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THE ACUTE TOXICITY OF SIX MONOCYCLIC AROMATIC CRUDE OIL COMPONENTS TO STRIPED BASS (*MORONE SAXATILIS*) AND BAY SHRIMP (*CRAGO FRANCISCORUM*)[1]

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The acute toxicities of benzene, toluene, ethylbenzene, p-xylene, m-xylene, and o-xylene were determined for striped bass and bay shrimp by static bioassay. The 96-hr LC_{50}, ranged from 2.0 to 11 μl/l (ppm) for striped bass and from 0.49 to 20 μl/l (ppm) for bay shrimp. Solubilities of these aromatics were determined by gas chromatography in 16 C (61 F) seawater with a salinity of 25°/$_{oo}$ as part of our procedure for dosing the animals. The solubilities were 1400, 330, 180, 180, 210, and 230 μl/l (ppm), respectively, which is high enough to be lethal to striped bass and bay shrimp. The toxic effect of the aromatics was more latent in shrimp than in fish as demonstrated by the difference in the 24- and 96-hr tests.

INTRODUCTION

Fish and aquatic invertebrates in San Francisco Bay are continuously being exposed to crude oil and its refined products as a consequence of the many petroleum spills occurring every year in the bay (U.S. Coast Guard Reports 1971–1975 [2]). Due to the petroleum chemicals in the bay and the programs initiated to clean it up, the acute and chronic effects of petroleum products on the marine biota in San Francisco Bay are of interest.

Aromatic hydrocarbons, being among the more toxic fractions in petroleum, are receiving much attention; this is especially true with the polycyclic aromatics some of which are carcinogenic. Aromatics are cyclic compounds containing one or more unsaturated rings, benzene being the simplest in the homologous series. Our research efforts were directed toward the monocyclic aromatics for several reasons: their mammalian toxicities (Stecher 1968; and American Petroleum Institute 1960*a*, 1960*b*, 1960*c*); relatively high composition in crude oils and its products (Goldstein and Waddams 1967); the excess production of aromatics by reforming crude oils (Horne and McAfee 1960); and water solubilities (Mackay and Wolkoff 1973; and Benville and Korn 1974). Six monocyclic aromatics were chosen for static bioassays with striped bass (*Morone saxatilis*) and bay shrimp (*Crago franciscorum*). The six aromatics—benzene, toluene, ethylbenzene, p-xylene, m-xylene and o-xylene—predominate in the aromatic fraction of many crude oils (Goldstein and Waddams 1967; and Ander-

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[2] Pollution Incident Reporting System (PIRS), computer printouts available through U.S. Coast Guard, 12th District, 630 Sansome St., San Francisco, CA 94111.

son et al. 1974). The striped bass was chosen, because it is an important recreational fish in this region; the bay shrimp is a popular bait and an important food organism for striped bass and other fishes in the bay. Bioassay information was needed as a preliminary step to studies of chronic effects of aromatics.

MATERIALS AND METHODS

Mature bay shrimp (mean weight 1.8 g, 0.063 oz) were acquired from a local bait dealer and juvenile striped bass (mean weight 6.0 g, 0.21 oz) from the Bureau of Reclamation fish diversion facility at Tracy, California. The animals were acclimated in seawater (Korn 1975) for one week before transferring the animals into bioassay tanks (180-liter or 48 gal fiberglass aquaria). The salinity and temperature of the seawater was 25°/$_{oo}$ and 16 C (61 F), respectively, which are representative of bay conditions.

