Bull. Environm. Contam. Toxicol. 20, 320-327 (1978)

# Toxicity and Sublethal Effects of No. 2 Fuel Oil on the Supralittoral Isopod *Lygia exotica*

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## INTRODUCTION

The ever increasing domestic requirements for petroleum products have resulted in a dramatic increase in the quantities of crude and refined petroleum shipped by tankers over coastal shipping lanes. As a result, concern has grown in recent years about the impact on marine organisms and ecosystems of petroleum hydrocarbons accidentally introduced into the marine environment. Accidental oil spills have occurred periodically in Galveston Bay, Texas over the past several years. Such a spill occurred in the Houston Ship Channel on 9 March, 1973 and resulted in the release of nearly 400,000 gallons of oil into upper west Galveston Bay from a barge carrying bunker C residual oil and No. 2 fuel oil. Visual observations 3 days after the spill along the shoreline of the Houston Yacht Basin, where most of the oil came ashore, did not reveal any obvious biological damage to the estuarine macrofauna except for a large mortality among the local population of the supralittoral isopod, Lygia exotica. Three days after the spill the few surviving individuals were still apparently affected by the oil in that they could easily be captured by hand, a situation not normally encountered.

Because of the ecological importance of this organism in the intertidal ecosystem (WHITTEN et al. 1950), it was of interest to determine the toxicity and sublethal effects of oil to Lygia from a nearby uncontaminated population. No. 2 fuel oil was selected as the test oil because one of the oils spilled was a No. 2 fuel oil and because it has been found to be generally more toxic to estuarine animals than some other oils similarly tested (ANDERSON et al. 1974a).

## MATERIALS AND METHODS

Three days after the spill, several specimens of living Lygia exotica were collected from the impact

0007-4861/78/0020-0320 $01.60

site at the Houston Yacht Basin. The specimens chosen were not obviously contaminated with a surface coating of oil. They were frozen immediately and stored frozen in glass vials with teflon-lined caps until analyzed by gas chromatographic technizues for petroleum hydrocarbons by a technique described previously (WARNER 1976). In these analyses, special attempts were made to identify and quantitate the sulfur-containing aromatics present (WARNER 1975).

The Lygia used in the laboratory studies were obtained from an uncontaminated population at 8 Mile Road, west of Galveston, Texas. They were maintained in the laboratory in a "semiterrestrial" tank which allowed a water-land excursion choice for the animals. The tank contained artificial seawater (Instant Ocean, Aquarium Systems, Inc.) at 30 o/oo salinity (S). Tetramin® (Tetra-Werke, W. Germany) was provided as food twice weekly and the seawater was changed every two weeks. Forty-eight hours prior to experimentation, selected individuals of uniform size were transferred to a tank with new seawater and no food. Seawater used in the maintenance of isopods, experimentation and in preparation of oil-seawater solutions was 30 o/ooS Instant Ocean. Lygia were maintained in the laboratory no longer than 17 days before experiments.

Water-soluble fractions (WSF) and oil-in-water dispersions (OWD) of No. 2 fuel oil (API reference oil No. III) were prepared fresh according to methods of ANDERSON et al. (1974a). The WSF was prepared by gently stirring 1 part oil with 9 parts seawater for 20 hours. The phases were allowed to separate for ½-1 hour before the aqueous phase (100% WSF) was drawn off. Subsequent dilution of the 100% WSF was with 30 o/ooS seawater. The OWD was prepared by vigorously shaking seawater-oil mixtures for 15 minutes on a shaker platform at approximately 100 cycles/minute. It should be recognized that, although the WSFs and OWDs prepared in this way contain hydrocarbon concentrations much higher than any that might be expected in the water column after a spill, they are representative of the hydrocarbon types present in solution or dispersion in seawater after a spill. All test mixtures were allowed to stand 30-45 minutes before the introduction of organisms. Because direct sampling of exposure mixtures was not always feasible, larger volume flasks containing the appropriate oil mixtures with equivalent surface: volume ratios were utilized for the hydrocarbon analysis of water. Total naphthalenes (naphthalene, methylnaphthalenes and dimethylnaphthalenes) in 200 ml seawater samples were measured by the ultraviolet spectrophotometric method of NEFF and

ANDERSON (1975) using a Pye-Unicam SP 1800 Ultraviolet recording spectrophotometer. Total hydrocarbons (carbon tetrachloride-extractable oily material) were determined by the infrared spectrometric method using a Wilks Miran I Infrared Analyzer (AMERICAN PETROLEUM INSTITUTE 1958).

Bioassays were carried out over a wide range of exposure concentrations: OWD - 1, 5, 10, 50, 100, 500, 1000 ppm oil added, and WSF - 5%, 10%, 25%, 50%, 75%, and 100% of the original water-soluble stock solution. Ten animals at each concentration were individually exposed in 125 ml Erlenmeyer flasks containing 7 mls of the desired exposure solution. The flasks were loosely covered with plastic wrap. The volume of the exposure mixtures, which did not change over 96 hours, was sufficient to allow continuous contact while not inducing forced swimming. Median tolerance limits (TLm) at 24, 48 and 96 hours were determined according to A.P.H.A. (1971) and based on nominal concentrations.

