Acute Toxicity of Benzene, a Component of Crude Oil, to Juvenile Striped Bass (*Morone Saxatilis*)

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As the biological effects of the volatile aromatic compounds in crude oil are not well documented, a systematic examination of these compounds was initiated at the National Marine Fisheries Service at Tiburon. One of the major constituents of these aromatic compounds is benzene. The acute toxicity of benzene to 1.5 ± 0.5-g juvenile striped bass (*Morone saxatilis*) was studied in a continuous flow laboratory bioassay system. At 17.4 C and 29 ppt salinity, the lethal threshold concentration and the 96-h LC50 for benzene were 10.9 μl/liter. The 95% confidence interval was 10.9 ± 0.2 μl/liter and the probit line slope, "S," was 1.1. Possible toxic mechanisms are discussed.

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Le peu de documentation sur les effets biologiques des composés aromatiques volatiles de l'huile brute nous a incité à entreprendre un examen systématique de ces composés au National Marine Fisheries Service, à Tiburon. Un des principaux constituants de ces composés aromatiques est le benzène. Nous avons étudié la toxicité aiguë du benzène sur de jeunes bars rayés (*Morone saxatilis*) de 1.5 ± 0.5 g dans un appareil d'analyse biologique à flot continu en laboratoire. A une température de 17.4 C et à une salinité de 29‰, la concentration de seuil létal et la CL50 après 96 h du benzène sont de 10.9 μl/litre. L'intervalle de confiance à 95% est de 10.9 ± 0.2 μl/litre, et la pente de la ligne des probits, « S », est de 1.1. Nous examinons les mécanismes toxiques possibles.

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INFORMATION concerning the potential effects of crude oil on aquatic organisms is of critical interest due to the increased incidence of oil pollution. However, in studies using crude oil as a toxicant, the data are difficult to analyze. Between batches of crude oil, any component may vary in quantity and, therefore, crude oil toxicity may vary. For this reason investigations are directed toward testing the biological effects of crude oil components. There is extensive documentation of the effects of the tar components on aquatic life (Bargmann 1971), but few reports dealing with the effects of the water soluble aromatic compounds. Therefore, a program was initiated at the National Marine Fisheries Service at Tiburon to systematically measure the effects of volatile aromatic compounds on aquatic species.

As a part of this program, the acute toxicity of benzene to juvenile striped bass (*Morone saxatilis*) was studied in a continuous flow laboratory bioassay. Benzene is an important aromatic compound because it naturally occurs in large amounts, at least 20% of the aromatics in crude oil (Brocksen and Bailey 1973), and is soluble in water, up to 1993 μl/liter in distilled water (Benville and Korn 1974). Benzene is lipid soluble and is absorbed across fish gill membranes directly into the blood (Brocksen and Bailey 1973). Striped bass were used in this experiment because they support an important recreational fishery in the Sacramento–San Joaquin Delta and the San Francisco Bay, a water system heavily used by oil tankers delivering to major refineries. Striped bass accomplish at least part of their juvenile development in this estuarine environment (California Department Fish and Game 1969). Since the respiration rate of striped bass increases as fish weight decreases (Brocksen and Bailey 1973), juvenile fish were used to attempt to obtain a sensitive measure of benzene toxicity to the species. Two bioassays were performed. The initial bioassay revealed the concentration range to be tested in the final bioassay. This report refers to the final bioassay only.

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Materials and methods — Underyearling striped bass used in this study were obtained from the fish screen retrieval system of the Bureau of Reclamation water diversion facilities near Tracy, California. By August, the fish, fed Oregon Test Diet (Lee et al. 1967), grew to the average weight (1.5 ± 0.5 g) and length (52 ± 5 mm) that they normally would attain at this time in the estuary (California Department Fish and Game 1969). Using a random numbers table, 640 of these fish were equally distributed into sixteen 70-liter aquaria and were allowed to acclimate for 1 wk. The glass aquaria used were rectangular and were approximately 30 cm wide, 60 cm long, and 40 cm high. Aquaria were covered to eliminate fish stress from laboratory disturbances. The fish were not fed during the acclimation and bioassay periods and showed a net loss of 3% of their wet weight (recorded for control fish).

