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DAPHNIA MAGNA AS A TEST ANIMAL IN ACUTE AND CHRONIC TOXICITY TESTS

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Abstract

Daphnia magna is a commonly used test animal in aquatic toxicology.

Test procedures for acute and chronic tests are described, together with the influence of several variables on their results.

The practicability of the methods was checked with four model compounds, viz. 1,1,2-trichloroethane, dieldrin, pentachlorophenol and 3,4-dichloroaniline. Toxicity data of these compounds for *Daphnia magna* are given.

Introduction

One reason why *Daphnia magna* is a commonly used test animal in aquatic toxicology is that it is easily cultured in the laboratory (ten Berge, 1978). Test animals of any desired age are available throughout the year, and tests of chronic toxicity can be extended to several generations of animals. Daphnids are of ubiquitous occurrence and form an important link in food chains (Gulati, 1978). There has so far been little research into the sensitivity of *D. magna* as compared with that of other invertebrates and fishes to xenobiotics, and that which has been done is not very suitable as a basis for comparison, because the testing times stood in poor relation to the life or generation span of the test animals used. Nevertheless, tests of long as well as short duration seem to show that daphnids are among the more sensitive organisms (Baudouin & Scoppa, 1974; Canton *et al.*, 1975; Jung, 1975; Morgan, 1972; Prawda, 1973; Rawash *et al.*, 1975). Of all species of daphnids, *D. magna* is the largest and easiest to handle.

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For a comparison of the sensitivity of *D. magna* with that of *D. pulex* and *D. cucculata*, as well as for the interreproducibility of tests which D. magna, the reader is referred to Canton and Adema (1978).

The aim of the investigation was to find optimum procedures for acute and chronic toxicity tests with *D. magna* as the test animal. The four model compounds used in the tests were chosen because of their differing physical and chemical properties, which require various modifications of the test procedures.

Principle of the method

A number of animals are transferred to vessels containing a synthetic medium, to which the compound to be tested is added in different concentrations kept constant for the duration of the test. The relationship between the effect of the compound, its concentration, and the exposure time is studied. Conditions such as lack of food or oxygen, unsuitable composition of the medium, and incorrect temperature should be avoided, unless the aim of the experiment is to test the effect of these variables.

Introductory considerations

In chronic tests, the aim of which is to study the effects of a toxic compound on the entire development of an animal, it is desirable to begin the tests with very young animals, a suitable age for daphnids being 16-24 hrs (ten Berge, 1978). In acute tests use can be made of animals of the same age or older, the latter having the advantage of being larger and more easily handled, but having the dis-

advantage that it takes time to culture them and that they may be less sensitive.

In chronic tests, the variables studied are the growth and reproduction of the animals, so that they should be well fed. In tests of acute toxicity, the animals may or may not be fed, either procedure having its advantages and disadvantages.

The composition of the medium and its temperature should allow normal development of the animals. A general rule is that toxicity tests should not be conducted under conditions differing from those under which the animals were cultured in order to avoid exposing them to an additional shock at the beginning of the test. The reason why a synthetic medium is chosen is that its composition is known and that it is known not to contain any contaminants. At least three such media have been described in the literature, all of them being suitable for the culturing of *D. magna* (standard reference water, Freeman, 1953; standard test medium, Frear & Boyd, 1967; and a test medium according to Flückiger & Flück, 1949).

The usual temperature at which toxicity tests are carried out is about 20°C, the only reason probably being that this never differs much from room temperature. It happens to be a suitable temperature for culturing (ten Berge, 1978). Ringelberg (1973) has also shown that a temperature of 20°C is a reasonable temperature for survival and an almost ideal one for reproduction. According to Kersting (1978), however, 20°C is slightly too high for optimum development of *D. magna.*

The number of animals employed in a test should be sufficiently large for the results to be statistically significant. In general, little is known about the number of animals required in toxicity tests. In addition, the number of animals that can be used in a test is restricted by experimental limitations. The usual number varies from 10 to 30 (Alabaster & Abram, 1965; Jensen, 1972; Doudoroff *et al.,* 1951; EPA, 1975; ISO proposed norm). Since reproduction proceeds by parthenogenesis, populations of daphnids cultured under standardized conditions are fairly homogeneous, so that a small number of them is as a rule sufficient. On the other hand, the animals are so small that there usually is no objection to using 20 to 30 of them in each testing vessel.