Saturated aromatic solutions were prepared by shaking seawater with an excess of the aromatics in a 20-liter (5.3 gal) polyethylene carboy for three 10-second intervals and allowing the mixture to settle for 1 hour. After gas chromatographic analyses of the saturated solutions, the proper volume was mixed into the bioassay test aquaria, each containing ten animals. A 100 ml (3.38 oz) water sample was taken from the bioassay test aquaria at 0-, 24-, 48-, 72-, and 96-hour intervals for analysis. The water samples were extracted with 9.7 ml (0.33 oz) TF Freon (trichlorotrifluoroethane) in a separatory funnel and a 3–4 µl aliquot of the Freon extract injected into a Micro-Tek 220 gas chromatograph equipped with a dual flame ionization detector. Two 6-foot columns were used at 105 C (221 F)—a 5% Bentone 34 and 10% didecylphthalate on 80/100 chromosorb PAW, and a 5% SP-1200 and a 5% Bentone 34 on 100/120 supelcoport. The first column was used for quantifying benzene, and the second was used for quantifying the other five aromatics. Aromatics were considered undetectable below 0.1 µl/l (ppm) for the concentrated extract or 0.01 µl/l (ppm) for the water sample, since the water sample was concentrated by extracting with 1/10th the amount of Freon. The concentration values were corrected by the percent recovery of the first extractions of a 100 ml (3.38 oz) water sample with 9.7 ml (0.33 oz) of TF Freon.[3] All aromatics used were 99+% pure.

TABLE 1. Acute Toxicity of Six Monocyclic Aromatics to Striped Bass and Bay Shrimp at 16 C and 25°/$_{oo}$ Salinity in Static Bioassays

Components	Solubility* (µl/l)	% Extracted w/TF Freon	Striped bass				Bay shrimp			
			24-hr		96-hr		24-hr		96-hr	
			LC_{50}	95% C.L.	LC_{50}	95% C.L.	LC_{50}	95% C.L.	LC_{50}	95% C.L.
Benzene	1400	90	6.9	**	5.8	—	22	20–24	20	19–22
Toluene	330	93	7.3	—	7.3	—	12	10–13	4.3	3.1–5.8
Ethylbenzene.....	180	82	4.3	3.9–4.7	4.3	3.9–4.7	2.2	—	0.49	0.21–1.2
m-xylene	210	78	9.2	8.3–10	9.2	8.3–10	4.8	3.6–6.3	3.7	2.9–4.7
o-xylene	230	74	11	9.4–12	11	9.4–12	5.3	4.4–6.5	1.3	1.1–1.6
p-xylene	180	78	2.0	—	2.0	—	2.0	—	2.0	—

*Solubility in 25°/$_{oo}$ seawater at 16 C (61 F).
** No confidence limits were calculated from tests without partial mortalities.

[3] Reference to a trade name does not imply endorsement by the National Marine Fisheries Service, NOAA.

The guidelines for the static bioassays were those outlined by Doudoroff et al. (1951), except for modifications mentioned in this section. The method used to compute the 24- and 96-hour LC_{50} and the 95% confidence limits was described by Litchfield and Wilcoxon (1949). Confidence limits were not calculated for tests without partial mortalities.

Water concentrations were analyzed with a curve-fitting program to describe the volatilization of aromatics over the elapsed time (Hewlett Packard BASIC program modified by Richard Faris at Tiburon Laboratory).

RESULTS

There were toxicity differences between the six aromatics for both the striped bass and the bay shrimp (Table 1). The range in the aromatic toxicities varied more for bay shrimp (.49–22 µl/l, ppm) than for striped bass (2.0–11 µl/l, ppm) at both time intervals (24- and 96-hr). The tolerance of these animals to the six aromatics within the 24-hr period indicated that benzene and toluene were more toxic to striped bass than to bay shrimp. Ethylbenzene, m-xylene and o-xylene were more toxic to bay shrimp than to striped bass for the 24-hr period. P-xylene exhibited the same toxicity for both animals at the 24- and the 96-hr intervals. The "toxicity order" of toluene reversed at the 96-hr interval, toluene being more toxic to bay shrimp. The toxicity of the other aromatics for the 96-hr period remained the same as for the 24-hr period.

