The Effect of a No. 2 Fuel Oil and a South Louisiana Crude Oil on the Behavior of the Soft Shell Clam, *Mya arenaria* L.

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Introduction

The toxic effects other than lethality of oils has often been treated as a secondary problem in bioassays. According to STIRLING (1975), there is a need to enlarge the concept of the routine bioassay test to include quantitative measurements of the effects of pollutants on behavior, physiology and metabolism. The inherent interspecific and intraspecific physiological differences of test species and various test conditions make direct comparison of bioassay results difficult. VAUGHAN (1973) and BEAN, et al. (1974) discussed these problems in detail.

Few studies have characterized the behavioral effects of oils on bivalves or examined the effects of temperature simultaneously. WILSON (1974) noted that bivalve molluscs have been avoided for toxicity tests because it is difficult to establish a simple criteria of effect. This study was therefore performed to determine the behavioral effects of a No. 2 fuel oil and a South Louisiana crude oil in bioassay tests conducted at winter temperatures. The winter temperatures were chosen because spills are more likely to occur during the inclement winter weather.

The oils were added in an emulsified form to simulate a potential naturally occurring condition. FORRESTER (1971), FOSTER, et al. (1971), GORDON, et al. (1973) and KANTER (1974) have recorded the formation of oil emulsions in sea water by various mechanisms. In the event of an oil spill during the colder months, it is probable that much of the oil would be dispersed and emulsified in the water column through turbulent wave action. Many of these emulsions tend to be relatively stable and a mechansim for the accumulation of emulsified oil by bivalves has been reported (STAINKEN, 1975).

Materials and Method

Behavioral observations were recorded during bioassay tests The tactile responses of the clams were examined by lightly tapping the shell with a glass stirring rod. Tests were conducted according to the "Standard Dispersant Effectiveness and Toxicity Tests" published by the U.S. Environmental Protection Agency (McCARTHY, 1973). An experimental concentration at which 50% of the experimental animals survived (LC_{50}) was determined during a 96 hr. exposure period. The method employed requires the use of a standard toxicant. Benzene was used initially but in subsequent tests phenol was utilized because benzene emulsions were not stable.

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Oil-in-water emulsions were ultrasonically prepared according to a procedure developed by GRUENFELD and BEHM (1973). Twenty thousand parts per million of Southern Louisiana Crude oil and No. 2 fuel oil were prepared and dilutions were made to achieve desired concentrations. The No. 2 fuel oil and Southern Louisiana were supplied by the U.S. Environmental Protection Agency, Industrial Waste Treatment Research Laboratory, Edison, N.J. The fuel oil was composed of 14% aromatics and 86% non-aromatics. Benzene was also sonified to yield a stock emulsion of 10,000 ppm. A 10,000 ppm stock solution of phenol was prepared.

All toxicants except benzene were used at $4^{o}C$ and $14^{o}C$. Benzene was tested at $14^{o}C$. The oils were added at concentrations from 50-800 ppm. The test concentrations of the oils were later increased to 1,600 ppm.

Clams for the experiments were collected from the Princes Bay - Sequine Point Area of Staten Island, N.Y. from December, 1973 - February, 1974. Clams were collected at water temperatures and salinity closely approximating test conditions. Young clams with a mean shell length of 25 mm or less were utilized. Prior to each experiment, clams were acclimated 24-48 hours in 1600 ml of artificial sea water (salinity - 26‰). Fifteen clams (15-24 mm) were used at each oil concentration. Five clams were placed in each aerated container. Five clams per container were also used for standard toxicant testing (benzene or phenol) at each concentration.

Results

Behavioural observations were made in each LC_{50} test. A noticeable reaction was not observed in clams exposed to benzene. The clams merely contracted upon tactile stimulation.

Clams exhibited an identical response pattern to oil exposure in all tests. Initially, at low oil concentrations (50 ppm) mucus was given off by the clam out the pedal opening and siphon. Higher oil concentrations resulted in proportionally higher mucus secretion. As more mucus was secreted, the clams increasingly shunted more out the pedal opening. All effects appeared to be both time and dose related, although crude oil effects were never as severe as the effect of fuel oil. Both oils depressed muscular contraction. A decrease in irritability and contractibility of the siphon was noticeable at 50 ppm. At concentrations greater than 100 ppm, the pedal opening musculature rapidly became totally relaxed and did not contract . The adductor muscles simultaneously lost the ability to contract rapidly. At concentrations greater than 400 ppm, the adductors began to relax in less than 15-20 sec and the animal consequently 'gaped'. It appeared that the anterior adductor was affected first, then the posterior.

The general relaxation of the musculature (adductors, siphon, pedal aperture) occurred at all oil concentrations. The intensity varied with time and dose.

The large amounts of mucus secreted at the 400 ppm concentrations appeared to be clogging the cavity between the valves by the end of the tests. The clams also appeared too weak to expel the mucus.

The response pattern of mucus secretion and muscular narcotization occurred in all tests with oil and seemed to be enhanced at 14°C compared to responses at 4°C.

The response of clams to phenol was not completely similar to that of oil. Mucus secretions were never as heavy in response to phenol, but phenol appeared to narcotize the musculature quicker. Phenol exposed clams reacted differently than oil exposed clams. The muscles remained at one length, turgid and lost their irritability. Adductor muscles remained half or sometimes fully contracted and the pedal aperture remained partially open. The muscles of the phenol exposed group rapidly became turgid at death. The tactile response of control clams remained normal during the experiments and mucus secretion was not evident.