In tests with volatile compounds the testing vessels cannot be aerated and may even have to be kept closed. In that case the volume of medium must be sufficient to keep the oxygen concentration at an acceptable value.

In chronic tests, inhibition of reproduction is a major ecological criterion in addition to mortality. Although the former is generally held to be the more important criterion, its accurate and reliable measurement is often time-consuming (Canton & Adema, 1978) and may not always be economically feasible. Little is known about possible differences between no-effect levels for the mortality criterion on the one hand and the reproduction criterion on the other.

The considerations set forth above raise the following questions:

1. What is the most suitable duration of acute toxicity tests with mortality as the criterion?
2. Can test animals subjected to acute tests be deprived of food?
3. Do daphnids of about one day old react differently in acute tests from daphnids of about a week old?
4. What is the most suitable duration of chronic toxicity tests with mortality as the criterion?
5. Is 20°C a suitable temperature for acute and chronic tests, and do slight deviations from this temperature significantly change the LC_{50} values?
6. What is the influence of the composition of the medium?
7. What is the minimum number of animals required per test for obtaining reliable LC_{50} values?
8. If aeration is impossible, what is the minimum amount of medium a test animal requires per unit of time for its oxygen supply? What kind of testing vessels would be suitable, and how many times should the medium be replaced? Would continuous-flow systems be preferable?
9. What is the minimum duration of a chronic test if reproduction is to be measured reliably?
10. Is inhibition of reproduction a more sensitive criterion than mortality?

General description of the method

Since the aim of the investigation here described was to find correct testing procedures, an exact description of methods cannot be given in advance. However, if not stated otherwise, the tests were carried out as described below.

The four model compounds in the tests were:

- 1,1,2-trichloroethane (TCE), a non-polar and volatile substance of low molecular weight;
- dieldrin (Dd), a non-polar compound of higher molecular weight, sparingly soluble in water but readily co-

distilling with it and readily absorbed by surfaces, particles, etc.;
- pentachlorophenol (PCP), a polar and acidic compound readily soluble in water in which at pH 8 it is almost totally dissociated;
- 3,4-dichloroaniline (DCAn), a polar and basic compound, readily soluble in water, in which at pH 8 it is almost totally undissociated.

The difference between acute and chronic toxicity tests is that the former are much shorter than the generation time of *D. magna,* whereas the latter extend over at least one generation.

The medium in which the tests were conducted was standard reference water (SRW) (Freeman, 1953) kept at a temperature of 20 ± 1°C during the tests.

The testing vessels were 1-litre glass beakers or conical flasks filled with 1 litre of medium to which the test compound was added with thorough stirring. Food was then added, whenever appropriate, in the form of a suspension of *Chlorella pyrenoidosa.* The algal cells had been grown separately, collected by centrifugation, and resuspended in standard reference water. As a rule 25 daphnids were used in each testing vessel. Twenty-five one-day-old daphnids were fed 10^8 algal cells a day, this supply being gradually raised to 1.0 - 1.5 x 10^9 cells for daphnids of 10 days or older. These amounts are the same as those supplied during culturing of daphnids (ten Berge, 1978) and are the optimum amounts for reproduction (Hueck & Adema, 1968).

Of the daphnids in each testing vessel, the dead ones were removed and counted at fixed intervals. The condition of the surviving animals was assessed, and any new-born young were counted and removed. In tests of long duration, the testing medium is usually replaced at fixed intervals, the fresh medium being charged (except for the blanks) with a new supply of test compound. Such replacements may be necessary to keep the oxygen concentration at a sufficiently high level (e.g., if aeration is impossible), to maintain the constant concentration of test compound or to remove excreta of the test animals. The oxygen concentration and pH were measured at fixed intervals in all testing vessels. Whenever necessary, vessels not containing volatile test compounds were moderately aerated. Those tests in which the animals had suffered of lack of oxygen or other secondary stress factors, were omitted in the final analysis of results. The actual concentrations of the test compounds were measured during all tests by chemical analysis. They never differed appreciably from the dosed concentrations. In general, the results are given as LC_{50} values.

