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# Utilization of Bacterial Colony Counters to Count Early Instar Water Fleas *(Daphnia magna)*

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Chronic toxicity studies utilizing the water flea (Daphnia magna) have proven to be a valuable tool in estimating safe levels of potential toxicants in the aquatic environment (BIESINGER et al. 1975; MACEK et al. 1976a,b; MAKI & JOHNSON 1975; NEBEKER & PUGLISI 1974; SCHOBER & LAMPERT 1977). In determining the impact of the test material on the reproductive capacity of the dahpnids, the number of offspring produced by the parental organisms must be assessed at specified time intervals, generally every weekday (U.S. EPA, 1975). Due to the small size of the animals and the large number of animals which must be counted (up to 70,000 per test), this task can be very tedious with a high error potential if counting is done manually. In addition, considerable time is expended when manually counting large numbers of organisms. A method has been developed by which a bacterial colony counter can be used to automatically count early instar D. magna in an accurate, time efficient manner.

## MATERIALS AND METODS

The colony counter used was a Fisher Count-All™ Model 600 equipped with a Hitachi video monitor. The video monitor is used to visually calibrate the instrument to detect and count daphnids of a specified size range, and to insure that all daphnids to be counted are located within the area being monitored.

During chronic toxicity studies, offspring were generally counted daily. The organisms were about 0.9 mm in size. The daphnids to be counted were generally in groups ranging from 20 to 150 individuals, a group consisting of the offspring produced in one aquarium. Individual groups were transferred to a 15% formalin solution and placed on a 9 mm diameter watchglass with 15 mL of formalin. The watchglass was placed on the colony counter stage and the instrument was calibrated to detect and count all organisms on the watchglass. The daphnids must be dispersed in the 15 mL solution since aggregates may not register all indivuals.

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Error Determination. The potential error of the counter was determined by automatically counting known sized groups of organisms as described previously by manually counting each group several times. Each group was automatially counted ten times with the organisms dispersed differently between each count. The organisms were dispersed by agitating the 15 mL solution with a spatula and care was taken to separate aggregates. The mean percentage error and standard deviation was calculated for each group.

## RESULTS AND DISCUSSION

Results of the error assessment for each group of daphnids counted are presented in Table 1. The instrument manufacturer considered the colony counter to be functioning normally if the error was not greater than 2%. Groups of daphnids up to 100 organisms were counted accurately within a 2% error, and groups of 125 organisms were counted with a slightly higher error. Groups larger than 125 daphnids were counted with an appreciably larger error (6.2 to 8.5%). The method was precise for all sized groups counted, although precision generally decreased with increased population size. The increased error with increased population size was due to the difficulty in preventing organisms from aggregating into groups.

TABLE 1

Error potential resulting from population counts of *Daphnia magna* utilizing a bacterial colony counter.

| Population size | Mean percentage error | Standard deviation | Number of counts |
|---|---|---|---|
| 50 | 1.4 | 1.0 | 10 |
| 100 | 1.6 | 1.0 | 10 |
| 125 | 2.7 | 1.3 | 10 |
| 150 | 6.2 | 1.9 | 10 |
| 175 | 6.7 | 2.4 | 10 |
| 200 | 8.5 | 2.1 | 10 |

These data suggest that the bacterial colony counter can be utilized in determining the numbers of offspring D. magna produced in chronic toxicity studies. Although a small error may exist when populations are counted, this error is probably less than the error incurred when counting the organisms manually. In addition, the time required to automatically count the daphnids is less than one half of that required in manual counting. The error incurred when counting large numbers of organisms can be minimized by dividing the groups into sub-groups of 125 organisms or less.

## REFERENCES


BIESINGER, K.E., R.W. ANDREW, and J.W. ARTHUR: J. Fish Res. Bd. Canada 31, 486 (1975).

MACEK, K.J., K.S. BUXTON, S.K. DERR, J.W. DEAN, and S. SAUTER: Chronic toxicity of lindane to selected aquatic invertebrates and fishes. U.S. Environmental Protection Agency. Ecological Research Series, No. EPA-660/3-76-046, 55 pp. (1976a).

MACEK, K.J., K.S. BUXTON, S. SAUTER, S. GNILKA, and J.W. DEAN: Chronic toxicity of atrazine to selected aquatic invertebrates and fishes. U.S. Environmental Protection Agency, Ecological Research Series, No. EPA-600/3-76-047, 55 pp. (1976b).

MAKI, A.W. and H.E. JOHNSON: Bull. Environ. Contam. Toxicol. 13, 412 (1975).

NEBEKER, A.V., and F.A. PUGLISI: Trans. Amer. Fish. Soc. 103, 663 (1974).

SCHOBER, U., and W. LAMPERT: Bull. Environ. Contam. Toxicol. 17, 269 (1977).

U.S. ENVIRONMENTAL PROTECTION AGENCY: Guidelines for registering pesticides in the United States. U.S. Government Printing Office, Washington, D.C. Federal Register 40, 26803 (1975).