# The Role of Dispersion in Fuel Oil Bioassay

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## INTRODUCTION

In recent years, due to proliferation in the use and transport of petroleum in the marine environment, an extensive literature has developed concerning the effects of accidental spillages. Literature is also available on long-term effects of repeated or chronic low-level discharges of petroleum-derived hydrocarbons. One approach toward assessment of potential effects, of both large scale spillage and low-level discharge, has been to conduct laboratory bioassay studies to determine short-term lethal and sublethal effects on individual marine species. While that approach is severely limited in predicting ecosystem effects, it does serve to bring into focus the complexity inherent in oil-seawater mixtures.

Oil and many of its products are a complex of volatile, water soluble, and insoluble compounds which comprise an as yet inadequately defined dynamic system in seawater. Apparent order of magnitude differences in concentrations required to produce lethal or sublethal biological response to petroleum in laboratory studies (JACOBSON and BOYLAN 1973; KITTREDGE 1971; SWEDMARK et.al 1973; TAGATZ 1961; MIRONOV 1970) may relate, in part, to the sensitivity of biological endpoint or organisms used. Most likely, reported differences are due also to expression of concentration in terms of volume-to-volume ratios of oil and seawater. As early as 1921, for example, dispersion was recognized as a primary determinant of mortality in laboratory studies which received identical volumetrically determined treatments (GUTSELL 1921).

Recently, (VAUGHAN 1973; ANDERSON, et.al. 1974) investigators have reported severity of treatment in petroleum bioassay in terms of the aqueous phase concentration of petroleum or selected components. Such an approach has allowed a more precise definition of the long-recognized (GUTSELL 1921; TARZWELL 1971) role of dispersion in petroleum bioassay. However, sampling requirements needed to adequately describe the dynamic system, even in simple bioassay chambers, have not been determined.

Mortality of coon stripe shrimp (Pandalus danae) has been investigated in flowing water aquarium systems receiving measured "spills" of fuel oil with differing methods of seawater delivery

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This work was conducted by Battelle, Pacific Northwest Laboratories, for the U.S. Atomic Energy Commission (now Energy Research and Development Agency) under contract AT(45-1)-1830.



Bulletin of Environmental Contamination & Toxicology,
Vol. 15, No. 1 © 1976 by Springer-Verlag New York Inc.

and analytical characterization. These studies stemmed from a need to develop a repeatable treatment for application to marine intertidal communities under continuous flow laboratory conditions. While the goal of repeatable treatment for long-term exposure was not realized in this endeavor, the results obtained offer a data base for discussion of the influence of seawater delivery methods and dispersion on bioassay systems. The specific objective in this research was to measure aqueous phase concentrations of No. 2 fuel oil and mortality of shrimp when three different methods of oil-seawater contact were used.

## METHODS

Three methods of oil and seawater introduction were evaluated. The first approach was intended to eliminate surface disturbance and subsequent dispersion of oil in the water column; the second method attempted to accomplish a moderate but uniform amount of oil-water mixing prior to exposure tank delivery; and the third was designed to enhance dispersion of oil droplets for the duration of the experiment.

Coon stripe shrimp, which served as the test species in each experiment, were captured from Sequim Bay, Washington by otter trawl, fed freshly cracked littleneck clams (Protothaca staminea) during pretest holding, and randomly assigned to tests and treatments. Shrimp size ranged from 5 to 8 cm in total length. Ten shrimp were used in each test chamber.

Test chambers were fiberglass aquaria (93 x 44 x 30 cm) with a solution volume of 75 l. Each chamber received a continuous seawater inflow of 2 l/min. The position of the inflow differed for each of the treatment conditions under investigation. For the first method, designed to eliminate surface turbulence, seawater inflow was through a 1/2-in (1.3 cm) polyvinyl chloride pipe with entry port 5 mm below the water surface. Oil was introduced at a 5 mm depth 4 cm from the point of water entry. To obtain mixing of oil and water prior to tank delivery, in the second method, seawater and oil were introduced through 1-in (2.5 cm) static mixers (Kenics Model 1-10-321-5) with the inflowing water released 1 cm above the test tank water surface. For that design, oil was pipetted into the top of the mixer in 5 ml aliquots at 5 min intervals until the total volume had been introduced. For the third method, designed to enhance dispersion throughout the test period, seawater was allowed to fall from a height of 20 cm above the test tank water surface in a stream 0.33 cm in diameter. Oil was introduced by pouring at the perimeter of the turbulence caused by the falling seawater.