Table 1. Effect of starvation on mortality of *D. magna.*

age	1 d								7 d							
feeding	-				+				-				+			
test duration (d)	1	2	4	7	1	2	4	7	14	21	1	2	4	7	1	2	4	7
average % mortality	0	1	9	42	0	1	1.5	2.5	5	12	0	1	9.5	26	0	2.5	5	11
standard deviation	-	2[5]	14	35	-	2.6	4.0	4.2	4.7	7.6	-	1.9	14	12	-	3[5]	6.3	9.5
lowest and highest values	-	0-8	0-24	12-100	-	0-12	0-16	0-15	0-16	0-24	-	0-4	0-48	8-44	-	0-8	0-20	0-24
number of observations	21	21	17	5	24	24	24	21	16	14	10	10	10	10	10	10	10	10

Trichloroethane was dissolved directly in the standard reference water. Dieldrin was dosed from stock solution in tert-butyl alcohol or acetone, the dilution factor being 1 in 10^4. Pentachlorophenol and dichloroaniline were dosed from stock solutions of the compounds in distilled water made, respectively, slightly basic or acidic, the dilution factor being 1 in 10^3.

The investigations, set-up, results, and discussion

Mortality resulting from lack of food

In order to determine the maximum practicable duration of tests with daphnids deprived of food, we compared the mortality of daphnids which were fed in the normal manner with that of daphnids which were not fed, under otherwise identical conditions (see 'General description of the method'). The experiments were conducted with one-day-old and 7-days-old daphnids. The results, many of which are derived from so-called blank tests conducted during the past few years, are collected in Table 1.

Table 1 shows that 48 hrs is the maximum time during which daphnids can be deprived of food without suffering increased mortality.

Lack of food as a secondary stress factor in toxicity tests

Even if daphnids do not succumb directly to lack of food, this may influence the LC_{50} values. To estimate this effect, we determined the LC_{50} values for the four model compounds in tests lasting 48 hrs with young (one-day-old) and adult (7-days-old) daphnids, one group of which was fed and the other not. These experiments were repeated at least once, each time in duplicate, and their results are collected in Table 2.

Table 2 reveals no clear-cut effect of the presence or absence of food on the LC_{50} values; the paucity of data allows no definite conclusion to be drawn. However, the

Table 2. Effect of feeding on results of acute toxicity tests with *D. magna* aged 1 day and 7 days, respectively.

	Daphnia magna one day old			
test compound	LC50 ($mg.l^{-1}$)			
	24 hrs		48 hrs	
	fed	not fed	fed	not fed
TCE	43	44	43	43
Dieldrin	> 0.2	> 0.2	> 0.2	> 0.2
PCP	1.7	1.2	1.0	0.6
DCAn	0.38	0.50	0.19	0.29

	Daphnia magna 7 days old			
test compound	LC50 ($mg.l^{-}$)			
	24 hrs		48 hrs	
	fed	not fed	fed	not fed
TCE	75	70	43	43
Dieldrin	> 0.2	> 0.2	> 0.2	> 0.2
PCP	2.8	1.3	1.5	0.8
DCAn	16	17	12	13

differences are comparable with the usual variability of those tests (Canton & Adema, 1978).

The presence of food in the medium may have a variety of effects. It may improve the condition of the animals, influence their physiology and so the uptake and metabolism of the toxic compound. The compound may also be absorbed by the food particles and in this way taken up by the animals. In view of all these complicating factors, it is impossible to predict the effect of adding food to a medium in which daphnids are subjected to toxicity tests. Therefore it would seem desirable to conduct acute toxicity tests in the simplest manner possible, i.e. without addition of food. The maximum duration of acute tests will therefore be 48 hrs. In important cases, acute tests are followed up with chronic tests, which in addition enable comparisons to be made between Lc_{50}. 48h values with and without food being added to the medium.

