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The Effects of a Simulated Refinery Effluent and Its Components on the Estuarine Crustacean, *Mysidopsis bahia*

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Abstract. The acute and chronic toxicities of a simulated refinery effluent and its components to *Mysidopsis bahia* were examined. The 96 hr LC50 for *M. bahia* was 4.7% of the Artificial Refinery Mixture (ARM). The mysid was more sensitive than an estuarine fish and grass shrimp, as well as 17 freshwater organisms previously tested. Fuel oil was the most toxic component of the ARM (96 hr LC50 0.73 mg/l) and contributed disproportionately to the toxicity of the mixture. Chronic exposure to 2.7% of the ARM formulation resulted in growth inhibition by day 8 and reproductive impairment. Long-term exposure to the 96 hr LC10 had deleterious effects on growth and/or reproduction for each component tested.

The potential for ecological damage from petroleum refinery wastes has long been recognized, and stringent guidelines for such discharges have been promulgated by the United States Environmental Protection Agency (USEPA 1973a). Laboratory toxicity tests provide the first approximation of the levels of discharge presenting the potential for harm. By screening discharges with "representative" or "sensitive" invertebrates rather than fish, substantial savings in time, space, and money may be possible in the preliminary stages of hazard evaluation. The crustacean *Mysidopsis bahia,* which Nimmo *et al.* (1977) suggest as a representative invertebrate of the estuarine system, was used in the evaluation of the acute and chronic effects of a simulated refinery effluent and its individual components.

Materials and Methods

The Artificial Refinery Mixture (ARM)

Refinery effluents are quite variable in composition. Consequently, defining and testing an "average" or "typical" effluent are impossible. To circumvent this variability, previous studies (Buikema *et al.* 1976; Hall *et al.* 1978; Rutherford *et al.* 1979) have utilized a simulated refinery effluent known as the Artificial Refinery Mixture (ARM). The ARM contains several components of refinery wastes but is unlike any specific effluent (Table 1). The concentration of each component in the ARM approximates the maximum allowable levels promulgated by USEPA (1973a) for refinery effluents. The ARM was prepared on the day of use according to Buikema *et al.* (1976).

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Table 1. Promulgated 1977 and 1983 refinery effluent guidelines for USEPA subcategory F integrated refineries (USEPA 1973a) and composition of the 1 X Artificial Refinery Mixture (ARM) (Buikema *et al.* 1976)

	US EPA guidelines (maximum concentration for any one day)		Artificial refinery mixture (ARM)	
Parameter	1977	1983	Concentration	Ingredient used
Ammonia nitrogen, mg/L	14.2	3.8	10.0	NH_4Cl
Cr total, mg/L	0.555	0.37	0.25	K_2CrO_4
Oil and grease, mg/L	10.8	1.5	10.0	No. 2 fuel oil
Phenol, mg/L	0.266	0.037	0.1	Phenol
Sulfide, mg/L	0.24	0.16	0.17	$Na_2S \cdot 9H_2O$
Total suspended solids, mg/L	21.6	7.4	20.0	Kaolinite [a]
pH	6.0–9.0	6.0–9.0	6.8–7.2	H_2SO_4/NaOH
Zn, mg/L	1.1	0.59	—[b]	—[b]

[a] Well-crystallized kaolinite from the Source Clay Mineral Repository, Department of Geology, University of Missouri, Columbia, MO

[b] Zinc was not used in the ARM, because at high concentrations it forms an insoluble complex with sulfide

Test Procedure

Mysidopsis bahia were obtained from USEPA, Narragansett, Rhode Island. These animals were maintained in recirculating culture systems consisting of 20 L all-glass aquaria equipped with undergravel filters, airlift tubes, and a filter layer of 7.6 cm of Pilot crushed oyster shell. Culture and test water were mixed from Dayno synthetic sea salts diluted with carbon dechlorinated Blacksburg tap water to 25 ± 1 ppt salinity. Cultures were held in an incubator at 25 ± 1° C under constant light from 15 watt fluorescent cool-white bulbs. Light intensity was ~50 ft-c at the air-water interface. Mysids were fed *ad libitum* daily with ≤ 48-hr-old San Francisco Bay brine shrimp nauplii.