Respiration measurements were made using a Gilson Differential Respirometer. Single side-arm, 20 ml respiratory flasks without center wells were used with 0.5 ml 10% KOH in each side arm and 1 ml of the appropriate test solution in the flask. Respiration of 20-21 individual isopods was measured for 3-4 hours in clean seawater to observe baseline oxygen consumption and to allow for experimental acclimation. Seawater media and KOH were then removed by aspiration without handling the animals. Appropriate exposure mixtures were added and within 30 minutes respiration measurements of control and exposed isopods were initiated and continued for 6-7 hours. The flasks were not shaken and the water bath temperature was maintained at 20°C. Oxygen consumption was calculated according to ARDITTI and DUNN (1969). Dry weights were determined after drying the animals to a constant weight (48 hours) at 100°C.

Differences between respiration rates of control and exposed animals were tested for significance at the 95% confidence level with Student's-test (STEELE and TORRIE 1960).

## RESULTS

Bioassay results, Table 1, indicate that Lygia is very resistant to No. 2 fuel oil prepared as either a WSF or OWD. TLm values are based on the amount of oil added (OWD) or % of stock solution (WSF), with actual exposure concentrations shown in Table 2.

TABLE 1

Acute toxicity to Lygia exotica of No. 2 fuel oil as either a water-soluble fraction (WSF) or an oil in water dispersion (OWD). TLm = concentration producing 50% mortality at the time given.

| | Median Tolerance Limits (TLm) | | |
|---|---|---|---|
| | 24 hr. | 48 hr. | 96 hr. |
| WSF | >100% | >100% | >100% |
| OWD | 73.0 ppm | 73.0 ppm | 36.5 ppm |

Mortality never reached 50% in the WSF bioassays although total hydrocarbon (TH) concentrations in the 100% WSF were consistently high (mean > 4.0 ppm, Table 2). The estimated concentration of total naphthalenes

TABLE 2

Measured exposure concentrations of #2 fuel oil in bioassay and respiration experiments. WSF and OWD concentrations are expressed as total hydrocarbons (TH) and/or total naphthalenes (TN).

Bioassay Exposure Concentration

| Preparation | Nominal Concentration | Concentration Found (ppm) 0 hr. | 24 hr. | 48 hr. | 96 hr. |
|---|---|---|---|---|---|
| WSF (TH) | 100% | 5.74 | 4.51 | 4.10 | 3.97 |
| (TN)* | | 2.50 | 0.41 | 0.35 | 0.13 |
| OWD (TN) | 1 ppm | 0.82 | | | |
| | 5 ppm | 0.38 | 0.82 | | |
| | 10 ppm | 0.99 | 0.32 | 0.07 | |
| | 50 ppm | 4.88 | 0.96 | 0.63 | 1.52 |
| | 100 ppm | 5.97 | 5.32 | 0.66 | 1.93 |
| | 500 ppm | 37.80 | | | |
| | 1000 ppm | 88.31 | | | |

Respiration Exposure Concentrations

| Preparation | Calculated Conc. | Found (ppm) Initial | Final |
|---|---|---|---|
| WSF (TH) | 100% | 4.69 | 4.43 |
| OWD (TN) | 30 ppm | 0.97 | 0.67 |
| | 60 ppm | 1.22 | 0.93 |

*From data of ROSSI et al. (1976).

in the 100% WSF dropped rapidly from 2.5 to 0.13 ppm in 96 hours. The concentration of total naphthalenes (TN) in No. 2 fuel oil, as OWD, causing 50% mortality in 96 hours was approximately 1.0 ppm TN. According to ANDERSON et al. (1974a), this concentration of OWD contains about 20 ppm TH, much more than in the 100% WSF. The observed increase in OWD concentrations at 96 hour could be due to microbial action.

Respiration rate of Lygia exposed to concentrations of WSF and OWD approximating TLm values are shown in Fig. 1. No statistically significant change in respiration rate due to oil exposure could be detected, although mean respiration rates of control animals were always lower than those of exposed animals. Analysis of initial and final (6-7 hours) water samples from respiration experiments are given in Table 2.

The Lygia samples from the spill site were heavily contaminated with petroleum hydrocarbons. A pooled sample of several animals (approximately 10g wet weight) contained approximately 1600 ppm total petroleum hydrocarbons. Especially noteworthy however, were the relatively high concentrations of sulfur-containing aromatics (mainly dibenzothiophenes) found in the tissues. The concentrations of the various dibenzothiophenes determined by GC coupled to a sulfur-specific flame photometric detector were 10 ppm dibenzothiophene, 50 ppm methyldibenzothiophenes, 60 ppm $C_2$-dibenzothiophenes, 25 ppm $C_3$-dibenzothiophenes, and 15 ppm $C_4$-dibenzothiophenes. Since the Lygia sample was not washed with an organic solvent to remove surface contamination, it is possible that a significant portion of the hydrocarbons detected were adsorbed to the surface of the carapace and not actually incorporated into the tissues of the animals.