Age of D. magna in acute tests

Table 1 shows that the maximum practicable duration of acute tests is independent of the age of the daphnids. Table 2 shows that adult daphnids (7 days old) generally are either as sensitive as, or slightly less sensitive than one-day-old animals during the first 48 hrs of the test. In addition to being more sensitive, young animals have the advantage that it takes less time to culture them and that they do not reproduce during the tests. Therefore, one-day-old daphnids are recommended for acute tests.

Duration of tests

A suitable duration of chronic tests can be derived from the course of LC_{50} as a function of time. This function is shown for the four model compounds in Fig. 1. It appears from this figure that the shape of the LC_{50} vs time curve strongly depends on the nature of the compound. In the case of TCE, the LC_{50} attains a constant value within 24 hrs. No LC_{50} is found for dieldrin within three weeks and within its limit of solubility in water. Constant LC_{50} values are reached for PCP and DCAn in 10-14 days, indicating that these substances are slow-acting poisons.

From the above it follows that a suitable duration of a chronic test with mortality as the criterion is about 14 days. In addition, Fig. 1 shows that within the maximum duration of acute tests (48 hrs), constant LC_{50} values may not be reached.

Temperature of the medium

In order to assess the effect of temperature on LC_{50} values, we carried out a series of three chronic toxicity tests

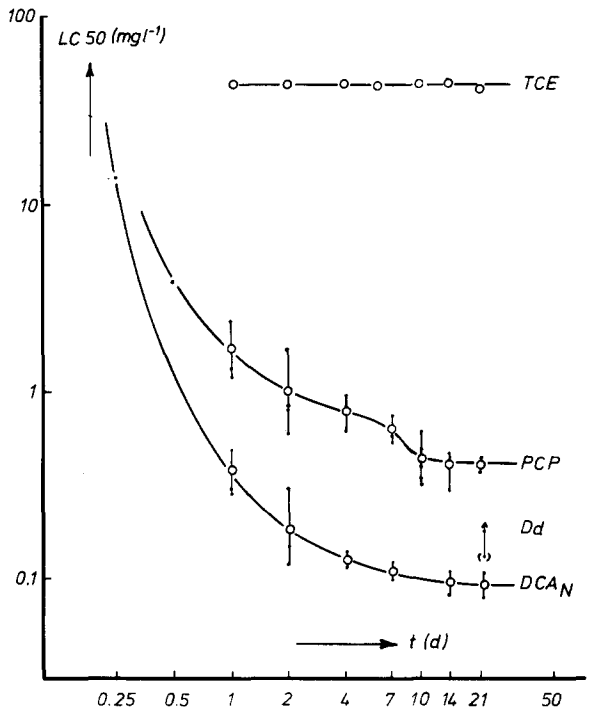

Fig. 1. The LC_{50} for four model compounds as a function of time.

at 15, 20, and 24°C, respectively, with PCP and DCAn as the toxicants. In addition to LC_{50}.21d values, we measured the mortality in blank tests. The results (Table 3) show that the temperature influences the LC_{50}.21d values only slightly. It does appear, however, that the blank mortality at temperatures higher than 20° is large enough to invalidate tests at such temperatures. A slight decrease in temperature to less than 20°C, or slight variations in temperature around 20°C, are not likely to greatly influence the LC_{50} values. However, in tests with growth and reproduction as the criterion, temperature fluctuations should be avoided, because they may change the rates of these processes.

Table 3 Effect of temperature on the LC50.21d[1] of PCP and DCAn for D. magna

test compound	LC50.21d[1] (mg.l^{-1})		
	15 °C	20 °C	24 °C
PCP	0.48 - 0.51	0.40 - 0.47	0.43 - 0.49
DCAn	0.07 - 0.10	0.10 - 0.12	0.10 - 0.10

1) Determined in duplicate; at 15, 20 and 24 °C, the mortalities in the controls were 6, 8 and 20 %, respectively.