Lethal concentrations of aromatics resulted in rapid mortalities of the striped bass. Almost all of the striped bass mortalities occurred within the first six hours of the bioassays. The latent toxic effects were more prevalent with bay shrimp than with striped bass during the testing period. Benzene was the only aromatic that showed a delay in its toxicity to both animals. P-xylene exhibited no difference in toxicity over the two time intervals to both species. Toluene, ethylbenzene, m-xylene and o-xylene were more toxic to bay shrimp at the 96-hr interval than at the 24-hr interval; however, these aromatics exhibited the same toxicity at both time intervals to striped bass.

There was a large difference in the solubility between the monocyclic aromatics in $25°/_{oo}$ seawater at 16 C (61 F). Benzene was the most soluble (1400 µl/l, ppm) and ethylbenzene and p-xylene the least (180 µl/l, ppm).

The extraction efficiency determined by multiple extractions of the six aromatics from seawater ($25°/_{oo}$ salinity) was high for a 1:10 Freon to seawater ratio. One extraction resulted in recoveries of 74.1 to 93.1%.

The concentrations of the six aromatics in the aquaria decreased linearly each day, in most instances. In some cases the concentrations followed a logarithmic decrease (Table 2). Average percent losses for the four time intervals (24-, 48-, 72-, and 96-hr) were 38, 61, 85, and 96%, respectively.

DISCUSSION

The monocyclic aromatics appear to be moderately toxic to fish relative to other pollutants tested in the past. For example, bioassays with fingerling rainbow trout in fresh water at 12 C (54 F) resulted in the following $LC_{50's}$: Antimycin 30–70 pptr, DDT 8–11 ppb, and acetone 8 ppth.[4]

Striped bass succumbed rapidly to a lethal aromatic concentration with most of the fish expiring within 6 hours. The rapid mortality rate with aromatics in the

[4] Benville, P. E. Jr., June 1976. *The Acute Toxicity of Nine Hydrophilic Solvents and Their Effect on the Acute Toxicity of DDT to Rainbow Trout.* Unpublished manuscript, pp. 1–13. NMFS, Tiburon Laboratory, 3150 Paradise Dr., Tiburon, CA 94920.

TABLE 2. Percent Loss of Aromatic Concentration in Test Tanks

Compounds	Initial conc. in µl/l	Percent loss			
	0 hr	24 hr	48 hr	72 hr	96 hr
Benzene	3.5	11	34	>99	>99
	5.5	35	53	80	>99
	10	28	51	64	82
	19	37	54	64	82
	22	27	55	66	78
	31	39	—	—	—
	34	38	—	—	—
Toluene	4.5	38	56	>99	>99
	12	41	51	96	>99
	16	44	—	—	—
	21	29	—	—	—
	29	31	—	—	—
Ethylbenzene	1.0	>99	>99	>99	>99
	4.9	67	>99	>99	>99
	5.9	>99	—	—	—
	12	38	—	—	—
	20	40	—	—	—
m-xylene	1.1	21	47	66	>99
	4.7	40	62	89	>99
	8.5	45	66	84	99
	13	35	—	—	—
o-xylene	1.3	32	82	>99	>99
	4.6	33	85	>99	>99
	9.3	23	49	95	>99
	18	33	—	—	—
p-xylene	1.0	19	38	59	>99
	3.9	18	—	—	—
	7.0	21	—	—	—

first few hours of the bioassay has been observed by other researchers (Pickering and Henderson 1966; and Morrow 1974). In contrast, a greater latent effect was exhibited in bay shrimp than striped bass as evidenced by the difference in $LC_{50's}$ between the 24-hr and 96-hr tests. This delayed effect may be characteristic of invertebrates or of the stage the invertebrates are in (Karinen and Rice 1974). These authors noted a toxicity difference between the 24-hr and 48-hr $LC_{50's}$ of crab to crude oil in the postmolt stage but no toxicity difference in the premolt stage

It appears that the location of the two methyl groups on the benzene ring affects the toxicity of the xylene isomers. P-xylene with the two methyl groups located at the opposite ends of the benzene ring makes the p-isomer the most stable and the most resistent to detoxification, whereas the ortho arrangement with the two adjacent methyl groups would be the least stable and would be most easily detoxified. This hypothesis held true for striped bass and for the 24-hr bay shrimp bioassays, except for o-xylene at the 96-hr interval. The 96-hr exception in bay shrimp may be explained by the delayed toxic effect of this aromatic on invertebrates.