The static bioassay data derived from the tests were inconclusive. The LC_{50} values were computed on semilog paper. Results of the tests are in Table I. Tests numbers 1A and 1B were run in natural filtered sea water to determine whether testing in natural sea water or artificial sea water would have an effect. An effect was not found and all other tests were conducted in artificial sea water. Scattered mortality was observed in the majority of tests but in most cases there was insufficient mortality after 96 hours to calculate a LC_{50}. Mortality was not found in the controls. Death was defined as a total lack of muscle response.

At 14°C, two 96 hour LC_{50} values for No. 2 fuel were obtained. These were 475 ppm (Test IIA) and 535 ppm (Test IIIA). Comparison by a t test revealed no significant difference and a mean LC_{50} value of 505 ppm was calculated. Some of the tests were continued 3 days beyond the 96 hour period, to see if there was a time effect. At 14°C, test IIIA and IIIP were continued. An LC_{50} (7 day) was found for No. 2 fuel oil to be less than 100 ppm, compared to test IIIA LC_{50} (96 hour) of 535 ppm. The LC_{50} (7 day) of phenol in test IIIB dropped to 535 ppm.

At 4°C, test IVA, IVP, VIA and VIP were also continued for 3 days (total 7 days exposure). In test IVA, a LC_{50} could not be found. In test IVP, the 7 day semilog plot had the shape of a backwards 'S' and two values were derived, 80 and 225 ppm phenol. In test VIP, the LC_{50} (7 day) for phenol was 450 ppm. It is probable that more mortality would have been encountered if the tests were continued beyond seven days.

TABLE I. Calculated toxicity (LC_{50}) during a 96 hour exposure period.

$14^{o}C$

(IA) So. Louisiana Crude	(IB) Benzene
conc: 50,100,200,400,800 ppm	10,20,30,40,50 ppm
LC_{50} = none	LC_{50} = none
(IIA) #2 Fuel Oil	(IIB) Benzene
conc: 50,100,200,400,800 ppm	50,60,70,80,90,100 ppm
LC_{50} = 475 ppm	LC_{50} = none
(IIIA) #2 Fuel Oil	(IIIP) Phenol
conc: 100,200,400,800,1600 ppm	50,100,200,400,800 ppm
LC_{50} = 535 ppm	LC_{50} = 565 ppm

$4^{o}C$

(IVA) So. Louisiana Crude	(IVP) Phenol
conc: 100,200,400,800,1600 ppm	50,100,200,400,800 ppm
LC_{50} = none	LC_{50} = 365 ppm
(VA) #2 Fuel Oil	(VP) Phenol
conc: 50,100,200,400,800 ppm	10,20,30,40,50 ppm
LC_{50} = none	LC_{50} = none
(VIA) #2 Fuel Oil	(VIP) Phenol
conc: 100,200,400,800,1600 ppm	50,100,200,400,800 ppm
LC_{50} = none	LC_{50} = 535 ppm

The Southern Louisiana Crude oil appeared to have a few acute toxic effects within the test parameters.

In the tests with No. 2 fuel oil, there seemed to be a temperature effect. At $4^{o}C$, a LC_{50} was not found in any tests (except VIA, 7 days), while at $14^{o}C$ a mean LC_{50} of 505 ppm was found in the two tests with No. 2 fuel oil. A temperature effect was not apparent in clams exposed to phenol.

Discussion

The behaviour effects found for M. arenaria were repeatable for both crude and refined oil. The increasingly greater concentrations of oil elicited greater mucus secretion and decreased tactile response. SWEDMARK, et al. (1973) reported the effect of crude oil and oil emulsions on bivalves and found a similar decrease in tactile response. The general behavior sequence they reported was: increased activity; successively imppaired activity; immobilization and death. The observations of this report are similar to their observations.

The copious production of mucus by M. arenaria had several effects. It imposed a steady drain on the energy reserves of the clams. The continual production of mucus clogged the gills and mantle cavity and would disrupt normal feeding mechanisms. The clogging mucus must be expelled by the clam by increased contraction of the adductors and mantle musculature which puts an additional strain on the metabolic rate. The increased metabolic demands for mucus production and excretion and the disruption of normal physiological and biochemical processes occurred at much lower concentrations of oil exposure than the LC_{50} indicates.

A problem encountered in comparing the results of this study was the lack of definition of death in bivalves in published reports. Terms such as "moribund" are found in the literature but the criteria of death was not defined. The definition of death in this report was the total lack of muscular response, though this was often difficult to determine. Stimulation of the musculature by pinching or light rapping with a stirring rod often elicited muscular twitches though the clam appeared "dead".

The bioassay LC_{50} values obtained for M. arenaria are greater in concentration (ppm) than those reported for other species exposed to No. 2 fuel oil (VAUGHAN, 1973; ANDERSON, et al., 1974). However, the values are lesser than those reported for many species exposed to crude oils. According to the ranking system of SPRAGUE and CARSON (1970), the No. 2 fuel tested in this report can be classified as moderately toxic to M. arenaria. An additional factor noted in the bioassay test was the time of exposure. Increasing the period of exposure from 96 hours to seven days decreased the LC_{50} values obtained. Similar increases in mortality of molluscs during longer exposure periods were reported by SPRAGUE and CARSON (1970), KANTER et al., (1971) and KASYMOV and ALIEV (1973).

Future bioassay work with molluscs, particularly bivalves, should be determined over a 7 day exposure period. However, behavioral observations appear to have advantages as a toxic criterion over lethality, and shorter exposure periods can be employed.

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