Surface oil was retained in the test chambers in all experiments by a U-shaped riser pipe discharge at the end opposite the infall. In each replicate identical volumes (0, 5, 12.5, 25, 50, and 100 ml) of No. 2 fuel (API standard reference oil, 40 percent aromatics) were used.

Temperature, dissolved oxygen, and salinity were monitored for each concentration in each replicate. Ambient test temperatures were measured by mercury thermometer and averaged 11.7°C. Salinity was measured with a temperature-compensated American Optical refractometer. Mean salinity was 30 parts per thousand.

Dissolved oxygen, measured by the seawater modification of the Winkler technique (STRICKLAND and PARSONS 1968) averaged 8.57 mg/l. To prevent sample contamination by surface oil, a pre-placed glass siphon at the midpoint of each test solution was used in sampling for water quality and fuel oil analyses. Concentration and time strata for oil samples are presented with the results.

Analyses of carbon tetrachloride (Burdick and Jackson, Analytical Grade) extracts of seawater for total oil were by infrared spectrophotometry (Perkin-Elmer 257) using the procedures described by VAUGHAN (1973). To evaluate the degree of solubilization produced by the mixing designs and identify compounds potentially contributing to observed mortality, additional samples were filtered under nitrogen pressure through 0.45-micron millipore filters and analyzed by gas-liquid chromatography. Rationale for considering such samples as representative of soluble oil as well as details of the chromatographic method are given by BEAN et.al. (1974).

## RESULTS

Two replicates were performed using the first method of oil and seawater introduction (oil and seawater below the surface). There was no shrimp mortality or behavioral response within 24 hours. Samples were taken for fuel oil analysis from all concentrations at 0.5, 1, 3, 6.5, and 24 hours. Additional samples in the 50 ml introduced volume replicates were taken at 30 min intervals for the first 11 hr of exposure. Measured total oil concentrations in the intensively sampled tanks (50 ml) were all less than 1 mg/l. Likewise, for the concentration stratified samples, measured total oil concentrations were negligible. Selected samples for soluble components revealed concentrations below the lower validated limit for the method (0.3 mg/l for benzene; 0.01 mg/l for other compounds).

The second method (static mixers 1 cm above water surface) was applied in two replicates. Shrimp mortality did not occur at any concentration in either replicate. In the first replicate, shrimp in the three highest fuel oil volumes (25, 50 and 100 ml) were noticeably "agitated" during the first hour of exposure. Similar behavior has been observed as a response to low concentrations of crude oil in earlier studies (BEAN et.al. 1974).

Samples were drawn from the tanks receiving 50 ml-introduced fuel oil at 1-hr intervals for the first 10 hr of exposure and at the 24-hr termination. Samples were taken at 1, 4, 6, and 24 hr for the remainder of the tanks. Measurable fuel oil concentrations were produced even at the lowest introduced volume. A summary of measured concentrations is listed on TABLE 1. Selected samples from control tanks did not reveal measurable total oil concentrations in any instance. In all tanks, concentrations for soluble components were below the lower validated limit for the method of analysis used.

