

Use of an Electronic Particle Counter to Determine the Number of Young *Daphnia magna* in Ecotoxicological Reproduction Tests

Hans-Günter Müller

*BASF AG, Emissionsüberwachung und Ökologie,
D-6700 Ludwigshafen, West Germany*

With the increased testing of new chemical substances resulting from different chemical regulations, often a chronic toxicity test with *Daphnia magna* will have to be undertaken. This test normally uses the parthenogenetic production of offspring as the major test criterion. (MAKI & JOHNSON 1975; U.S. EPA 1975; SCHÖBER & LAMPERT 1977). Therefore in the course of this test, often every other day, the offspring produced in all test vessels will have to be counted. In general the reproducibility of the test will become better when the number of parent animals, and hence the number of young animals, increases.

MATERIALS AND METHODS

The particle counter used was a HIAC PC 320 with a CMH 600 sensor. With this equipment it is possible to count particles from 10 to 600 μm in size and to differentiate between different particle sizes. The measurable concentration range is from 0 to 300 particles per mL.

Prior to counting, the young animals were separated from the adults using a 0.9 mm sieve. This raw preparation contained a lot of exuviae and food particles and was therefore suspended in a 1 % formaldehyde solution. Most of the extraneous particles however are small and thus will not be counted if the particle size of young daphnids is set correctly on the particle counter; for most practical purposes this will be sufficient.

In the 1 % formaldehyde solution the dead daphnids settled down very quickly and the supernatant containing the exuviae and the food particles could be discarded. The offspring were then resuspended in 300 mL of particle free tap water and then counted.

RESULTS AND DISCUSSION

The number of daphnids were counted by five different people and the mean values of these counts were taken as the best possible estimations of the exact numbers. These numbers were then compared with the results from counts with the particle counter. The results are given in table 1.

Table 1. Error potential for counting different population sizes of D. magna with an electronic particle counter.

No. of samples (5 times manually counted)	Population size	Mean of particle counts	Range of particle counts
10	100	99	98 - 100
10	500	501	491 - 517
7	1000	1003	983 - 1025

The range of the particle counts shows that it is possible to count the number of young D. magna with a deviation of less than 3 % for one single count and that there is no systematic error resulting in generally too high or generally to low numbers.

Increasing the number of samples up to ten leads to a mean percentage error of less than 1 %. This holds true for populations in the range of 100-1000 animals, but is also expected to be true for bigger population sizes, under the condition that the concentration of animals will not be greater than 300/mL.

These data show that counting of young D. magna from ecotoxicological tests with an electronic particle counter is possible and will lead only to a small error. Compared with the natural deviation in the production of offspring this small error can be neglected. It should be noted that counting of a series of 50 samples with some ten thousands of *Daphnia* will take no more than 0.5 hour.

REFERENCES

- MAKI, A.W. and H.E. JOHNSON: Bull. Environ. Contam. Toxicol. 13, 412 (1975)
- 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)
- SCHOBER, U. and W. LAMPERT: Bull. Environ. Contam. Toxicol. 17, 269 (1977).

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