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New Approach to the Seven-Day *Ceriodaphnia dubia* Test with Additional Comments Pertaining to the Same Test for *Daphnia magna*

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A large number of aquatic toxicology laboratories have encountered problems in the successful execution of the seven-day Ceriodaphnia test. This test was originally designed by Mount and Norberg (1984) and was later included in the short term methods for estimating chronic toxicity of effluents of Horning and Weber (1985). Their main intent was to develop a test that was easy to carry out and at the same time was as efficient as possible. We have redesigned this test with the inherent requirements of the test animals in mind and have increased, as a result, both the efficiency and the success rate. The purpose of this paper is to describe this redesigned test.

MATERIALS AND METHODS

Table 1 presents the test conditions for Ceriodaphnia dubia J. Richard 1894 (Berner 1986) and for Daphnia magna Straus 1820. The conditions for culturing C. dubia have been previously published (Cowgill et al 1985a; 1985b; Takahashi et al 1987) and their new diet has been recently described (Cowgill et al 1988). Daphnia magna culturing conditions have been reported elsewhere (Cowgill et al 1985c; Cowgill et al 1986). The major changes that have been installed have in no way changed the basic tenets of the original test (Mount and Norberg 1984) but have emphasized the fundamental needs of the animals in terms of space and diet. The test vessel consists of a wide mouth clear glass jar graduated in milliliters to contain 150 mL. Into this jar is fitted a glass tube, 3.5 cm in diameter which has affixed to one end a nytex screen of 243μm mesh for C. dubia or 1000μm mesh for D. magna. These screens have been affixed to the glass tubes with silicone glue. In addition, after the screens have been glued to the glass tube, three glass beads, 8 mm in diameter, are affixed to the underside, equidistant from each other, to each of the screens. This setup is covered with a glass petri dish 5.5 cm in diameter. The jar containing the screened tube is filled with double distilled water and autoclaved for 10 min at a pressure of

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Table 1. Test conditions for Ceriodaphnia dubia and Daphnia magna

Variable	Condition
Test vessel capacity (mL)	150
Content (mL)	100
Screen composition	Nytex
Screen mesh size (μm)	
C. dubia	243
D. magna	1000
Light (lux)	
C. dubia	670 ± 72
D. magna	2150 ± 250
Photoperiod (h)	16 light/8 dark
Temperature (°C)	25 ± 2
Dissolved oxygen (mg/L)	8.5 ± 0.5
pH	8.1 ± 0.2
Dilution water	
Hardness, calculated as $CaCO_3$ mg/L	
C. dubia	90-110
D. magna	160-180
Alkalinity, calculated as $CaCO_3$ mg/L	
C. dubia	55-75
D. magna	40-52
Habitat	Environmental chamber
Changing frequency	Daily/every other day
Diet	
Algae	Ankistrodesmus convolutus Corda Nitzschia frustulum Kutzing
Feeding rate	
	A. convolutus
C. dubia (cells/vessel)	9×10^6
D. magna (cells/vessel)	18×10^6
	N. frustulum
C. dubia (cells/vessel)	1.8×10^6
D. magna (cells/vessel)	3.6×10^6
Feeding frequency	daily
Test requirements	
Age of organisms (h)	<12
Number of broods	3
Permitted control loss (%)	20
Number of organisms/vessel	1
Number of organisms/concentration	10
Test length (d)	
C. dubia	7
D magna	9
Variables monitored	
Daily	Light, temperature, dissolved oxygen, progeny, survival
Test termination	Survival, total progeny, adult weight
Endpoints	Survival, total progeny, adult weight, loss of controls limited to 20%

124 kPa. This procedure is repeated three times, renewing the distilled water each time, before the setup can be used for a test. This is done to remove all effects of the silicone glue. Two times through the autoclave proved insufficient.

These vessels are filled with 100 mL of dilution water and toxicant or test effluent. A comparison was made studying the effects of dissolved oxygen and test organism longevity among volumes of 15 mL, 50 mL, 100 mL and 150 mL. Only glass vessels were used in this study, as the "disposable plastic salad dressing cups" (Horning and Weber 1985: 61) in contact with some effluents may leach toxic amounts of phthalates.