Six experimental replicates were performed using seawater in a stream 20 cm above the water surface, the third introduction method. Shrimp mortality was observed at 50 ml and 100 ml volume introductions in every replicate (TABLE 2). Measurable total

TABLE 1.
Total fuel oil concentrations (mg/l) measured by infrared spectrophotometry in bioassay using static mixers.

| Volume of Fuel Oil (ml) | Hours from Introduction | Mean Concentration | Standard Deviation |
|---|---|---|---|
| 5 | 1 | 3.16 | 2.62 |
| 5 | 4 | 2.40 | 1.36 |
| 5 | 6 | 2.49 | 0.59 |
| 5 | 24 | 0.65 | 0.49 |
| 12.5 | 1 | 4.48 | 0.99 |
| 12.5 | 4 | 3.76 | 0.62 |
| 12.5 | 6 | 3.95 | 2.35 |
| 12.5 | 24 | 3.51 | 0.32 |
| 25 | 1 | 2.48 | 0.70 |
| 25 | 4 | 0.78 | 0.03 |
| 25 | 6 | 5.12 | 0.19 |
| 25 | 24 | 6.25 | 1.04 |
| 50 | 1 | 4.84 | 0.05 |
| 50 | 2 | 3.80 | 3.84 |
| 50 | 3 | 3.60 | 0.52 |
| 50 | 4 | 1.80 | 1.59 |
| 50 | 5 | 2.40 | 1.70 |
| 50 | 6 | 3.91 | 3.22 |
| 50 | 7 | 2.87 | 1.47 |
| 50 | 8 | 3.29 | 1.42 |
| 50 | 9 | 3.46 | 2.03 |
| 50 | 10 | 3.34 | 4.33 |
| 50 | 24 | 4.63 | 1.96 |
| 100 | 1 | 7.98 | 9.16 |
| 100 | 4 | 8.47 | 0.61 |
| 100 | 6 | 3.08 | 0.40 |
| 100 | 24 | 1.57 | 1.00 |

TABLE 2.
Summary of mortality in experiments receiving continuous dispersion during shrimp exposure (n = 10).

| | Volume of Fuel Oil Introduced (ml) | | | | | |
|---|---|---|---|---|---|---|
| Replicate | 0 | 5 | 12.5 | 25 | 50 | 100 |
| 1 | 0 | 0 | 1 | 0 | 9 | 10 |
| 2 | 0 | 0 | 2 | 5 | 4 | 9 |
| 3 | 0 | 0 | 0 | 2 | 4 | 10 |
| 4 | 0 | 0 | 0 | 2 | 6 | 9 |
| 5 | 0 | 0 | 0 | 0 | 2 | 4 |
| 6 | 0 | 0 | 0 | 0 | 2 | 4 |
| Means | 0 | 0 | 0.5 | 1.5 | 4.5 | 7.7 |
| Std. Dev. | 0 | 0 | 0.84 | 1.90 | 2.66 | 2.87 |

TABLE 3.
Total fuel oil concentrations (mg/l) measured by infrared spectrophotometry in bioassay with continuous dispersion.

| Volume of Fuel Oil (ml) | Hours from Introduction | Mean Concentration | n | Standard Deviation |
|---|---|---|---|---|
| 5 | 1 | 3.02 | 6 | 1.50 |
| 5 | 4 | 1.53 | 4 | 0.71 |
| 5 | 6 | 0.84 | 2 | 0.07 |
| 5 | 24 | 0.25 | 4 | 0.29 |
| 12.5 | 1 | 12.21 | 6 | 5.68 |
| 12.5 | 4 | 5.90 | 4 | 1.65 |
| 12.5 | 6 | 3.51 | 2 | 0.51 |
| 12.5 | 24 | 0.42 | 3 | 0.13 |
| 25 | 1 | 17.63 | 6 | 15.48 |
| 25 | 4 | 11.42 | 4 | 7.80 |
| 25 | 6 | 4.35 | 2 | 0.72 |
| 25 | 24 | 4.00 | 3 | 4.45 |
| 50 | 0.5 | 51.13 | 4 | 41.29 |
| 50 | 1.0 | 65.03 | 4 | 31.05 |
| 50 | 1.5 | 53.46 | 4 | 31.08 |
| 50 | 2.0 | 51.31 | 3 | 11.07 |
| 50 | 2.5 | 40.81 | 2 | 2.35 |
| 50 | 3.0 | 34.25 | 4 | 10.20 |
| 50 | 3.5 | 35.71 | 4 | 11.61 |
| 50 | 4.0 | 33.88 | 4 | 6.72 |
| 50 | 4.5 | 25.65 | 4 | 14.19 |
| 50 | 5.0 | 29.51 | 4 | 15.54 |
| 50 | 5.5 | 35.12 | 2 | 11.64 |
| 50 | 6.0 | 26.84 | 4 | 9.75 |
| 50 | 6.5 | 20.80 | 2 | 5.56 |
| 50 | 7.0 | 21.71 | 3 | 11.08 |
| 50 | 7.5 | 21.72 | 2 | 14.60 |
| 50 | 8.0 | 19.34 | 2 | 4.23 |
| 50 | 8.5 | 21.11 | 2 | 19.71 |
| 50 | 9.0 | 25.91 | 2 | 2.50 |
| 50 | 9.5 | 15.30 | 2 | 5.45 |
| 50 | 10.0 | 13.50 | 4 | 6.02 |
| 50 | 10.5 | 11.14 | 2 | 8.20 |
| 50 | 24.0 | 2.96 | 6 | 3.26 |
| 100 | 1 | 83.19 | 6 | 58.17 |
| 100 | 4 | 38.47 | 4 | 28.15 |
| 100 | 6 | 9.53 | 2 | 0.72 |
| 100 | 24 | 8.29 | 3 | 2.33 |