Aromatics appear to be more toxic to striped bass than to other fingerling fish. Pickering and Henderson (1966) reported on the following LC_{50} values in soft and in hard water for bluegill, flatheads, goldfish and guppies; 22–37 ppm for benzene; 24–59 ppm for toluene; 32–97 ppm for ethylbenzene; and 21–37 ppm for xylene. Our values for 96-hr test ranged from 2.0 to 11 ppm for striped bass. Possible causes for the differences in toxicity values ($LC_{50's}$) could be attributed to bioassay technique. The bioassay technique must be adapted to the chemical and physical properties of the toxicant being tested. This is especially true with compounds that have a high volatility and a low solubility as in the case of the aromatics. In the past, toxicity values ($LC_{50's}$) for aromatics were calculated from the amount of the aromatic poured into the test aquaria. This procedure assumes all of the aromatic goes into solution which we found has not been the case. Only a small portion of the aromatic will dissolve, resulting in a higher LC_{50} value. This problem was overcome by making a saturated solution of the aromatic, analytically determining the concentration of the aromatics, and diluting the saturated solution to the desired concentration. The concentration of the saturated aromatic solution can be duplicated when the salinity and temperature are constant.

It is interesting to note that an increase in salinity lowers the amount of aromatic that will dissolve. For example, the $25°/_{oo}$ salinity used in the bioassays reduced the concentration of benzene in a saturated solution from 1993 ppm in distilled water to 1400 ppm, and of toluene from 401 to 330 (Benville and Korn 1974). The solubility of benzene was affected the most by the increase in salinity with a 30% reduction in dissolved benzene; toluene solubility was affected less, with an 18% reduction. It appears that, as aromatics become more struturally complex, their solubility is less affected by salinity. The solubility of ethylbenzene and the xylene isomers would propably be even less affected by salinity than toluene. We do not have the solubility data to support this hypothesis, and the reported solubility data cannot be used because of the inconsistencies which are encountered with compounds below the 10,000 ppm in solubility. Gunther, Westlake, and Jaglan (1968) have suggested many reasons for the inconsistencies in solubility data.

Meyerhoff (1975) has reported slightly higher LC_{50} values for benzene and striped bass (10.9 μl/l or ppm for 96-hr). One reason for a higher value is a different technique. The benzene was introduced at a slow rate until the desired concentration was reached. This would reduce the trauma experienced by the fish when they are placed in high aromatic concentrations.

Later work by Neff et al. (1976) which includes quantitative analyses of bioassay solutions yields 96 hr LC_{50} results higher than results we found using a similar type of shrimp (*Palaemeontes*). Their LC_{50} values compared to our results were 27 to 20 μl/l (ppm) for benzene, 9.5 to 4.3 μl/l (ppm) for toluene and 7.4 to 1.3–3.7 μl/l (ppm) with xylenes. The difference in results may be explained by temperature and species differences between the two studies.

The current experiment was based on a single dose test where the concentrations were decreasing over time. The loss of these aromatics in most instances was a linear function when compared to five other functions (exponential, power function, and three hyperbolic functions). The aromatic concentration could not be accurately predicted at any time interval because of uncontrolled variables such as change in biomass from mortalities during the test and fungal

and bacterial degradation. The magnitude of these and other losses (volatilization, sorption, etc.) can be determined only by measuring the amount of toxicant left in the water. Then an accurate toxicant exposure level and the persistence of the toxicant in the water are known. Our analysis showed that more than half of the aromatic usually volatilized within 48 hours, with an average biomass of 0.43 g/l (0.056 standard deviation).

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