Forty-eight hr before a test was to begin, 100 to 200 ovigerous females were isolated from culture tanks and pipetted into 3 L battery jars. Holding conditions were identical to those in culture tanks. The jars were checked at 24-hr intervals for released young. All tests used young ≤ 48-hr old.

Definitive static acute toxicity tests were set up with ARM and selected ARM components in ranges defined by preliminary tests. Methods for acute tests closely followed those described by Borthwick (1978). All tests were set up in duplicate with 10 animals in one L at each of the four concentrations and a control. Photoperiod, light intensity, temperature, and dilution water were identical to culture conditions. Tests were conducted without aeration or renewal. Dissolved oxygen remained ≥ 70% saturation.

Test solutions were mixed by adding appropriate amounts of a standard solution of toxicant to a one-L volumetric flask and synthetic sea water for dilution. These solutions were dispensed in two-L Carolina glass culture dishes and stirred. In tests with a No. 2 fuel oil component, the oil was added separately as an oil-water dispersion (OWD) after the solutions were dispensed. Fifty ml of the dispensed solution were placed in a stainless steel Waring Blender. The appropriate amount of oil was added by Hamilton Syringe pipette and emulsified for 20 sec. Then the OWD was returned to the container and allowed to form a film.

In tests with an oil component, animals were pipetted underneath the oil film. In those tests without oil, 10 mysids were distributed randomly to 60 mm petri dish bottoms. These dishes were submerged in the test containers, and the number of mysids in each container was verified. Culture dishes were covered with plastic wrap to retard evaporation and placed in an incubator. Mysids were fed 10 to 20 brine shrimp nauplii per mysid per day. Test organisms were observed after 24,

48, and 96 hr. Death was defined as no movement after the animal was prodded. Occasionally, no carcass for a test animal could be found. These missing animals were considered dead because mysids in exposure containers were occasionally observed eating dead animals. This problem was not encountered in control groups. The LC50s and 95% confidence limits were calculated by Finney's probit procedure in SAS76 (Barr *et al.* 1976).

Chronic toxicity tests were conducted in triplicate. Ten neonate mysids were exposed in 1 L of control and the 96 hr LC10 and LC30 concentrations of the ARM and ARM components as determined from acute toxicity data. Chronic tests were set up and conducted under the conditions described above except that test solutions were renewed three times weekly, and food supply was increased as animal size increased. Survival, egg deposition, and release of young were checked daily. The number of deposited eggs was determined by examination under a dissecting microscope. Animals were measured weekly with an ocular micrometer. Tests lasted 3-4 weeks. Analysis of variance and Duncan's New Multiple Range Test were used to determine differences among groups.

Results and Discussion

Acute Tests

The LC50 values for mysids exposed to ARM and its components are presented in Table 2. The 96 hr LC50 for mysids exposed to the total ARM is approximately 5% of the ARM formulation. This is lower than those values observed for the estuarine fish *Lagodon,* 70%, and the grass shrimp *Palaemonetes*, 19% (Hall *et al.* 1978). Our tests indicate that one-day old *Palaemonetes pugio* larvae are nearly as sensitive to the ARM as neonate mysids (96 hr LC50 is 5.4%). Of 18 freshwater organisms tested (Buikema *et al.* 1976), *Daphnia pulex* was the only species as sensitive as the mysid. The relative sensitivity of mysids to other toxicants has also been established. Mysids were the most sensitive of any organism concurrently tested for oil (Anderson *et al.* 1974; Neff *et al.* 1976), kepone, and cadmium (Nimmo *et al.* 1977). When our data on the acute toxicity of ARM components were compared to published toxicity data, the mysids generally were as, or more, sensitive than other estuarine invertebrates (*e.g.*, Eisler and Hennekey 1977; Epifanio and Srna 1975; Portmann 1972; Tatem *et al.* 1978).

