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Renewal Device for Test Solutions in *Daphnia* Toxicity Tests

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Acute toxicity tests provide information about lethality of toxic agents or mixed wastes when test organisms are exposed. Tests follow basically three types of laboratory designs: static, static with renewal, and continuous flow. The type of test method that is selected depends on the objective of the test. Static tests are sufficient to assess the acute toxicity of effluents and pure compounds or to determine effectiveness of various effluent treatment strategies for reducing toxicity. However, if a test material is volatile or rapidly degrades, renewal or continuous flow tests are recommended (Buikema et al. 1982). An estimate of the degradation rate of the test material using first order rate kinetics can be used to determine which method (static, static with renewal, or flow through) is to be utilized. We suggest that test material degradation should be less than 10% within a 4 day period for the static method, less than 10% in a 24 hour period for the static with renewal method, and greater than 10% in 24 hour period for the flow through method.

The common practice in static renewal tests is based on transferring live organisms to a chamber containing the same concentration or dilution of test material as that from which organisms were removed, and counting live and dead organisms in each test chamber (Standard Methods 1980). The usual practice for aquatic invertebrate toxicity tests in our laboratory includes the use of five concentrations and a control with five replicates per concentration. Therefore, for an acute test with Daphnia pulex with 50 animals per concentration, about 4 hours labor are involved in renewing the solutions for one test when the procedure from Standard Methods (1980) is followed. Because of the extensive time required to renew test solutions and the possibility of miscounting animals during renewal, especially in colored effluents, we developed a simple device that can reduce the time of the whole renewal operation to 10 minutes without removing organisms from test chamber. The renewal device described in this paper has been tested and evaluated using industrial effluents and potassium chromate. To date, this apparatus has been used to conduct at least 30 renewal tests with various toxicants using Daphnia pulex as the test organism.

MATERIALS AND METHODS

The renewal system consists of two flasks and a renewal device connected by means of surgical tubing and operated under a vacuum. Each flask contains a stopper with a glass tube passing through it. One flask contains the withdrawn solution, and the other flask is used to maintain an even flow rate and acts as a trap to catch any overflow from the first flask in the series (Figure 1A).

The renewal device is constructed from 25-mm and 20-mm diameter PVC pipe (Figure 1B). The 25-mm pipe is used as a handle and contains a stopper with a glass tube passing through it for attachment to the vacuum system. The 20-mm pipe is covered on one end by 250-µm mesh Nitex screen (Tetko, Inc., Elmsford, New York) to prevent removal of organisms during use. The other end is attached to the opened end of the handle. Located at the upper end of the 20-mm pipe is an opening (5 mm diameter) which is closed to create a vacuum during the renewal process. A piece of 25-mm pipe is attached to the screen end of the device and used to estimate the remaining solution level in each beaker. When a 90 percent solution has been withdrawn, the opening in the 20-mm piece is uncovered. A residual 25-mL volume is then released back into the beaker. Fresh solution is then added to each replicate to yield a total volume of 200 ml.

To evaluate the renewal device, acute toxicity tests were conducted in the North Texas State University, Institute of Applied Sciences (NTSU-IAS) aquatic toxicology laboratory. Tests were conducted in a controlled environmental chamber at a temperature of 20 $\pm$ 2°C and 16:8 light and dark photoperiod. Each test consisted of five concentrations or dilutions and a control with five replicates. Each replicate contained approximately 10 animals. The tests followed standardized toxicity test methodology (Peltier and Weber 1984). The EC_{50} values and their 95% confidence limits were determined using a computer program developed by Stephan (1977). The Kruskal-Wallis statistical tests were performed using the Statistical Analysis System (SAS 1982) on the NTSU mainframe AS5000 computer to determine significant differences of *Daphnia pulex* mortality within each acute toxicity test and between static renewal and static tests.

Mortality of daphnia was recorded after 48 hours. Test solution with organisms from each replicate test chamber at the end of the test was poured through a 250-µm mesh Nitex screen. Organisms caught on the screen were quickly placed in a petri dish containing moderately hard water and were counted under 10x magnification. Daphnia were considered dead if they failed to move or respond to gentle probing. Alkalinity, pH, hardness, and

conductivity were measured in the controls and in the highest test concentrations before the test started and at the completion of the test. Dissolved oxygen was recorded at the initiation of tests and every 24 hours. The test solutions were not aerated.

