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A *Chironomus tentans* Bioassay for Testing Synthetic Fuel Products and Effluents, with Data on Acridine and Quinoline

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Sensitive tests of toxicity to aquatic insects are useful for assessing the effects of potential pollutants on aquatic ecosystems. Within the last few years, research emphasis has included the toxicity of coal-related synthetic fuel products and effluents to aquatic biota. As part of an Oak Ridge National Laboratory (ORNL) program to evaluate the environmental effects of synthetic fuel products and effluents, a series of tests is being used to measure the toxicity of whole and fractionated synthetic fuel products and effluents and representative pure compounds (polycyclic aromatic hydrocarbons, phenols, and azaarenes) to aquatic organisms. Synthetic fuel materials are complex mixtures of inorganic and organic substances, including aliphatic hydrocarbons, aromatic hydrocarbons, and N-, S-, and O-substituted aromatics (HERBES et al. 1976; MILLEMANN et al., in preparation). By testing fractions and components of whole materials, we can begin to explain observed toxic effects and recommend further treatment (i.e., wastewater treatment or produce upgrading) to reduce toxicity (e.g., PARKHURST et al. 1979). Test organisms include freshwater crustaceans (Daphnia pulex and magna), green algae (Selenastrum capricornutum), blue-green algae (Microcystis aeruginosa), snails (Physa heterostropha and Helisoma sp.), fathead minnows (Pimephales promelas), bluegill sunfish (Lepomis macrochirus), and rainbow trout (Salmo gairdneri) (SOUTHWORTH et al. 1978; GIDDINGS 1979; PARKHURST et al. 1979; PARKHURST et al., in press; MILLEMANN & PARKHURST, in press; MILLEMANN et al., in preparation).

To our knowledge, an appropriate test method has not been demonstrated for aquatic insects. To be considered appropriate, the method must be precise, simple, economical, and valid for testing the complex mixtures found in synthetic fuel products and effluents. Our purpose was to develop a toxicity testing method for the midge Chironomus tentans that would meet these criteria. Some of the methods appropriate in earlier C. tentans bioassays are not optimal for synthetic fuels testing. In some cases, the tests used a soil substrate (DAD et al. 1980), or the animals were fed during the test (KARNAK & COLLINS 1974). An

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organic substrate or food could have a confounding effect by changing water quality and/or by adsorbing components of the test materials. Organisms could be exposed to lower-than-expected water concentrations and/or higher-than-expected concentrations in food or substrate. Aeration (KARNAK & COLLINS 1974) and exposure to light are not desired in a synthetic fuels test, because volatilization (SOUTHWORTH 1979) and photodegradation (ZEPP & SCHLOTZHAUER 1979) could change concentrations of some toxic materials.

To test the C. tentans bioassay, we chose to measure the toxicity of two azaarenes, acridine and quinoline. Azaarenes, for which relatively few toxicity data exist, have been identified as components of coal-derived synthetic fuel products and effluents (HERBES et al. 1976; PARKHURST et al., in press).

MATERIALS AND METHODS

Chironomus tentans egg masses were shipped to ORNL from Battelle, Pacific Northwest Laboratory, Richland, Washington, and aquarium-reared in a manner similar to that reported earlier (BIEVER 1965). Each 35-liter aquarium was filled to a depth of approximately 8 cm with well water [pH 7.8, alkalinity 120 mg/L, hardness 140 mg/L (PARKHURST et al., in press)]. A substrate of shredded paper towel was provided, and each aquarium was aerated. Cultures were fed weekly a suspension of ground dry dog food in water. Water temperature in the aquaria was 20 to 25 C. Aquaria received about 12 h of light daily.

Glass beakers (100-mL capacity) were filled with 80 mL of filtered (0.45 m) well water. To each beaker approximately 0.3 g of glass wool (KARNAK & COLLINS 1974) was added as a substrate. All beakers were covered with cardboard boxes to exclude light throughout the tests; no aeration was provided. Tests were run at 23 to 26 C.