Table 2. LC50 values obtained for neonate mysids exposed to the ARM and selected ARM components. Concentrations are expressed as mg/L except for the ARM which is expressed as % of its 1 X formulation (Table 1). Ninety-five % confidence limits are in parentheses

	Time (hr)		
Toxicant	24	48	96
ARM	17.0 (7.9–[a])	7.0 ([a]–19.1)	4.7 (3.9–5.5)
Ammonia Nitrogen	35.4 (31.0–39.5)	22.8 (19.6–26.5)	16.8 (14.8–19.3)[b]
Chromate	>10.0[a]	8.88[a]	6.59[a]
Fuel Oil	1.8 (1.38–2.65)	0.94 (0.74–1.13)	0.73 (0.43–0.81)
Phenol	14.4 (12.6–17.1)	12.8 (11.1–15.5)	12.5 (10.7–15.6)
Sulfide	2.41[a]	2.22 (1.85–2.66)	1.92 (1.56–2.34)

[a] Confidence limits could not be calculated

[b] Estimated 24, 48, and 96-hr LC50 concentrations of un-ionized ammonia 2.4, 1.6, and 1.2 mg/L

Several researchers have conducted acute toxicity tests on *Mysidopsis almyra* with OWD of No. 2 fuel oil. Anderson *et al.* 1974; Cox 1974; Tatem *et al.* 1978 reported 48-hr LC50 values for No. 2 fuel oil of 1.3, 1.1, and 0.9 ppm, respectively. These values are quite comparable to the 1.1 ppm (0.94 mg/l) value obtained for *M. bahia* in our study. To our knowledge, these are the only mysid toxicity data available for interlaboratory comparisons. The similarity between the 48-hr LC50 values indicates that these acute mysid tests are reproducible, but the overall reproducibility of mysid tests has not been examined specifically and needs to be established.

The major contributor to the toxicity of the ARM to *M. bahia* appears to be No. 2 fuel oil. It is the only component for which the LC50 concentrations, alone and in the mixture, are of the same order of magnitude. This ratio can be expressed as a toxic unit (Table 3). These values are generally used to predict the toxicity of an untested mixture based upon toxicity data on individual components. If the lethal effects of the individual toxicants in a given mixture are additive, when the sum of the toxic units is 1 or more the mixture will be lethal (USEPA 1973b). We have used this approach to examine the contribution of each component and the additivity of the components in a tested mixture rather than to predict the toxicity of an untested mixture. The sum of the experimentally determined toxic units for the LC50 concentration of the ARM is less than 1, indicating synergy rather than addition of components. If toxic unit values below 0.1 are eliminated from consideration, the fuel oil is the only component left to contribute to the toxicity of the mixture. The importance of the fuel oil component in the toxicity of the ARM was confirmed by Hall *et al.* (1978) and Lee (1976), who found that the toxicity of the ARM minus its oil component decreased by an order of magnitude.

Chronic Tests

Mysidopsis bahia was exposed to the 96-hr LC10 and LC30, 2.7% and 3.8% of the total ARM, for three weeks. A high attrition rate due to the handling stress involved in testing was experienced. Older animals occasionally jumped from the test solutions and were stranded on the sides of the test containers. Of 90 mysids tested, 39 were alive on day 22. More controls than experimental animals survived the duration of the test although the means of days survived were not significantly different among the groups (Table 4).

Table 3. The contribution of individual components to the toxicity of the ARM

Toxicant	A mg/L of individual component in LC50 concentration of ARM	B LC50 concentration in mg/L of individual component	Toxic units A/B
Ammonia Nitrogen	0.36	16.8	0.02
Chromate	0.012	6.59	<0.01
Fuel Oil	0.47	0.73	0.64
Phenol	0.005	12.5	<0.01
Sulfide	0.008	1.92	<0.01

Table 4. Effects of ARM exposure on the survival and growth of *M. bahia*. Means connected by the same line are not significantly different at the 0.05 level using Duncan's New Multiple Range Test

	Control	LC10 (2.7%)	LC30 (3.8%)
Days survival	15.5	14.5	12.4
Length (mm) on day 8	4.2	3.5	3.6
Length (mm) on day 15	6.5	6.1	5.8
Length (mm) on day 22	7.4	7.2	6.8

Weekly length determinations (Table 4) indicated that animals exposed to both levels of ARM were significantly smaller than controls by day 8. This length difference was maintained through the test for animals exposed to LC30. Animals exposed to LC10 remained significantly smaller through day 15. On day 22, they remained smaller than controls, but the difference was not significant.