Table 4. Effect of composition of medium on the LC_{50}.21d of PCP and DCAn for *D. magna*.

test compound	LC50.21d[1] (mg.l^{-1})		
	100 % SRW	50 % SRW	25 % SRW
PCP	0.40 - 0.47	0.17 - 0.19	(0.08 - 0.06)
DCAn	0.10 - 0.12	0.02 - 0.03	(0.005)

1)Determined in duplicate; in 100, 50 and 25 % SRW, the mortalities in the controls were 8, 6 and 30 %, respectively.

Composition of the medium

The media used for culturing daphnids are of a well-defined composition. Most of them contain Na^+, K^+, Ca^{+2}, Mg^{+2}, Cl^-, SO_4^{-2}, and HCO_3^- in amounts which differ from one medium to another. The testing of pure compounds in media of well-defined composition in which daphnids grow well is more or less a matter of routine, but difficulties may be met in the testing of undiluted or only slightly diluted aqueous solutions, e.g., waste waters which may lack essential elements, so that the conditions needed for optimum growth of the daphnids are upset.

In order to find out whether the composition of the standard reference water used in all tests described in this paper is critical, we conducted several toxicity tests with PCP and DCAn in standard reference water, and in the same medium diluted 1 : 1 and 1 : 3, respectively, with distilled water. The results of these tests are summarized in Table 4.

Table 4 shows that the blank mortality is too high in standard reference water diluted with three volumes of distilled water. The figures also show that the LC_{50} values depend strongly on the composition of the medium. This emphasizes the need for some knowledge of the composition of the material to be tested if this is a dilute aqueous solution (e.g., waste water). Upper and lower limits of hardness, potassium and sodium concentration, etc., which daphnids will tolerate, are not known; all that is known is the composition of those media in which daphnids grow well (see 'Introductory considerations').

Table 5. Effect of number of test animals on the LC_{50}.14d of PCP.

number of daphnids per testing unit	LC50.14d (mg.l^{-1})	lowest and highest values (mg.l^{-1})
10 in 400 ml	0.44	0.34 - 0.58
25 in 1000 ml	0.46	0.37 - 0.54

Number of test animals per testing vessel

In the 'Introductory considerations' a number of arguments for using 25 daphnids in each testing vessel are presented and the results of duplicate tests with this number of animals always agree well (Canton and Adema, 1978). However, the use of fewer animals is sometimes unavoidable. We therefore attempted to establish whether the results of tests with 10 animals differed appreciably from tests with 25 animals. To this end the LC_{50}.14d of daphnids towards PCP was determined in two series of ten experiments, one with 25 daphnids in 1000 ml of medium, and one with 10 daphnids in 400 ml of medium. The results are presented in Table 5.

Although these tests were no more than a pilot experiment, it appears to be quite possible to obtain reliable LC_{50} values with 10 animals per testing vessel.

Amount of medium required for adequate supply of oxygen in the absence of aeration

The minimum amount of medium a test animal requires in the absence of aeration depends on its oxygen consumption per unit of time. The oxygen consumption of daphnids of various ages was measured with a respirometer. At 20°C, 25 daphnids of 1-2 days old consume about 100 μg of oxygen a day. If it is assumed that the oxygen concentration should be in excess of 80%[1] of the saturation level for the entire duration of an acute test (48 hrs), 25 daphnids will need a minimum of 110 ml of medium initially saturated with oxygen. Since some additional oxygen may be consumed in processes other than the respiration of the daphnids, we adhere to the standard practice of providing a minimum of 250 ml of oxygen-saturated medium for 25 daphnids.

The oxygen consumption of 25 adult egg-bearing daphnids is about 850 μg of oxygen a day. The 150 young they produce on average in 24 hrs consume an additional 600 μg of oxygen a day. If one does not wish to replace the medium more often than once every other day in chronic tests, the minimum amount of oxygen-saturated medium required by 25 adult daphnids for their oxygen supply is 2.5 l. If 25 daphnids are to be kept in 1 l of medium in chronic tests this should be replaced every day.