Filtered and aerated water, drawn from the San Francisco Bay was used for the experiment and was monitored for physical and chemical characteristics. Temperature was recorded continuously on a Bacharach Instrument Co. Tempscribe Chart. Dissolved oxygen, pH, and salinity were measured daily with a Yellow Springs Instrument Co. Model 54 D.O. meter, a Corning Instrument Co. pH meter, and a temperature compensated A. O. Goldberg refractometer. Dissolved carbon dioxide, alkalinity, nitrate-nitrogen, and nitrite-nitrogen were determined daily with a Hach Kit.[2]

The physical and chemical parameters of water used in this bioassay closely represented those which striped bass would normally encounter when they reach the San Francisco Bay estuary. The average and range of the measured parameters were: temperature 17.4 (16.9–17.9) C, dissolved oxygen 7.7 (7.2–8.2) mg/liter, pH 7.7 (7.6–7.8), salinity 29 (28–30) ppt, dissolved carbon dioxide 6.0 (5.5–6.5) mg/liter, nitrate-nitrogen 1.5 (0.0–2.0) mg/liter, nitrite-nitrogen 0.0 (0.0) mg/liter, and alkalinity 115 (110–120) mg/liter $CaCO_3$. Banks of fluorescent lights, operated through a time switch, provided controlled illumination. Day length was adjusted weekly to correspond to the natural photoperiod.

Benzene (reagent grade) was introduced into aquaria from gas wash bottles (Benville and Korn 1974). Constant air flows were maintained through gas wash bottles filled with benzene. Volitalized benzene was bubbled through an air stone near the bottom of each aquarium. The water inlet for each aquarium was directed toward each air stone from a position 3–5 cm above. This prevented the appearance of any localized areas of high concentration. Benzene concentrations were regulated by controlling the air flows.

Initially, benzene was metered into aquaria at a high rate and stabilized test concentrations were reached within 90 min. Water in each aquarium was exchanged at a rate of 2 liters/min to achieve thorough toxicant mixing, stabilize the toxicant concentration, and prevent accumulation of nitrogenous waste. A 99% replacement of water in each aquarium was attained in 2.8 h (Sprague 1973). Benzene was removed from the effluent water by an activated charcoal filter.

Benzene concentrations were determined by a gas chromatography method suggested by P. E. Benville (personal communication 1973). Each 100-ml water sample was extracted twice with 10-ml of TF Freon (triflurotrichloroethane). A small aliquot of the 20-ml extract was injected into a gas chromatograph to yield freon and benzene peaks on a strip-chart record. The benzene peak height (PH) was then compared to that of a previously prepared benzene standard. The concentration of benzene in the water sample was computed using the following equation:

$$\text{sample } (\mu\text{l/liter}) = \frac{\text{standard } (\mu\text{l/liter}) \times \text{injected } (\mu\text{l}) \times \text{sample PH (mm)}}{5 \times \text{extract injected } (\mu\text{l}) \times \text{standard PH (mm)}}$$

The number 5 is a factor that accounts for concentrating the benzene from 100 ml of water into 20 ml of freon. Water samples with known benzene concentrations were extracted by this method and 99% of the benzene was recovered.

The aliquot of the freon–benzene extract was injected into a 6-ft column packed with 5% Bentone-34 and 10% didecylphthalate on 80/100 mesh Chromosorb PAW. For this bioassay, the precision and accuracy of the gas chromatography were 0.1 μl/liter and, normally, ±5%.

Benzene concentrations and fish deaths were monitored at the same times (2, 4, 8, 20, 24, 32, 48, 72, and 96 h). Average test concentrations ± standard deviations were 23.1 ± 0.5 μl/liter, 20.8 ± 1.0 μl/liter, 15.6 ± 3.4 μl/liter, 14.1 ± 2.0 μl/liter, 12.9 ± 2.1 μl/liter, 11.6 ± 1.5 μl/liter, 11.2 ± 2.3 μl/liter, 10.3 ± 0.6 μl/liter, 9.6 ± 1.3 μl/liter, 7.7 ± 1.5 μl/liter, 5.9 ± 1.7 μl/liter, and 4.4 ± 0.6 μl/liter. For each test concentration, the median effective time (ET50) was estimated on log-probit paper (Sprague 1973). Median effective time confidence limits (95%) were estimated according to the method of Litchfield (1949). The 72-h LC50 and the 96-h LC50, the 95% confidence limits, and probit line slope, "S," were computed by the method of Litchfield and Wilcoxon (1949).