Control and exposed mysids were compared on the basis of number of days to egg deposition, the number of eggs deposited, time from egg deposition to the release of live young (embryonic development), time from newborn to newborn (length of life cycle), the number of live young produced in the first brood, the total number of broods deposited, and the total number of eggs deposited throughout the duration of the test (Table 5).

The most striking result of exposure to ARM was the negative relationship between ARM concentrations and the number of eggs deposited in brood one. These differences are also reflected in the number of live young produced. However, live counts may not be as accurate a measure of reproductive capacity due to the possibility of cannibalism of newborn young by adults before counts can be made (Clutter 1969; Nimmo, personal communication).

The number of days until egg deposition was not significantly different among groups. In the LC30 exposed females, two instances occurred in which the eggs were gone from the brood chamber one day after deposition. This may have been due to lack of fertilization (Clutter 1969). This loss of eggs was not observed in the control or LC10 exposed animals. Only one female exposed to the LC30 successfully completed a brood and produced live young.

Differences in the number of eggs produced over the entire 21-day test period reflect both the decreased egg production and slight delay of egg deposition in exposed groups. The majority of control females completed two broods each with about six eggs. However, exposed animals completed only one brood with fewer eggs, and the number of eggs was negatively proportional to concentration. The total eggs produced over the test is the most comprehensive index of reproductive effects and shows a significant difference between the control and exposed animals.

The effects of chronic exposures to selected ARM components are summarized in Table 6. Control animals survived longer than experimental animals in all tests. These differences were significant for exposures to all components

Table 5. Effects of ARM exposure on the reproductive capacity of *M. bahia*. Means connected by the same line are not significantly different at the 0.05 level using Duncan's New Multiple Range Test

Parameter	Control	LC10 (2.7%)	LC30 (3.8%)
Days to egg deposition	15.2	17.6	16.4
Eggs deposited in first brood	6.2	4.7	3.2
Embryonic development time (days)	4.6	5.0	5.0
Length of life cycle (days)	20.0	21.6	18.0
Live young in first brood	5.8	4.0	0.7
Total broods deposited in 21 days	1.8	1.3	1.0
Total eggs deposited in 21 days	13.5	7.1	4.0

except phenol. The survival of animals exposed to ammonia was seriously affected. No exposed animals survived to reproductive maturity, precluding determination of effects on reproduction.

Growth inhibition on day 8 was the most sensitive, general indicator of chronic effects and occurred in response to all levels of all toxicants tested. In the case of the LC10 exposure to phenol (6.97 mg/l), significant growth inhibition occurred without a corresponding decrease in reproductive success, which indicates different thresholds for the two effects. Although a linear relationship generally exists between length of a female and the number of eggs she produces in malacostracans, including mysids (Clutter and Theilacker 1971), this type of relationship can be disrupted by sublethal toxicant exposure (Buikema *et al.* 1980). The growth inhibition by day 8 provides a more sensitive index of sublethal toxicant stress than reproductive impairment in a very short time period.

Reproductive impairment, as indexed by total number of eggs produced per female, was observed in animals exposed to chromate, fuel oil, and phenol. Reproduction in mysids exposed to the LC10 and LC30 of chromate and the LC30 of phenol was completely repressed. These animals never formed yolk invested ova. Mysids exposed to both concentrations of No. 2 fuel oil showed a seven day delay in egg deposition but no decrease in number of eggs deposited or young produced per brood.

An additional sublethal effect was observed in chromate exposures. Animals exposed to both levels of chromate were observed swimming in spirals with the ventral surface up. This upset in equilibrium became apparent on the third day of exposure and continued for the remainder of the test.