The above calculations are based on the assumption that, apart from the respiration of the daphnids, there are

[1] This concentration has been chosen arbitrarily. In practice it never causes any harmful effects. Even a concentration of 70%, which may occur in the chronic tests here described is still considered acceptable.

no other oxygen sinks, such as food remnants, excreta, biologically degradable or easily oxidizable test compounds, and biologically degradable organic solvents used for dosing the test compound. No such solvents (e.g., acetone) should be used for dosing test compounds into non-aerated testing vessels, which should moreover be thoroughly cleaned during each replacement of the medium. Instead of acetone, tert.butanol may be used, this being poorly degradable. The oxygen concentration in all testing vessels should be measured regularly; in acute tests at least at the beginning and end of the experiment, and in chronic tests at least just before each replacement of the medium.

Testing vessels; replacement schemes and aeration

During the testing of volatile compounds, or of compounds which easily codistil with water (such compounds usually being non-polar) the medium cannot be aerated without loss of test compound. For very volatile compounds it may even be necessary to keep the testing vessels closed. During the testing of polar compounds, it is generally possible to aerate the medium without loss of test compound. However, aeration can be harmful to young daphnids. For this reason aeration is as a rule not started before the 4th day of chronic tests. For the same reason, and also to keep their set-up as simple as possible, acute toxicity tests with one-day-old daphnids are carried out without aeration. These considerations together with the calculations of the preceding paragraph result in the scheme below (Fig. 2), which has proved to give reliable results.

Instead of regularly replacing the medium in chronic tests, one might use continuous-flow systems, In our experience, these give the same results as do adequate replacement schemes, but they have the disadvantage of being rather complex and expensive for the small volumes of medium required for daphnids. Moreover, the gain of time they afford is offset by the intensive biological monitoring that is in any case required.

test compound type	type of tests	number of daphnids per volume	testing vessel	replacement of medium	aeration
non-polar	acute tests	25/ 250 ml or 25/1000 ml[1)]	conical or round-bottomed flask (closed and completely filled)	-	-
	chronic tests	25/1000 ml	conical or round-bottomed flask (closed and completely filled)	once in 24 hrs	-
		25/2500 ml[2)]	ibid	once in 48 hrs (e.g., Mondays, Wednesdays, Fridays)	-
polar	acute tests	25/ 250 ml or 25/1000 ml[1)]	glass beaker or tank	-	-
	chronic tests	25/1000 ml[3)]	glass beaker or tank	Mondays, Wednesdays, Fridays[4)]	+[3)]

1) In acute tests it may for several reasons be better to use 25 daphnids in 1 l of medium instead of 250 ml:
   a) the results of the tests can be compared directly with those of the chronic tests;
   b) the equipment can be standardized (1-litre vessels being by far the most common);
   c) the available amount of oxygen is on the very safe side.

2) This scheme is to be preferred for reproduction tests, because too frequent replacement of the medium may adversely influence reproduction.

3) In tests conducted with aeration a suitable ratio of number of test animals to volume of medium is 25 daphnids to one litre. In this kind of test, aeration is not started before the fourth day, because it is not necessary and may even be harmful to young daphnids.

4) Even if the medium is well-aerated, and the test compound stable and polar, the medium should be replaced regularly in order to remove excreta and to replace any test compound that may have been lost from the medium.

Fig. 2. Set-up of acute and chronic toxicity tests.

Table 6. Mortality and inhibition of reproduction as criteria in chronic toxicity tests.

test compound	no-effect level for mortality ($mg.l^{-1}$)	no-effect level for reproduction ($mg.l^{-1}$)	ratio (mortality:reproduction)
TCE	32	18	1.8
Dieldrin	0.10	0.032	3.2
PCP	0.18	0.32	0.56
DCAn	0.056	0.0056	10

Tests with reproduction as the criterion

If reproduction is to be measured quantitatively in addition to mortality, a test duration of more than two weeks is recommended. From the age of 7 days onwards, and under optimum conditions, daphnids give birth to new young about three times a week. A test duration of two weeks may therefore be too short, for if the last of three broods is delayed until just after this period in some of the testing vessels, the results will be biased. However, tests lasting three weeks give practically the same results (reproduction expressed as a percentage of the blank reproduction) as tests lasting four weeks, and can therefore be considered reliable.