fuel oil concentrations were found in all experimental tanks (TABLE 3). Samples for soluble components were processed for two of the replicates (replicates 5 and 6, TABLE 2) and soluble oil was detected in all samples with quantifiable amounts in five of the samples (TABLE 4).

TABLE 4.
Concentrations (mg/l) measured by
gas chromatography for soluble compounds
in bioassays receiving continuous dispersion.

| Compounds | Hours from Introduction | Introduced Volume (ml) 50 ml Rep. 5 | 50 ml Rep. 6 | 100 ml Rep. 5 | 100 ml Rep. 6 |
|---|---|---|---|---|---|
| Benzene* | | | | | |
| Toluene | 1 | * | 0.017 | * | * |
| Ethyl benzene* | | | | | |
| m, p-xylene | 1 | * | 0.023 | 0.015 | 0.019 |
| | 2 | 0.015 | 0.019 | * | * |
| O-xylene | 1 | * | 0.029 | 0.015 | 0.018 |
| | 2 | 0.019 | * | * | * |
| 1,2,3 Trimethyl benzene* | | | | | |
| Tot. Sol. Aromatics | 1 | * | 0.069 | 0.030 | 0.037 |
| | 2 | 0.034 | 0.019 | * | * |

* Traces detected which were below the lower validated limit for the method. (0.3 mg/l for benzene; 0.01 mg/l for others)

## DISCUSSION

These studies point out the importance of standardizing the conditions under which oil and water are mixed prior to use in bioassay studies and the amount of dispersion that occurs during the exposure period. Under three sets of mixing conditions identical volumes of oil and water resulted in significant differences in observed mortality and measured amounts of oil in the water column, either as total or soluble oil. Minimized surface disturbance and mixing resulted in no measurable concentration of fuel oil in the water column. When oil and water were mixed initially and disturbance minimized, measurable amounts of fuel oil were found at all introduced volumes, but the correlation between the introduced oil volume and the average measurable oil concentration in the water column was poor ($r = 0.81$). In addition, soluble oil was not detectable in any of the selected samples. Where mixing occurred on introduction and surface disturbance continued throughout the test, water column concentrations of total oil were linearly related to volume introduction ($r = 0.99$). Mortality correlated well with introduced volume and mean measured total oil ($r = 0.99$ in each instance).

A second factor of importance is the depuration of fuel oil from the system. The close interval sampling of the 50-ml-introduced volume treatments demonstrates the rapidity of depuration. Even though the variability between experiments in measured total oil concentrations is high, a generalized logarithmic curve of depuration is apparent for the continuously mixed systems (FIGURE 1). It is interesting that the rate of depuration is comparable to that observed by ANDERSON et.al. (1974) for oil-in-seawater dispersions of South Louisiana crude oil in "static" systems receiving continuous aeration.

