Hatching Rate as Bioassay. Proposal for a Standard Technique

by KURT JENSEN
BIOKON A/S
Prinsesse Maries Alle 14
1908 Copenhagen
Denmark

Introduction

The bioassay technique may offer an easy way to get some information of the toxicity of a particular matter. Though it is well known that the sublethal effects are at least as damaging as the acute toxicity, the controlling authorities often have to turn to a simple toxicity test to get any impression of the actual quality of a particular waste water. This is the background of the proposal for this experimental method.

Some authors have suggested standard bioassay techniques for toxicity tests: (AKESSON, 1970; BROWN and AHSANULLAH, 1971; CAIRNS et al., 1969; CAIRNS and SPARKS, 1971; CAIRNS and WALLER, 1971; PERKINS, 1972; SMITH et al., 1973; TARZWELL, 1969).

Experimental Animals

Eggs of the brine shrimp Artemia salina (1.) are easily available as they are sold in dried condition as diet to aquarium fish. The ability to hatch is high, even after the eggs have been stored dry for years. This means that between the tests the experimental animals can be stored simply as dried eggs.

Experimental Set-Up

The eggs of Artemia salina is most easily hatching at salinities between 30 to 35 o/oo. As test media use either synthetic seawater or water from the recipient if the salinity of this is sufficient. A method for producing synthetic seawater is given by LaROCHE et al., 1970.

Before use of recipient water this must be purified by filtering over graphite.

The test can be done in small flasks (e.g. 50 ml). Pour for example 25 ml of experimental media into the flasks and add the toxic matter until the different concentrations of your investigation is obtained.

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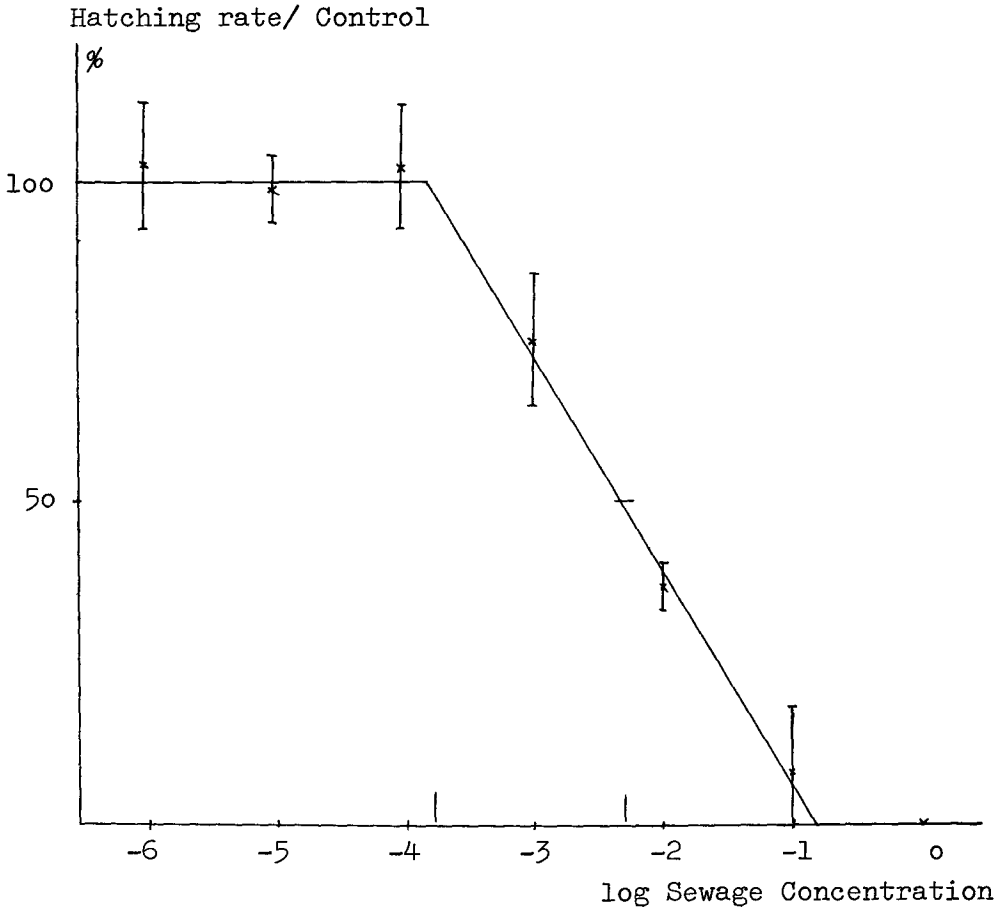

Table 1. Results of an actual test series.

From the results can be calculated the concentration where the hatching rate is half the initial. This C_{MHR} (concentration of the medium hatching rate) is well fitted for comparison of different test results.

After producing the wanted number of test flasks add a small amount of Artemia eggs. For 25 ml is used what we might call a pinch.

If a large quantity of low salinity liquid is added to the test media, salinity must be raised to assure a sufficiently high one in the hatching media. Between 30 and 35 o/oo.

The flasks must be aerated in the test period. Be aware that the aeration must be very slow, otherwise water will evaporate from the flask.

The flasks are stored at 20°C for 5 days. Another experimental period can be used, but as BOD_5 tests are done frequently in connection with sewage control it seems likely to use five days as a standard. The results with this test period are fine. At the end of the experimental period the rate of eggs which have hatched can be counted and compared to the hatching rate in control flasks.

It might be difficult to count the jumping up and down larvae of Artemia. This is easily avoided by shaking the flasks and filtering the suspensions. The filter paper is spread out and placed under a microscope or more easily photographed and enlarged.

Results

The experimental results of the hatching tests show a characteristic graph typical to many toxicity tests. The curve falls in three parts. At low concentrations of the toxic matter there is no noticeable effect on the hatching rate. At a particular concentration the curve suddenly decreases. This point is homologeous to incipient lethal level in experiments with larvae and adult animals. The curve is falling until the hatching rate is zero.

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