Bull. Environm. Contam. Toxicol. 24, 656-661 (1980)

# Acute Toxicity of a #6 Fuel Oil to Marine Organisms

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The oil tanker Argo Merchant broke up in shoal waters off Nantucket, Massachusetts, in December 1976 and spilled a #6 fuel oil containing a #2 cutter stock. The U.S. Coast Guard attempted to burn the oil slick at sea, but heavy seas contributed to an unsuccessful operation. Shortly after the spill, we performed acute toxicity tests with five test materials and three saltwater organisms--an alga, a copepod, and a fish. The test materials included a #6 fuel oil, Tullanox® 500 - a wicking agent, and lighter fluid. The materials were tested singularly and in combination. The three materials were also combined according to instructions from the U.S. Coast Guard, ignited, and the resulting residue tested.

## MATERIALS AND METHODS

### Test materials

1. The sample of #6 fuel oil was received at EG&G, Bionomics Marine Research Laboratory (BMRL) on 12 January 1977. The sample was received in a five-gallon metal container.

2. The sample of Tullanox 500, a wicking agent, was received at BMRL on 17 January 1977. The test material was an extremely fine white powder and was contained in an unlabeled plastic bag.

3. The lighter fluid tested was purchased from a local convenience store. The container was labeled "Gulf Lite® charcoal starter". The manufacturer was the Gulf Oil Corporation, Houston, Texas.

4. A combination of #6 fuel oil plus Tullanox 500 was prepared according to instructions in a Mailgram® from the U.S. Coast Guard dated 14 January 1977. Specifically, oil and Tullanox were combined in a 1,000:1 ratio, weight:weight. The resultant mixture, a black liquid, was tested.

0007-4861/80/0024-0656 $01.20
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5. A residue was prepared according to the instructions mentioned above. Oil and Tullanox 500 were combined in a 1,000:1 ratio, weight:weight in a cut-off 55-gallon steel drum bottom for the fish test and in a glass Petri dish for the alga and copepod tests. Then, 1 mℓ of lighter fluid per 20 $cm^2$ of oil surface was added. The dish and steel drum and their contents were heated to 30 and 40°C, respectively, before igniting the oil/Tullanox 500/lighter fluid mixture with a Bunsen burner. Both mixtures burned until they extinguished. The resultant residue was tested. The residue was a tar-like material from the Petri dish burning and a more carbon-like material from the steel drum burning.

No solvents were used with any test material. Concentrations of each test material are reported here as mg of test material per ℓ of seawater or ppm.

Test organisms

The alga tested was the chain-forming diatom Skeletonema costatum. The culture was obtained from the U.S. Environmental Protection Agency's Environmental Research Laboratory, Narragansett, Rhode Island, and maintained in stock culture at BMRL.

The marine copepod tested was Acartia tonsa. The copepods, collected by plankton net from Big Lagoon, a Gulf of Mexico estuary adjacent to BMRL, were acclimated at 20±1 °/₀₀ salinity and 20±1°C for 48 hours prior to testing. Mortality was estimated to be <5% during the acclimation period and the animals appeared to be in excellent condition at initiation of the tests.

Atlantic silversides, Menidia menidia, were collected from Little Talbot Inlet near Jacksonville, Florida, and transported to BMRL. Fish were acclimated for a minimum of four days prior to testing. Mortality was <10% during the acclimation period. The fish appeared to be in good condition at initiation of the tests. Standard length of the fish was 5-7 cm. Test animals were not fed during the tests.

Test conditions

All test methods followed those described by U.S. Environmental Protection Agency (1975), except as noted.

The alga, S. costatum, was tested in flasks with beginning cell numbers of 2.0 x $10^4$ cells/mℓ. The cultures were incubated at 20±1°C under 2,000 lux illumination. Salinity of the medium was 30 °/oo. Test concentrations and controls were triplicated. All counts were made with a hemacytometer and Zeiss Standard 14 compound microscope.

All water used for holding and maintenance of A. tonsa and for test solution preparation was filtered (1.2-µm), natural seawater at a salinity of 20 °/oo. Test water temperature was maintained at 20±1°C. The test was conducted in 50 x 100-mm glass crystallizing dishes, each of which contained a total volume of 100 mℓ of test solution and 10 test animals. Test concentrations and controls were triplicated.

All M. menidia tests were conducted in 19-ℓ uncovered glass jars which contained 15 ℓ of filtered (5-µm), natural seawater. Salinity was 29±1 °/oo and temperature was 20±1°C for all tests. Each jar contained 3 test animals. All concentrations and controls were quadruplicated, resulting in 12 fish per treatment, except in the residue test where only 6 fish were tested per treatment because of a limited amount of test material.