Table 6. The effects of chronic exposure to selected ARM components on *M. bahia*. Nominal concentration is expressed as mg/L. Survival is expressed as mean number of days. Growth is expressed as length in mm on day 8. Reproduction is expressed as mean total number of eggs produced per female. Means connected by the same line are not significantly different at the 0.05 level using Duncan's New Multiple Range Test

Chemical and parameter	Control	LC10	LC30
Ammonia nitrogen			
concentration (mg/L)[a]	0.0	12.5	14.9
survival	14.5	6.4	3.1
growth	4.9	4.4	—[b]
reproduction	11.0	—	—
Chromate			
concentration (mg/L)	0.0	4.68	5.73
survival	17.7	11.7	7.9
growth	5.0	4.3	3.9
reproduction	14.5	0.0	0.0
Fuel oil			
concentration (mg/L)	0.0	0.53	0.63
survival	19.8	14.8	11.6
growth	5.3	3.9	3.7
reproduction[c]	10.0	3.8	4.4
Phenol			
concentration (mg/L)	0.0	6.97	9.84
survival	16.1	14.0	12.8
growth	4.6	4.1	3.5
reproduction	6.5	4.6	0.0
Sulfide			
concentration (mg/L)	0.0	0.96	1.45
Test terminated due to poor control survival			

[a] Estimated concentrations of un-ionized ammonia are 0.0, 0.9, 1.0 mg/L
[b] No observations made
[c] 28 day test

Comparisons of mysid sensitivity in chronic exposures to those of other organisms are difficult, because no threshold levels of toxicants were determined. Chronic effects were evident at all concentrations of all toxicants tested. The failure to bracket no effect concentrations also precluded the estimation of maximum allowable toxic concentrations (MATCs). Future tests may be able to use short-term growth impairment to establish a concentration range bracketing an effect/no effect level for chronic tests. The chance of determining threshold levels of toxicant for life time growth inhibition and reproductive impairment would be increased by including exposure concentrations that do not produce a growth impairment by day 8.

Summary

The simulated refinery effluent was acutely toxic to *M. bahia* (96-hr LC50 ≈ 5%), and chronic effects were observed at 3%, the lowest level tested. Except

for No. 2 fuel oil, the 96-hr LC50 concentrations of ARM components were greater than the promulgated 1980 guidelines (Tables 1 and 2). Based on acute toxicity data, the individual guidelines for ammonia, chromate, phenol, and sulfide appear to be protective during short term exposures. However, our analysis of toxicant contribution to the ARM toxicity indicates synergistic interactions are possible. Except for No. 2 fuel oil, the chronic studies were conducted at concentrations greater than the promulgated 1983 guidelines (Tables 1 and 6). In all instances, chronic effects were observed at the concentrations studied. Because we did not identify no-effect levels at the concentrations studied, a discussion of whether the promulgated guidelines for ammonia, chromate, phenol, and sulfide will be protective is not possible.

Number 2 fuel oil was the most acutely toxic component, and it individually contributed most of the toxicity to the ARM. The 96-hr acute value was 50% of the promulgated 1983 guideline for oil and grease. Chronic effects were observed at 35% of the promulgated 1983 guideline for oil and grease, the lowest concentration studied. These data indicate that the promulgated oil and grease guideline would not be acutely or chronically protective if No. 2 fuel oil constituted the oil and grease component. However, the No. 2 fuel oil used to represent the oil and grease component of the simulated effluent is a refined oil which is more toxic than many substances (Anderson *et al.* 1974; Tatem *et al.* 1978) likely to be found in the oil and grease component of a refinery discharge.

Several factors contribute to the potential importance of *M. bahia* as a test animal in establishing water quality criteria. *M. bahia* is sensitive, easily cultured and handled, and has a short life cycle with several easily observed parameters reflecting sublethal toxicant stress. However, biological background information is lacking for *M. bahia*. Additional information about the life history and natural physioecology of *M. bahia* is necessary in order to maximize the applicability of laboratory toxicity data to the environment (Buikema and Benfield 1979). For example, information on the isosmotic point, field fecundity, molting frequency, and effects of environmental factors on biology and toxicity for *M. bahia* is needed. Current and light intensity appear to be important for the orientation of other genera of mysids and may be important factors in sexual behavior (Clutter 1969) and, thus, in chronic test design. The utility and defensibility of *M. bahia* as a test organism would be increased with this type of information.

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