Results and discussion — All 160 fish used as controls were alive when the experiment was terminated. There were no fish deaths past 4320 min (72 h), so the 96-h LC50 and the 72-h LC50 (median lethal concentrations) were the same, 10.9 μl/liter. The 95% confidence interval was 10.9 ± 0.2 μl/liter and the probit line slope, "S," was 1.1. A regression was fitted to points representing the log of the average test concentration versus the log of the ET50 and is shown in Fig. 1. Since there were no fish deaths past 72 h, the asymptotic LC50 was assumed equal to the 96-h LC50, 10.9 μl/liter. A vertical asymptote was drawn in Fig. 1 connecting the calculated LC50

[2]Use of this equipment does not constitute an endorsement of the manufacturers.

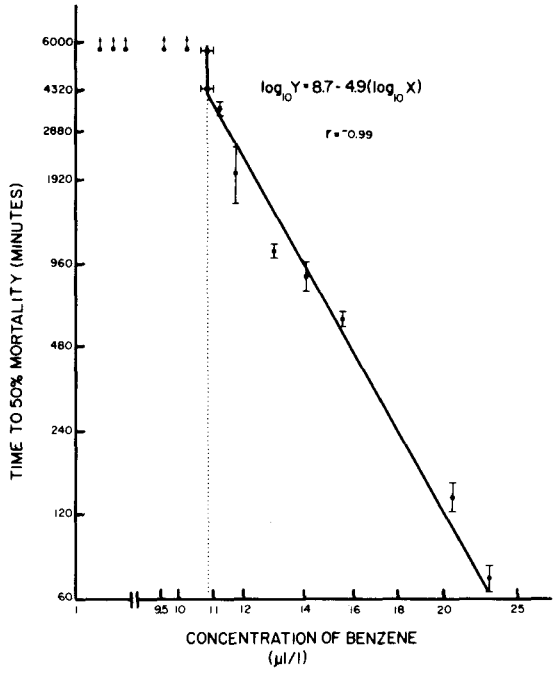

FIG. 1. Benzene toxicity curve for juvenile striped bass (*Morone saxatilis*). Confidence intervals (95%) for each point are shown.

values for 72 h and 96 h. According to a scheme listed by Sprague (1973), benzene should be considered a "toxic" substance to juvenile striped bass.

In mammals there are at least three ways benzene may cause death. Well-documented benzene-induced narcosis (Meyers 1970; Thienes and Haley 1972) is a symptom of central nervous system (CNS) depression. This CNS depression can lead to respiratory depression and collapse. Large amounts of benzene have also been found associated with erythrocytes resulting in acute anemia (Thienes and Haley 1972) and some loss of oxygen transport capacity of the blood. Benzene is known to sensitize the heart to epinephrine and any stress sends the heart into ventricular fibrillation (Thienes and Haley 1972). During ventricular fibrillation, muscle tissue of the ventricle is not contracting in a coordinated manner. Erratic contractions pump very little blood and cardiac failure rapidly follows (Guyton 1971). By all of the mentioned toxic modes of benzene, death eventually results from anoxia.

Some substantiation for these toxic modes of benzene in fish can be given. Brocksen and Bailey (1973) postulated a biochemical pathway for the detoxification of benzene. They speculated that once the detoxification capacity of the pathway was surpassed, benzene accumulated and eventually caused narcosis in the fish. This narcosis was evidenced by decreased levels of respiration in fish exposed to benzene. Also, the effects of stress on fish preconditioned to benzene can be demonstrated. When preconditioned fish are chased with a dipnet for a few seconds, they pass through a period of excitation and quickly die.

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