ameroid and 5000 ppm for Corexit were obtained for the *Penaeus* shrimp. For *Palaemonetes*, the Ameroid remover was up to 10,000X

TABLE 3. Approximate 48-hour LC₀, LC₅₀ and LC₁₀₀ values for *Penaeus* shrimp and oil spill removers (ppm).

Oil Spill Remover	LC ₀	LC ₅₀	LC ₁₀₀
Corexit	500.0	5,000.0	>7,500.0
Ameroid	<0.5	2.5	5.0

more toxic than Corexit (Table 4) at all LC values obtained. This is shown well with LC₅₀ values, Ameroid being 1.0 ppm and Corexit being 10,000 ppm. The Ameroid oil spill remover was highly toxic and the LC₀, LC₅₀ and LC₁₀₀ values were similar for both genera of shrimp (Tables 3 and 4). *Palaemonetes* were more tolerant to Corexit than

TABLE 4. Approximate 48-hour LC₀, LC₅₀ and LC₁₀₀ values for *Palaemonetes* shrimp and oil spill removers (ppm).

Oil Spill Remover	LC ₀	LC ₅₀	LC ₁₀₀
Corexit	<5,000.0	10,000.0	50,000.0
Ameroid	<0.5	1.0-2.5	5.0

Penaeus. For *Palaemonetes*, the LC₅₀ concentration was 10,000 ppm but the *Penaeus* value was only 5000 ppm, a 2X difference. *Palaemonetes* was over 6X more tolerant to the LC₁₀₀ level. At the LC₁₀₀ value, the *Palaemonetes* tolerated 5X the LC₅₀ concentration while the *Penaeus* shrimp tolerated 1.5X the LC₅₀ concentration.

Crude Oil/Oil Spill Remover Mixtures

The response of the *Palaemonetes* to the oil/Corexit mixtures is shown in Table 5. Corexit was added at a concentration of 10% (v/v) of the oil. Ranges of LC₅₀ values for the oils are from 0.5 ppt to 5.0 ppt. Comparison of the oil LC₅₀ values of Table 5 with Table 2 shows that the toxicity of the crude oil/Corexit mixture was up to 13.3X greater than the crude oil alone. Comparison of the LC₅₀ values with Table 4 shows that the toxicity of the mixture was up to 200X greater than Corexit alone.

TABLE 5. Approximate 48-hour LC₀, LC₅₀ and LC₁₀₀ values for *Palaemonetes* shrimp and crude oil (ppt)/Corexit (ppm) mixture.*

Oil Number	LC ₀		LC ₅₀		LC ₁₀₀	
	Oil	(Corexit)	Oil	(Corexit)	Oil	(Corexit)
G-30	0.5	(50)	0.75	(75)	5.0	(500)
W-30	5.0	(50)	1.00	(100)	5.0	(500)
W-4-D	2.5	(250)	5.00	(500)	10.0	(1000)
Q-4-D	0.25	(25)	0.50	(50)	5.0	(500)

* These figures indicate crude oil concentration (ppt). Corexit concentration is 10% of the crude oil concentration (ppm).

The same trends are noted with the LC₁₀₀ values. The oil concentrations causing 100% mortality when mixed with Corexit were from 2 to 10X more concentrated than the amount of crude oil alone required to produce 100% mortality (Table 2). The Corexit concentrations mixed with oil causing 100% mortality were from 50 to 100X less concentrated than the amount of Corexit alone required for 100% mortality (Table 4).

At the LC₀ level the oil/Corexit mixture was from 2 to 5X more toxic than the crude oils (Table 2) and from 20 to 200X more toxic than Corexit alone (Table 4).

TABLE 6. Results of physical and chemical analysis performed by the E. W. Saybolt Company, Inc. on the test crude oils.

Property	Oil Number			
	Q-30	W-30	Q-4-D	W-4-D
Gravity, API				
at 60 F	33.5°	29.3°	32.3°	22.4°
Viscosity, S.U.				
at 100 F	45.4 sec	63.5 sec	50.4 sec	188.1 sec
Asphaltenes	NIL	NIL	NIL	NIL
Naphtha	23%	26%	14%	15%
Gas Oil				
390-620 F	36%	24%	40%	22%
Heavy Distillate				
620-760 F	27%	36%	36%	13%
Residuum	14%	14%	10%	50%
Sulfur				
ASTM D1551	0.22%	0.33%	0.30%	0.83%

Oil Q-4-D when mixed with Corexit, was more toxic than the other oil/Corexit mixtures, followed by oil Q-30, W-30, and W-4-D.