## DISCUSSION

TLm values from bioassays indicate that under the experimental conditions used here, No. 2 fuel oil prepared as either WSF or OWD is not very toxic to Lygia. ANDERSON et al. (1974a) reported median lethal concentrations for three crustaceans and three fish tested under similar conditions. All three of the crustaceans and two of the fish were substantially more sensitive than Lygia to No. 2 fuel oil. The third fish, Fundulus similis, had TLm values similar to those of Lygia. Such high resistance to No. 2 fuel oil does not agree with the field observations noted earlier. The high field mortality may have been aggravated by other factors such as more complete and vigorous coating of oil in the active intertidal area or by

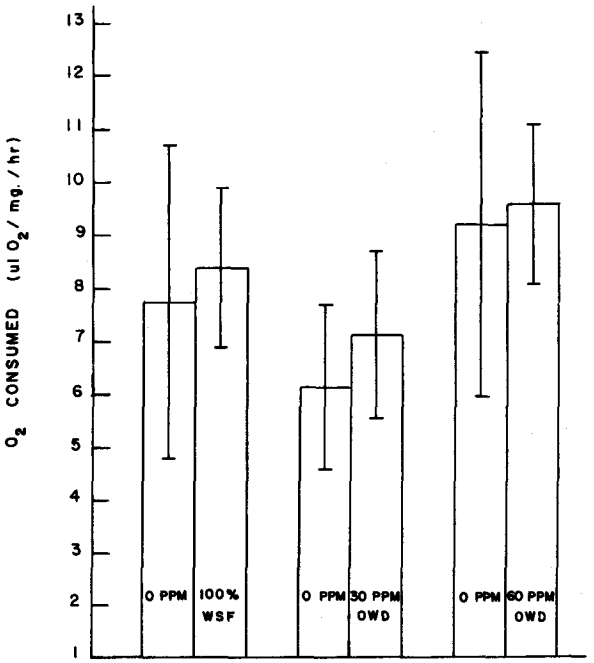


Figure 1. Respiratory rates of Lygia exotica during exposure to No. 2 fuel oil. Respiratory rates of each group of amphipods was determined immediately before exposure (0 ppm) and then during exposure to 100% WSF, 30 or 60 ppm OWD. Vertical lines are standard deviations.

recent stressful temperature-salinity conditions. In addition, the other oil involved in the spill, Bunker C residual oil, may have contributed to the high field mortality.

This latter possibility tends to be supported by the GC data (WARNER 1975). The gas chromatogram from fraction 3 of Bunker C residual oil (API reference oil #IV) closely resembles qualitatively the gas chromatogram of fraction 3 of the Lygia sample. The main difference between the two is that the Lygia sample is greatly enriched in dibenzothiophenes, suggesting selective accumulation of these compounds. Practically nothing is known about the uptake kinetics and acute toxicity of dibenzothiophenes in marine animals. In light of the results reported here and by WARNER (1975) these topics deserve further attention.

Respiration has been used in prior investigations to examine the sublethal effects of oil on marine organisms (ANDERSON et al. 1974b; NEFF et al. 1976). COX (1974) found increased respiration rates, similar to those reported here for a number of shrimp-like crustaceans after short term exposure to No. 2 fuel oil as either WSF or OWD. However, TATEM (1975) reported depressed rates with longer exposure (21 hours) to the WSF of No. 2 fuel oil by the grass shrimp Palaemontes . In shorter exposures, he observed respiration rates to either increase or decline. Many factors affect the oxidative metabolism of invertebrates (NEWELL, 1970), often making the interpretation of respiration data difficult. Unless supplemented with specific information such as biochemical respiratory adjustments, oxygen consumption data should be used only in general comparisons.

## SUMMARY

1. No. 2 fuel oil was of relatively low toxicity to the intertidal isopod Lygia exotica as indicated by the TLm values of over 100% for the WSF and 73 ppm at 24 and 48 hours and 36.5 ppm at 96 hours for the OWD.

2. Respiration was not significantly affected by short term exposure to several concentrations of No.2 fuel oil prepared as either a WSF or OWD.

3. Lygia contaminated by a spill of No. 2 fuel oil and Bunker C residual oil contained high concentrations of dibenzothiophenes. It is not known whether the dibenzothiophenes were accumulated by the Lygia tissues or adsorbed to the exoskeleton. Therefore, the high mortality of Lygia following the spill cannot yet be attributed to the dibenzothiophenes.

## ACKNOWLEDGEMENTS

This research was supported by grant #206-76(170) from the American Petroleum Institute to J.M. Neff.

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