Fourth-instar larvae were collected for testing either by disturbing the substrate or by scooping up small quantities of it. Larvae were removed with a curved dissecting needle with minimum handling, then randomly distributed among test vessels. Each beaker contained seven larvae. A 24-h acclimation period with filtered well water was used, after which any dead or pupated C. tentans were replaced (extra beakers were stocked with larvae at the same time as the test vessels). The criterion for mortality was failure to respond to probing with a dissecting needle by directed avoidance behavior (swimming or crawling from the needle). During the tests, any pupating individuals were noted and excluded from the test population (but not replaced), so that mortality during pupation would not be included in the data.

Four beakers were used at each toxicant concentration. Acridine was tested by first dissolving the chemical in methanol, and then in filtered (0.45 µm) well water; quinoline was dissolved directly in filtered well water. Filtered well water was used for all subsequent dilutions. For acridine, a control was run with methanol at approximately its highest tested concentration (4 mL methanol per liter well water). Toxicant concentrations in stock solutions, and in the test vessels at the beginning and end of the tests, were measured spectrophotometrically (acridine at 250 nm; quinoline at 312-313 nm after addition of 5% phosphoric acid to sharpen the absorbance peak). The arithmetic mean (of initial and final concentration) of concentration was used in the calculation of LC50 values and fiducial limits using a computerized probit analysis with correction for control mortality (BARR et al. 1976).

RESULTS AND DISCUSSION

The calculated 48-h LC50 values for acridine and quinoline are 1.96 mg/L and 57.2 mg/L, respectively (Table 1). The 95% fiducial limits are within 3 to 12% of the LC50 values. Neither mortality nor pupation during the 24-h acclimation period exceeded 1% in either test. Control mortality was 11.5% or less in each test; the overall value of 8.5% is within the 10% level recommended by the U.S. ENVIRONMENTAL PROTECTION AGENCY (1975). Control mortality should be held to a minimum, so that the possible interacting effects from stress and toxicant are avoided (SPRAGUE 1969). The pupation rate was 8.3% or less in each test; the overall value was 7.1%. Exclusion of pupating individuals from the test population can appreciably reduce the sample size and must be taken into consideration. Final concentrations of acridine and quinoline were about 70 and 90% of initial concentrations, respectively.

TABLE 1. Acute Toxicity of acridine and quinoline to Chironomus tentans, with data on percent pupation and mortality during acclimation and on percent pupation and control mortality during the 48-h test

	Concentration (mg/L)						
		95% fiducial limits		Acclimation		Test	
Toxicant	48-h LC50	Lower	Upper	% pupation	% mortality	% pupation	% control mortality
Acridine	1.96	1.74	2.19	1.2	1.2	8.3	4.8
Quinoline	57.2	54.8	59.2	1.0	1.0	6.1	11.5
			Pooled	1.1	1.1	7.1	8.5

Table 2 summarizes data on the toxicity of acridine and quinoline to Daphnia spp. as well as our results with C. tentans. Daphnia spp. are commonly used in toxicity testing and are considered sensitive aquatic organisms (ADEMA 1978; LEEUWANGH 1978). The data for C. tentans are comparable to those for Daphnia spp. The C. tentans test system appears to be a sensitive and simple (no organic substrate, feeding, light, or aeration; small test vessels; ease of stock culture and handling) method for evaluating the potential toxicity of synthetic fuel products and effluents to aquatic biota. Verification of the usefulness of the method awaits further testing with whole synthetic fuel materials and their components.

TABLE 2. Comparison of toxicity to Chironomus tentans and Daphnia spp. of acridine and quinoline

		Toxicant concentration (mg/L)		
Test organism	End point	Acridine	Quinoline	Reference
Chironomus tentans	48-h LC50	1.96	57.2	This study
Daphnia magna	48-h LC50	2.3	28.5	PARKHURST et al., in press
Daphnia pulex	24-h LC50	2.92	----	SOUTHWORTH et al., 1978
Daphnia sp.	"Toxic threshold"	----	52	MCKEE & WOLF 1963
Daphnia sp.	"Kill"	5.0	----	MCKEE & WOLF 1963

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