It is interesting to note that in all tests involving the crude oils, oil Q-4-D was most toxic at nearly all LC values, oils Q-30 and W-30 were second in toxicity, and oil W-4-D was least toxic.

DISCUSSION

Crude Oils

Since oil is a complex mixture it is not surprising to find chemical differences in individual oils and different biological responses to each different oil. Several investigations have indicated that the most toxic components of crude oils may be in the lightest fractions (Habault, 1936; Shelford, 1917; Tagatz, 1964; Holcomb, 1969). Tagatz (1964) reports that the gasoline fraction was the most toxic to juvenile American shad, *Alosa sapidissima* (48-hour TL_m 91 ppm). Diesel fuel was somewhat less toxic (48-hour TL_m 167 ppm) and heavy bunker oil the least toxic (48-hour TL_m 2417 ppm). When testing the toxicity of crude oil to oysters, Collier (1953) reported that when petroleum was poured onto the water surface, most of the toxic components evaporated into the air, indicating these were the lightest fractions. A recent review by a private research laboratory (Battelle Memorial Institute, 1967) reported literature indicating that fresh oil contains some toxic, volatile compounds that have deleterious effects upon molluscan and crustacean shellfish. Smith (1968) also reports similar results with oil from the "Torrey Canyon".

A preliminary test was conducted to observe the effects of a light fraction of crude oil on a species of marine shrimp. Number 2 diesel oil (similar to the lighter fractions of most crude oils) was tested with the Brown shrimp (*Penaeus aztecus*) to obtain a 48-hour LC_{50} value. Due to rapid evaporation of the toxicant, no in-depth study was conducted with the product during this project. However, the preliminary test on 15 Brown shrimp per concentration gave a 48-hour LC_{50} value of approximately 100 ppm. This value is similar to that reported by Tagatz (1964) for American shad, and may indicate a similar response to these light fractions by different aquatic organisms.

The two oils, Q-4-D and W-4-D showed great differences in toxicity with both genera of shrimp. Table 6 indicates that oil Q-4-D, the most toxic, had a gas oil fraction percentage almost twice that of oil W-4-D, the least toxic and the heavy distillate fraction of oil Q-4-D was nearly three times that of oil W-4-D. Some tests in this study were maintained up to 72 hours. Little mortality occurred after 48 hours. The major cause of death after 48 hours appeared to be low oxygen concentration due to heavy bacterial growth. This leveling off of mortality indicates that the most toxic components of the crude oil evaporated rapidly after exposure to the open air and that components causing acute toxicity responses are the lighter fractions of the crude oil, particularly gas oil. The sulfur residuum of Q-4-D was one-third that of W-4-D, indicating sulfur at least in oil has little influence on the toxicity.

Oils Q-30 and W-30 were intermediate in toxicity and also had similar chemical and physical properties in the lighter components. Oil Q-30 was observed to have a higher percentage of the lighter fractions. Q-30 was also slightly more toxic than W-30 for all genera of shrimp tested (Tables 1 and 2). This higher toxicity may have been due to the higher gas oil fraction. The elemental sulfur component apparently had little toxic effect since the least toxic oil W-30 had the highest concentration. The same relationship appears to be true for the heavy distillate fraction.

The Naphtha fraction concentrations were almost identical in most toxic and least toxic oils. This should indicate that the difference in toxicity does not lie completely in this fraction.

If the toxic components of the crude oils tested are not in the lighter fractions they may be found in various compounds that were not

measured by the Saybolt analysis. These could be compounds that are slightly volatile or that leach out of the oil into the water, but either evaporate or are broken down into harmless compounds by biodegradation or oxidation. These may be the "water soluble" components reported by several investigators (Chipman and Galstoff, 1949; Tagatz, 1964; McKee, 1956; North, 1961; Portmann and Connor, 1968).

