Individual and Combined Toxicity of Some Petroleum Aromatics to the Marine Amphipod *Elasmopus pectenicrus*

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Abstract

Toxicity of 4 components of petroleum oils to the marine amphipod *Elasmopus pectenicrus* (Bate) has been assessed. Two ephemeral aromatic hydrocarbons, naphthalene (A) and 1, 2, 4-trimethylbenzene (B) were more toxic than two persistent aromatics, o-cresol (C) and o-toluidine (D). The acute toxicity concentrations obtained for individual aromatic compounds were always greater than the actual concentrations found in the water-soluble fractions (WSF) of fuel oils. Results from mixtures of 2 or more components indicated that the LC50 levels were primarily determined by the more toxic substances, A and B. Naphthalene and 1, 2, 4-trimethylbenzene became more toxic to the *E. pectenicrus* when present in a mixture of more than 2 components, and the toxicity increased with increasing numbers of components present. Synergistic effects, therefore, possibly occur in the whole WSF. No antagonistic effects were observed among the 4 petroleum aromatics.

Introduction

As evidenced by relative rates of evaporation, the major chemical components of the water-soluble fractions (i.e., benzene-extracted organics) of fuel oils can be classified into the two categories of volatile and non-volatile. The former are represented by naphthalene, 2-methylnaphthalene and 1, 2, 4-trimethylbenzene, and the latter by o-cresol, o-toluidine and some dimethylphenols (Anderson et al., 1974; Winters et al., 1976). Although the boiling points of the first group are slightly higher than those of the second group, the non-hydrocarbons (o-cresol and o-toluidine) were lost from an exposed solution at a much slower rate. This slow rate of evaporation is probably caused by the formation of hydrogen bonds between these compounds and water molecules (Lee et al., 1978). Toxicity tests in the laboratory showed that the volatile or ephemeral components were much more toxic than the non-volatile or persistent ones (Moore and Dwyer, 1974; Donahue et al., 1977; Lee et al., 1978). Anderson et al. (1974) and Rossi et al. (1976) suggested that their results, in which fuel oil proved to be more toxic than crude oil, could be explained by the higher concentrations of di- and tri-aromatic hydrocarbons in the fuel oil. The toxicity of naphthalenes (including substituted naphthalenes) and benzenes (including substituted benzenes) to various marine organisms is well established (Anderson et al., 1974; Dunstan et al., 1975; Morrow et al., 1975; Calder and Lader, 1976; Korn et al., 1976; Donahue et al., 1977; Struhsaker, 1977). In contrast, studies of the possible biological effects of the non-volatile aromatic hydrocarbons are fewer, possibly they have attracted less attention because they are less toxic. However, using an algal lawn technique, Winters et al. (1976) found that some non-volatile aromatics (e.g. p-toluidine) were highly toxic to the microalga *Aegagropila quadruplicatum*. Emery (1970) also reported that cresol at a concentration of 8.6 ppm was toxic to immature stages of *Gammarnus fasciatus*. Toxicity testing of more than two combinations of petroleum hydrocarbons has been almost completely neglected. The only paper which dealt with the effect of mixtures of individual aromatic compounds was by Donahue et al. (1977).
We purposely selected 4 aromatics dissolved in seawater as the pollutants; two are very volatile (naphthalene and 1,2, 4-trimethylbenzene), and the other two are less volatile (o-cresol and o-toluidine). The latter two components were chosen because they evaporate slowly from seawater and thus may have long-term effects on marine biota. By employing a marine amphipod as a test animal, we wished to examine (1) the relative toxicity of the 4 selected petroleum components and (2) the combined toxicity (antagonistic and synergistic) of all possible combinations of the 4 compounds. We also wished to examine the hypothesis that the toxicity of fuel oil can be ascribed to naphthalene alone, as suggested by some earlier laboratory results.