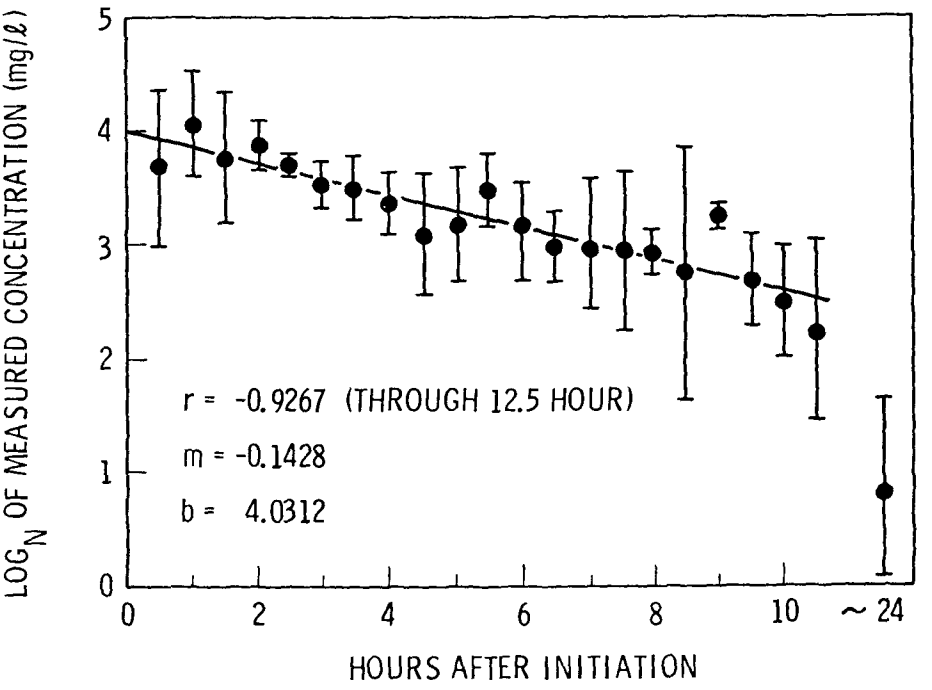


FIGURE 1.
Purge of aqueous phase fuel from system receiving continuous mixing.

The occurrence of such rapid depuration, in "static" systems or in the "batch" treatment of continuous flow systems, demands the development of complex expressions for effective treatment concentration. Although the development of such expressions should not be difficult (SPRAGUE 1970), it appears that given the dynamic nature of oil-seawater mixtures, a more attractive approach would be an alternative that allows for continuous delivery of contaminant and seawater.

The general level of toxicity of No. 2 fuel oil to shrimp concurs with findings by ANDERSON et.al. (1974) for marine crustaceans in Texas. ANDERSON et.al (1974) commented on the similarity between toxicities of crude and refined oils when expressed as measured concentration. They pointed out that it was much more difficult to achieve high aqueous phase concentrations for crude oil compared with fuel oil which, in part, explains a higher apparent toxicity of fuel oil. Our studies have clearly demonstrated that differing measured concentrations of fuel oil in the water column may be produced depending on the method of fuel oil-seawater contact. The dependence of water column concentration on the method of contact is so great that data presented in terms of oil-seawater ratios alone preclude meaningful discussion of toxicity. In view of the findings of ANDERSON et.al. (1974) concerning the relative difficulty of getting crude oil into aqueous phase, the conclusion may be judiciously extended to crude oil

as well.

In earlier studies (BEAN et.al. 1974) the best correlation between shrimp mortality and crude oil was to the soluble aromatic fraction. The data from these studies do not discount that finding; however, mortality was produced in these studies at soluble aromatic concentrations an order of magnitude below concentrations in which similar shrimp survived in the earlier studies. Thus, the current findings emphasize the need to account for a wider range of compounds in the bioassay of petroleum.

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