Oil Spill Removers

Most investigators agree that oil spill removers are very toxic to aquatic life (Corner, Southward and Southward, 1968; Portmann and Connor, 1968; George, 1961; O'Sullivan and Richardson, 1967; Smith, 1968). In all cases the oil spill removers tested during this project tended to substantiate these other investigators. Even the most "non-toxic" oil spill remover caused mortality to all test organisms at low concentrations when mixed with crude oil (Table 5).

The Ameroid brand remover caused a loss of equilibrium which resulted in the shrimp swimming upside down and sideways. They would eventually lie prostrate on the bottom of the test containers. Extreme excitation was also noted and often, premature molting occurred by comparison with the control animals. At concentrations of 0.5 to 1.0 ppm it took several hours for these distress symptoms to appear. Above 5.0 ppm these symptoms were noted in a few minutes.

The Corexit brand oil spill remover, though less toxic than the Ameroid brand, and labeled "non-toxic" by its manufacturer, was lethal to our test organisms at concentrations much lower than reported for other *Penaeus* species. The manufacturer of Corexit claims that it has been shown to be "non-toxic" to Pink shrimp (*Penaeus duodrum*) at a concentration of 1.0% (Anonymous, 1969). For the two species of *Penaeus* shrimp tested in our study a 48-hour LC_{50} value of 5000 ppm (0.5%) was obtained (Table 3) and a non-toxic response at 0.05%. One possible explanation for this observed difference could be species difference.

Crude Oil/Oil Spill Remover Mixtures

The Ameroid brand oil spill remover appears to be similar to the types used on the Torrey Canyon spill off the English coast in March, 1967. Corner, Southward and Southward (1968) report that the removers used in the Torrey Canyon spill contained 80-90% kerosene and other light petroleum components. If this is the case, then the major component of this oil spill remover is similar to what appears to be the most toxic component of the crude oil itself. The two apparent toxic fractions of crude oil represent 33% to 76% of the total volume of the crude oil. If an oil spill remover is added at the recommended 10% of the volume of the crude oil then in effect, the toxic component will be increased by 8% to 9%. This would be the effect of the addition of an Ameroid type remover with an 80-90% light oil fraction or kerosene base. Only preliminary tests were conducted with a crude oil/Ameroid mixture because all concentrations that would be effective in removing oil were far in excess of the lethal limits of the Ameroid alone. All attempts to test with this mixture killed all test organisms within a few minutes.

Portmann and Connor (1968) report that the addition of certain emulsifiers to crude oil reduces the level of toxicity for the emulsifier. An increase in toxicity was observed during this project. Other studies have also reported an increase in toxicity (Zillich, 1969). Though some investigators also reported a decrease or little change in toxicity with the addition of oil spill removers (Portmann and Connor, 1968; Dowden, 1962), results from the present study indicate a synergistic effect between the two products. The oil was broken into many small droplets which did not coalesce easily. This allowed a greater amount of the emulsified oil to come into contact with the test organisms. Perhaps

this fine emulsion allowed greater interference with natural functions, such as gill action and oxygen exchange, motion or metabolism. The remover may also have allowed a greater amount of toxicants to be released from the oil into the water. Any of these factors should tend to increase the toxicity of the mixtures over the observed toxicity of the individual products. Thus it would seem that toxicity should increase rather than decrease when an emulsifier is added to crude oil.

The expressed purpose of these oil dispersants is to make the oil more susceptible for degradation by natural processes. By making these hydrocarbon products more available to the environment a greater proportion of potentially harmful products may enter the food chain through deposition in biological organisms (Blumer, Souza, Sass, 1970). These products are evidently not eliminated by these organisms but are stored and may be passed along the food chain. Since some of these products are carcinogenic (Blumer, 1970) the chance of these contaminated organisms reaching man should preclude the use of chemical dispersants except under very special circumstances.

Though most authorities agree that removal of floating oils with chemical agents may cause more problems than the oil alone, these agents are still widely used. On the basis of this study it may be said that the use of the chemical removers could have serious economic and ecological implications in shallow fishing and nursery areas where toxicant concentrations could build up easily. These effects might not be noticed as easily in areas having a deeper water column. Most authorities would probably agree that the best solution to the ever increasing problem of oil pollution is in prevention. With effective preventive measures the smaller number of oil spills that occur could probably be taken care of by natural physical and biochemical processes, as has been observed even in cold Alaskan waters (Kinney, Button and Schell, 1969).

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