Initially, the neonates of each species are placed individually in each jar where they remain for the first two days, depending upon renewal frequency. The reason for this is to provide the neonate with greater access to food. On the third day, each organism is gently poured into its screened tube (screen size is species dependent, cf Table 1) that will be retained for the remainder of the test period. This tube is partially immersed in dilution water so that in the process of pouring, the organism is always in contact with a liquid. After this has been accomplished, each renewal day, the screened tube containing the organism is simply placed in a new jar containing the renewal test solution. Upon being placed in the renewal solution the organisms are fed at once. Should the screen become clogged with algae, the adult organism can easily be poured into a fresh tube. The screen size has been selected to permit the passage of neonates to the bottom of the jar while the adult remains within the confines of the screened tube. In the process of screen transfer, only the adult is transferred leaving the neonates in the old jar. Great care must be exercised when transferring organisms to assure that they do not remain away from liquid for any length of time. Ceriodaphnia respond poorly to constant handling. They are quite fragile animals and can easily be damaged by a glass pipette. This suggested procedure reduces the handling of these organisms to an absolute minimum. The neonates are relatively easy to count if a bright light is employed as a background. This is especially true as the new diet (Cowgill et al 1988) has produced larger adults and hence bigger neonates. In the absence of the adult, the neonates are much more easily removed if their dry weight is desired.

RESULTS AND DISCUSSION

Table 2 presents comparative data for dissolved oxygen and longevity in relation to ambient medium volume. Twenty animals, each maintained in its own glass vessel, comprised the number of replicates for each volume. The ambient medium was renewed daily in this study. Cowgill et al (1985a) reported a longevity of 125 days in 200 mL

for C. dubia sustained on a single green algal diet. The study results presented in Table 2 were obtained with the mixed algal diet recently described by Cowgill et al (1988). This project continued for 55 days.

Table 2. Data for dissolved oxygen and C. dubia longevity in relation to ambient medium volume at 25 ± 1°C (N = 50)

Volume mL	Dissolved Oxygen mg/L	Longevity (d)
15	3.9 ± 0.8	9.5 ± 1.4
50	5.0 ± 0.8	11.0 ± 1.4
100	8.5 ± 0.8	45.6 ± 3.2
150	8.5 ± 0.8	46.0 ± 2.0

It has been discovered that C. dubia exhibits greater tolerance to low levels of dissolved oxygen on mixed algal diets (2.9 ± 0.3 mg/L) than on a single green algal diet (4.0 ± 0.2 mg/L) or trout chow + yeast + cerophyll (≤ 5.0 mg/L dissolved oxygen - Horning and Weber 1985: 64). In Table 2, it is clear that 15 mL and 50 mL are too stressful for these organisms, which have a rather high metabolic rate, as the dissolved oxygen content is too low and the stress of inadequate space and low dissolved oxygen reduces the longevity. The dissolved oxygen content and the longevity are essentially the same for both 100 mL and 150 mL. The latter is the size jar that was selected and the test volume of 100 mL has proved adequate.

The changes that have been installed in the seven-day cladoceran test in no way alter the basic tenets of the original test (Mount and Norberg 1984). The diet has been changed from the trout chow + yeast + cerophyll to a mixed algal diet, the latter being closer to what C. dubia consumes in nature. C. dubia maintained on a mixed diet has an adequate supply of lipids as neonates are able to survive axenically in the absence of algal or any other source of food for 10 days. The synthetic diets do not supply the animals with a sufficient lipid supply to sustain them in the absence of food for the length of the 48 h static acute test. For further discussion of this point please see Cowgill et al (1985a). The second major change that has been instituted is that of an increased test solution volume which allows the organism more living space. The third change and probably the most important one is that of reduced handling of the test neonate. We have found that the increase in space for C. dubia, coupled with reduced handling, and a mixed algal diet has permitted us to carry out this seven-day test efficiently and easily in compliance with the test requirements.

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