Separate tests were conducted with all three test organisms in which they were exposed to the reference toxicant dodecyl sodium sulfate (DSS) under the same conditions stated above for each organism. The DSS was Fisher Scientific Company Laboratory Grade, lot number 742584.

## Statistical analyses

Each concentration was converted to a logarithm and the corresponding percentage mortality to a probit (FINNEY, 1971). The 96-hour LC50's and 95% confidence limits were then calculated by linear regression. No correction was made for control mortality.

## RESULTS AND DISCUSSION

In all cases except for the residue, A. tonsa was the most sensitive test organism. S. costatum and M. menidia were similar in sensitivity to #6 fuel oil, Tullanox, oil + Tullanox, and oil + Tullanox + lighter fluid (Table 1).

TABLE 1

Calculated 96-hour EC50's and LC50's for *Skeletonema costatum*, *Acartia tonsa*, and *Menidia menidia* exposed to five materials plus a reference toxicant (dodecyl sodium sulfate) in static seawater.

| Test material | Test organism | EC50/LC50 (mg/ℓ) | 95% confidence limits (mg/ℓ) |
|---|---|---|---|
| #6 fuel oil | *S. costatum* | 160 | 80-320 |
| | *A. tonsa* | 5.1 | 3.4-11 |
| | *M. menidia* | 130 | 50-330 |
| Tullanox 500 | *S. costatum* | 2,700 | 1,100-7,100 |
| | *A. tonsa* | 240 | 100-580 |
| | *M. menidia* | 2,300 | 760-6,800 |
| Oil & Tullanox | *S. costatum* | 180 | 60-560 |
| | *A. tonsa* | 7.4 | 3.4-14 |
| | *M. menidia* | 570 | 390-840 |
| Oil & Tullanox & Lighter Fluid | *S. costatum* | 180 | 50-660 |
| | *A. tonsa* | 4.4 | 1.3-8.8 |
| | *M. menidia* | 360 | 120-1,000 |
| Residue | *S. costatum* | 9,600 | <1,000->10,000 |
| | *A. tonsa* | 10,000 | 3,000-40,000 |
| | *M. menidia* | 4,000 | 260-62,000 |
| DSS | *S. costatum* | 2.4 | 1.7-3.6 |
| | *A. tonsa* | 0.12 | 0.04-0.34 |
| | *M. menidia* | 1.2 | 0.9-1.4 |

The order of test material toxicity was essentially the same for all test organisms--#6 fuel oil > oil + Tullanox + lighter fluid > oil + Tullanox > residue. The lone exception was A. tonsa which exhibited slightly greater sensitivity to the oil + Tullanox + lighter fluid than to the oil alone.

After combustion of the three materials, toxicity of the residue to M. menidia was approximately 30 times less than the oil alone; S. costatum, 60 times less toxic; and A. tonsa over 2,000 times less toxic. M. menidia seemed to be the most sensitive species to the residue, but erratic mortality and fewer test animals per test concentration could have produced a biased result. The cause of the erratic mortality in the residue test is unknown. It is hypothesized, however, that incomplete combustion of the materials could have resulted in concentrations of toxic materials disproportionate to the amount of residue. The much higher EC50's and LC50's for S. costatum and A. tonsa may have occurred because of better combustion of the smaller amounts mixed and ignited for those tests. The M. menidia test could not be repeated because all test materials (fuel oil and Tullanox) had been expended.

The laboratory results indicate that if a fuel oil spill occurs at sea, as was the case with the Argo Merchant, the greatest impact would probably occur on the zooplankton in the immediate vacinity of the spill. The laboratory results were confirmed by visual observations at the Argo Merchant spill site where free-floating plankton appeared to have been affected by the oil, both dissolved and emulsified in the water (KERR 1977).

Since the ignition components alone or mixtures of them plus the oil were no more toxic than was the oil alone, and because the residue resulting from the burning of this mixture was less toxic to the test organisms by 30 to more than 2,000 times, the burning of oil at sea would seem to be a reasonable alternative to the use of dispersants or sinking agents. The use of this method for cleaning up oil spills at sea, however, would be dependent on weather and sea conditions.

The calculated 96-hour EC50's and LC50's for the reference toxicant for all three test organisms were within limits established for each organism by numerous reference toxicant tests performed at BMRL over several years (Table 1). This confirmed that the organisms were in satisfactory condition for testing.

## REFERENCES

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