Materials and Methods

Test Amphipods

The amphipods Elasmopus pectenicrus (Bate) used in this study were collected from the south jetty of Port Aransas, Texas, USA. In the laboratory E. pectenicrus were removed from the red seaweed Gelidium sp. and placed in a culture bowl (20 cm in diameter) containing 1.5 l of filtered seawater (30% S). Adult amphipods were selected and acclimated at 23°C for 7 days before experiments began.

Test Aromatic Solutions

In preparing the test solutions, two factors were considered, namely, solubility of the aromatics, and their concentrations in the WSF of fuel oil. The 4 aromatic compounds used in this study were naphthalene, 1, 2, 4-trimethylbenzene, o-cresol and o-toluidine. According to Eganhouse and Calder (1976), naphthalene and 1, 2, 4-trimethylbenzene are barely soluble in seawater. At 25°C, the solubilities of these two aromatics in 30% S seawater are about 24 and 40 ppm, respectively. The other two aromatics, o-cresol and o-toluidine, are very soluble in seawater (solubility >5 g l⁻¹).

The water-soluble fraction (WSF) of No. 2 fuel oil (Exxon, Baytown) contains about 0.75 ppm of naphthalene, 0.56 ppm of 1, 2, 4-trimethylbenzene, 1.0 ppm of o-cresol and 0.34 ppm of o-toluidine (Winters et al., 1976). Based on these data, stock solutions of 20 ppm were prepared for naphthalene and 1, 2, 4-trimethylbenzene, and 40 ppm for o-cresol and o-toluidine. These stock solutions were then diluted to various concentrations as required. The stock solutions were made daily. A solvent was not used in the preparation of stock solutions because, first, a solvent such as methanol or ether could be toxic to the test animals and thus further complicate the situation and, second, the stock solution prepared for each compound was always less than the saturation for that compound.

From the 4 selected aromatics, a total of 15 kinds of possible combinations can be made. For 1 component, they are naphthalene (A), 1, 2, 4-trimethylbenzene (B), o-cresol (C), and o-toluidine (D); for 2 components, AB, AC, AD, BC, BD, and CD; for 3 components, ABC, ABD, ACB, ACD, BCD, and BCD; for 4 components, ABCD. In addition, the whole WSF of a No. 2 fuel oil was used (see Table I).

For mixtures of two or more components, the following rule was adopted: the relative proportions of any 2, 3 or 4 compounds were always the same as in the WSF. For example, a mixture of naphthalene and o-cresol should have the ratio (A to C) of 0.75:1.00. Therefore, 3.50 ppm of AC solution will contain 1.50 ppm of naphthalene and 2.00 ppm of o-cresol. The whole WSF was made from a No. 2 fuel oil (Exxon, Baytown) and the method has been described elsewhere (see Pulich et al., 1974; Lee et al., 1978).

Bioassay Procedures

Twenty adult amphipods were used for each concentration, but the total number of individuals used for each solution varied (see Table I). Test amphipods were placed in a culture bowl (10.5 cm in diameter) containing 200 ml of test solution, and were fed with mixtures of ground sea lettuce and fish flake. The bowls were covered with glass plates to reduce evaporation. Test media were changed daily, and experiments lasted at least 7 days. The concentrations of the 4 aromatics and combinations of aromatics in the test solutions were not monitored. We assumed that concentration changes were similar to those determined in the WSF of fuel oil (Lee et al., 1978).

In such case, the amphipods would have experienced a concentration change of naphthalene and 1, 2, 4-trimethylbenzene from 100% at the beginning to trace amounts at the end of 24 h; o-cresol, from 100 to 92%; and o-toluidine, from 100 to 77%. The cycles were repeated every day for a week. During this period, mortality in each concentration was determined and recorded daily. All experiments were carried out at room temperature (ca. 23°C ± 1.7 °C) and in 30% S seawater.

To estimate the (24 h, 48 h and 96 h) LC50 for each solution, the method of