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Acute Toxicity of Toluene, Hexane, Xylene, and Benzene to the Rotifers *Brachionus calyciflorus* and *Brachionus plicatilis*

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A large number of studies on the biological effects of oil pollution in the aquatic environment deal with the effects of whole crude or refined oils or their water-soluble fractions. However, low boiling, aromatic hydrocarbons, which are probably the most toxic constituents of oil, have until now not been examined in sufficient detail.

Toluene, benzene and xylene, constitute a major component of various oils. They may be readily lost by weathering but are toxic in waters that are relatively stagnant and are chronically polluted. Korn et al. (1977) have stated that toluene is more toxic than many other hydrocarbons such as benzene, though the latter are more water-soluble.

Reports of the effects of exposure to organic solvents like hexane or toluene are still limited although organic solvents are a well-known group of neurointoxicants (Ikeda et al. 1986). Various benzene derivates continue to be used as chemical intermediates, solvents, pesticides, so on, in spite of incomplete knowledge of their chronic toxicity (Pagano et al. 1988).

The majority of toxicity studies about the effects of pollution on aquatic organisms under controlled conditions have used either fish or the cladoceran *Daphnia magna* and there are few studies reported using rotifers. The effects of herbicides on population variables of laboratory rotifer cultures have been investigated (Couillard et al. 1989; Snell and Persoone 1989; Serrano et al. 1986; Fernandez et al. 1991; Ferrando and Andreu 1991).

Rotifers are one of the main sources of zooplankton production and they have an important ecological significance in the aquatic environment.

The present work was designed to investigate the effect of short-term exposure to some petroleum derivates which might be expected to occur immediately under an oil-slick, on freshwater and brackish environment rotifers.

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MATERIALS AND METHODS

Brachionus calyciflorus cysts were stored at 6°C in the dark. Cysts used in the experiments were produced in mass cultures maintained under rigorously controlled conditions (Dr. T.W. Snell, University of Tampa, USA). Cysts hatching was initiated by transferring to warmer temperatures and light. A standard synthetic freshwater (EPA) was used as hatching, culture and dilution medium (EPA, 1985). This medium is prepared from reagent grade chemicals and composed of 96 mg $NaHCO_3$, 60 mg $CaSO_4.2H_2O$, 60 mg $MgSO_4$, and 4 mg KC1 per liter of deionized water. Standard environmental conditions for these bioassays were: temperature 25°C; pH 7.4-7.8; hardness 80-100 mg $CaCO_3$/L; alkalinity, 60-70 mg/L, and darkness.

Brachionus plicatilis cysts are produced under rigorously controlled laboratory conditions (Dr. T.W. Snell, University of Tampa, USA) and stored in 55 ppt salinity, synthetic seawater at 6°C in the dark. Cyst hatching is initiated by transferring the cysts to lower salinity, warmer temperature and light (Snell and Persoone 1989b). Standard environmental conditions for acute toxicity bioassays were: temperature 25°C; salinity 15 ppt; pH 7.7, and darkness.

Bioassays were conducted in sterile, 24-well polystyrene tissue culture plates which were used once and discarded. Because of the short duration of the test (24 hr), rotifers were not fed and the medium was not renewed during the bioassay. Control survival was almost always 100% after 24 hr. A 24-well plate has six concentrations for one acute toxicity test. Ten neonates were placed in 1 mL in each of 3 replicate wells in a column, for a total of 30 animals per concentration (column), so the control and five concentrations for each toxicant. We carried out a total of nine replicates for each toxicant. Toluene, hexane, xylene and benzene (86% purity, AFRASA) were dissolved in ethanol at 1mg/L. Bioassay plates were placed in an incubator under standard conditions, the percent dead in each chemical treatment was recorded after 24 hr, and median lethal values (24 hr-LC50s) and 95% confidence limits were calculated from survival data using "moving-average" analysis, with an IBM computer.

RESULTS AND DISCUSSION

The results of 24 hr LC50 values for both species of rotifers for all tested hydrocarbons are listed in Table 1. Comparisons of the LC50 values indicate that hexane was the most toxic of the compounds tested to both *B. calyciflorus* and *B. plicatilis*. Hexane was followed in order of decreasing toxicity by toluene, xylene and benzene.

B. calyciflorus was more sensitive to all tested toxicants than was *B. plicatilis* Sensitivity factors, derived by dividing the 24 hr-LC50 value for *B. plicatilis* by the 24 hr-LC50 for *B. calyciflorus*, ranged from 4.8 for toluene to 1.9 for xylene (Table 1).

Comparisons of LC50 values from the present tests with those previously available should be useful for elucidating whether any large differences between LC50 derived using various test methods and different species.