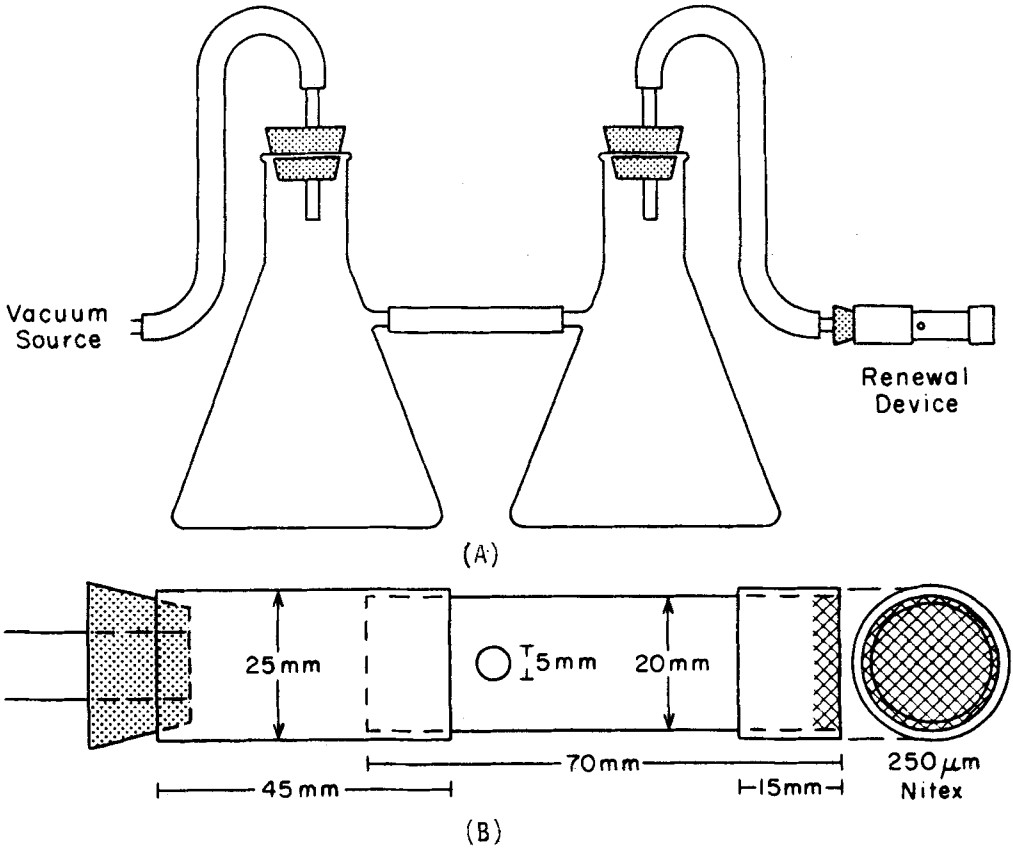

Figure 1. Illustration of Vacuum System (A) and Device for Renewal Test Solution (B).

The Daphnia pulex culture was developed and maintained in the laboratory at NTSU-IAS. The culture system consists of four rows with fifteen 600-ml beakers each containing 500 ml of moderately hard water and eight daphnids. All beakers were examined daily in each of the four rows for the presence of neonates. The neonates were culled individually using a glass pipet and transferred into a 600-ml holding vessel. Daphnia pulex were fed vitamin-enriched Selenastrum capricornutum at densities approaching 2 x 10^6 cells/ml (Waller and Lazorchak, personal communication). Ten millilters of algal culture were added daily to each beaker containing 8 adults in 500 mL water. Dilution water consisted of laboratory prepared, EPA moderately hard water (Peltier and Weber 1984). Hardness of this water ranged from 75 to 100 mg/L as $CaCO_3$; alkalinity was 45 $\pm$ 10 mg/L as $CaCO_3$; pH was 7.5 $\pm$ 0.5; and dissolved oxygen ranged from 7.5 to 8.5 mg/L.

Table 1. Mortality of Daphnia pulex in static and static-renewal acute tests for nontoxic industrial effluents and hexavalent chromium.

	Renewal of Test Solution		Non-Renewal of Test Solution	
	(No. of tests)	Average Mortality (%)	(No. of tests)	Average Mortality (%)
Effluent Dilutions (%)		Effluent		
Control[1]	21	8.1	5	4.4
6.25	21	9.4	5	4.7
12.5	21	10.8	5	5.0
25.0	21	10.4	5	4.8
50.0	21	10.0	5	6.3
100.0	21	10.5	5	11.2
Concentrations (mg Cr^{+6}/L)				
Control[1]	15	8.9	15	6.6
0.09	1	3.0	1	2.0
0.14	1	22.4	1	22.7
0.19	1	17.9	1	16.3
0.23	1	64.4	1	60.9
0.30	1	100.0	1	100.0

[1]Each dilution of test concentration had approximately 50 Daphnia pulex.

The stock potassium chromate solution for reference toxicant tests was prepared from reagent-grade potassium chromate - K_2CrO_4 (Fisher Scientific Company). Each concentration (chromium) and dilution (effluent) was prepared independently in a separate chamber and then delivered to the test vessels. The initial total chromium concentrations in the test chambers were confirmed by chemical analyses according to Standard Methods (1980) and were measured using a Perkin-Elmer 2380 Atomic Absorption Spectrophotometer.

RESULTS AND DISCUSSION

The average Daphnia pulex mortality in a series of dilutions for the industrial effluent and a series of concentrations for hexavalent chromium in static and static renewal tests is illustrated in Table 1. The average neonate mortality among dilutions of nontoxic effluents in either static and static renewal tests was not significantly different ($p = 0.89$ and 0.88, respectively) demonstrating that the effluents were not toxic. Also, average mortality at the same effluent dilution between static renewal and nonrenewal tests was not significantly different (p value ranged from 0.11 to 0.87) demonstrating that mortality was not attributed to the renewal device.

For comparative purposes, static and static renewal tests with Daphnia pulex and potassium chromate were conducted simultaneously. The 48-h EC_{50} values for both tests were 0.22 mg Cr^{+6}/L, and average mortality for each concentration was not significantly different.

In order to detect any stress on Daphnia pulex caused by the renewal apparatus, 300 neonates distributed in 30 beakers were tested for 48 h with EPA moderately hard water. In 15 beakers containing approximately 150 individuals, the test solution was not renewed. The mortality in beakers where solution was renewed averaged 8.9 percent compared to 6.6 percent mortality in non-renewal beakers (Table 1). The values were not significantly different ($p = 0.93$).

Results obtained in this study indicated that even a sensitive species like Daphnia pulex is not stressed when the renewal apparatus is used. The renewal apparatus described in this paper can be satisfactorily used for short-duration static tests. The average mortality during testing was similar whether the test solutions were renewed or not; however, time needed to renew test solution was significantly reduced. Therefore, when the renewal method is necessary, the time saved using this renewal apparatus is particularly advantageous when several tests are conducted simultaneously.

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