The sensitivity of reproduction as a criterion depends on the nature of the test compound, as follows from Table 6.

In view of the major ecological importance of reproduction, tests of reproduction inhibition are recommended. In carrying out reproduction tests, it appeared that, depending on the quality of the freshly prepared medium, this may have to be filtered over activated charcoal, as is the standard practice in preparing medium for culturing daphnids (ten Berge, 1978). However, the medium so purified should be absolutely free from particles of charcoal, because these may absorb the test compound.

Conclusions

1. If no food is supplied, the duration of toxicity tests with *D. magna* should not exceed 48 hrs.
2. If the test compound is a slow-acting poison, a constant LC_{50} may not be reached before the lapse of 10-14 days, during which the daphnids should be supplied with sufficient food and will therefore grow during the experiment.
3. Adult daphnids may be less sensitive to certain toxicants or react more slowly than young ones.
4. Acute toxicity tests should be carried out with one-day-old daphnids, without food and with a maximum test duration of 48 hrs.
   Chronic toxicity tests with mortality as the criterion should be started with one-day-old daphnids, that are fed normally, and should last at least two weeks.
5. The temperature of the medium should not exceed 20°C. Since the rate of growth and reproduction increases with temperature (within limits), a relatively high temperature is recommended for chronic tests with reproduction as the criterion. If the only criterion is mortality, slight temperature fluctuations do not greatly influence the results.
   A temperature of 18-20°C is quite suitable for acute as well as for chronic tests.
6. The composition and pH of the medium should allow the animals to develop normally. No difficulties are encountered in this respect in the testing of known compounds in standard media. However, if the compound(s) to be tested are in dilute aqueous solution (e.g., waste water), care should be taken to keep the concentration of essential elements and the pH of the medium within suitable limits.
7. In view of their small size, a number of 25 daphnids per testing vessel will seldom give rise to problems of handling, and is moreover sufficient for reliable results to be obtained. If, however, the number of daphnids per testing vessel is limited to 10, the resulting LC_{50} values will generally be approximately correct.
8. A frequent source of error is lack of oxygen in the test medium, particularly in the closed and non-aerated systems needed for the testing of volatile compounds. The set-up of the test (ratio of number of daphnids to volume of medium, aeration or no aeration,

replacement scheme) should be so chosen that lack of oxygen is not likely to occur. The oxygen concentration in all testing vessels must be measured frequently.

Continuous-flow systems offer little economical advantage, because the gain in time is insignificant compared with the time needed for counting the daphnids, etc.

9. The duration of chronic tests in which reproduction is quantitatively measured in addition to growth and mortality should be at least three weeks.
10. In general, reproduction is a sensitive criterion.

Summary

Daphnia magna is a much used test animal in aquatic toxicilogy, providing useful data about the acute as well as chronic toxicity of chemicals.

If toxicity tests are to be reproducible, so that they provide a firm basis for comparison, testing methods should be standardized, and the extent to which variations in test parameters can influence the results should be known. The influence of such variations has been investigated in tests with four model compounds, namely 1,1,2-trichloroethane, dieldrin, pentachlorophenol, and 3,4-dichloroaniline.

Several investigations have been carried out with the aim of finding a suitable duration of acute and chronic tests, of assessing the influence of the age of the test animals and the food given to them, of determining the influence of slight temperature variations and of the composition of the medium, of finding a suitable number of test animals per testing vessel and of finding the optimum ratio of number of test animals to amount of medium taking into account the latter's rate of replacement.

In the chronic tests, the inhibition of reproduction was taken as a test criterion and compared with the mortality.

The present paper ends with ten conclusions regarding the questions raised above. These conclusions have been condensed into standard procedures for acute and chronic toxicity tests, published by the Centraal Laboratorium TNO (1977), and by the Dutch Standardization Institute as proposed norms (NEN 6501 and NEN 6502, 1976).

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