Toluene 24 hr-LC50 values were 113.3 and 552 mg/L for *B. calyciflorus* and *B. plicatilis* respectively, in the present study. The median lethal concentration (LC50) for both species are much higher than those reported in the literature for the same aromatic hydrocarbon in other aquatic invertebrates and fishes. Johnson and Finley (1980) determined a 24 hr-LC50 of 60 ppm for *Daphnia magna* exposed to toluene. Fish are more sensitive to this kind of compound, for example the LC50 of toluene on *Salmo gairdneri* and *Lepomis machrochirus* were 0.82 and 2.70 ppm respectively (Johnson and Finley 1980).

The 24 hr-LC50s for hexane in the present study were 68.3 ppm for *B. calyciflorus* and 154.3 ppm for *B. plicatilis* There are few works about the lethal effect of this organic solvent on aquatic organisms. Most of them showed the toxic consequences of it on the growth of algae (Dhargalkar and Bhosle 1987), mutagenic activity (Vandermeulen and Lee 1986), oxygen consumption by invertebrates (Cotta and Zanzottera 1986) or metabolic levels in rats (Ikeda et al. 1986). Morrow (1974) found that the 96 hr-LC50 for hexane in the fish *Oncorhynchus kisutch* was greater than 100 ppm. No studies were found about acute toxicity of this compound on aquatic invertebrates.

Xylene 24 hr-LC50 values from the present tests were 252.7 ppm and 495.9 ppm for for *B. calyciflorus* and *B. plicatilis* respectively. Neff et al. (1976) also tested xylene with the aquatic crustacean *Palaemonetes pugio* They determined 96 hr-LC50 value of 7.4 ppm. In other work with *Daphnia magna* (Verschueren 1983) the authors determined a 24 hr-LC50 between 100 and 1000 ppm. And Caldwell et al. (1977) derived 96 hr-LC50 value of 6 ppm for *Cancer magister*

Benzene was the least toxic compound tested on both rotifer species. We found LC50 values > 1000 mg/L. *Daphnia magna* was exposed to benzene, a 24 hr-LC50 of 250 ppm was found (Verschueren 1983). This compound was also tested by Caldwell et al (1977) with *Cancer magister*, their 96 hr-LC50 was 108 ppm. In the fish, *Gambusia affinis*, Morrow (1974) determined a 24 hr-LC50 of 395 ppm using benzene.

B. calyciflorus was more sensitive to all compounds tested than *B. plicatilis*, and species sensitivity differences were quite small for benzene and xylene during the present study. Previous studies with pesticides (Ferrando and Andreu 1991) conducted with *B. calyciflorus* and *B. plicatilis* using static tests also substantiates that *B. calyciflorus* is more sensitive than *B. plicatilis*

The present tests involved the use of a solvent carrier (ethanol) to help solubilize the hydrocarbons into stock solutions. There is a possibility that test

Table 1. Mean *Brachionus calyciflorus* and *Brachionus plicatilis* 24 hr-LC50 (mg/L) values ($\bar{X}$) for some hydrocarbons.

Compound		*B. calyciflorus*	*B. plicatilis*	Sensitivity factor LC50 B.p./LC50 B.c.
Toluene	$\bar{X}$	113.3	552.6	4.8
	95% CL	94.5-132.1	519.5-585.7	
	CV(%)	16.5	5.9	
	N	9	9	
Hexane	$\bar{X}$	68.3	154.3	2.2
	95% CL	57.9-78.7	145.5-160.0	
	CV(%)	15.2	5.7	
	N	9	9	
Xylene	$\bar{X}$	252.7	495.9	1.9
	95% CL	203.9-301.5	461.8-530.1	
	CV(%)	19.3	6.8	
	N	9	9	
Benzene	$\bar{X}$	>1000	>1000	

concentrations may have exceeded the water solubility of chemicals in tests conducted with the solvent carrier. When water solubility is exceeded, it is unkown what proportion, if any, of the chemical present as a suspension or in a colloidal state is available to the test organism and how this, in turn, affects toxicity. Thus, with some of these compounds, water solubility may have been exceeded in trying to achieve levels that produced mortalities in tested organisms.

The present tests indicated that rotifers were tolerant to toluene, hexane, xylene and benzene. The LC50 values determined for rotifers indicate that these species were not among the most sensitive species to these chemicals. The highest acute values reported in the literature for these compounds during several tests with aquatic species were 60 ppm toluene with the cladoceran, *Daphnia magna* (Johnson and Finley 1980); 100 ppm hexane with the fish, *Oncorhynchus kisutch* (Morrow 1974); 100 ppm xylene with *D. magna* (Verschueren 1983); and 395 ppm benzene with the fish, *Gambusia affinis* (Morrow 1974).

Finally, our data demonstrated that *B. plicatilis* (an euryhaline species) is more resistent species to hydrocarbons than *B. calyciflorus*. And both species are more tolerant to these chemicals than most of the aquatic invertebrates and fishes. Differences in bioassay methods between investigators may contribute to the observed differences.

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