

## Interference of Insoluble Particles with the Reproduction Toxicity Test Using *Daphnia magna*

Hans-Günther Müller

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

According to different international activities in most of the industrialised countries ecotoxicological testing of new chemical substances will have to be done. For this purpose the *Daphnia* reproduction test is of major importance being a sublethal but not extremely time consuming test system. In the OECD-recommendations it is required even in the basic test level.

Unfortunately experience with this test is still very limited. There is especially a need for further investigations in the technical difficulties, the interaction between the main parameters, and the limitations of the test (KÖNIG & MÜLLER 1981; MÜLLER 1981; MÜLLER in press).

Poorly water-soluble substances are one of the problems ecotoxicologists are very often confronted with. This paper deals with the influence of insoluble particles being present in the *Daphnia* reproduction test.

### MATERIALS AND METHODS

Test species was *Daphnia magna* Straus. The reproduction tests were carried out in 2 L glass-beakers, filled with 1.5 L of a reconstituted fresh-water (GODE et al. 1981) and the test substance. In every beaker 30 test animals with a maximum age of 24 h were introduced. We used 4 parallels resulting in 120 animals per substance. The test duration was 21 d with changing of the test water as well as counting and discharging of offspring every other day. The tests were carried out at 20°C. A day to night rhythm of 16:8 h was used. The daphnids were fed green algae and baker's yeast once a day. For all substances and the control we calculated the mortality rate and the reproduction rate per surviving female adult after 21 d. Silicagel and aluminium oxide as test substances were of analytical grade, the dextrane gel Sephadex-G 10 was washed with water prior to use. The cellulose particles were extracted with methylene-chloride, washed with diluted hydrochloric acid and rinsed with water until the effluent became neutral.

## RESULTS AND DISCUSSION

The particle sizes of the test substances measured with an HIAC PC 320 particle counter are given in table 1. These sizes assume spherical shape for all particles.

Table 1

Size ( $\mu\text{m}$ )	Number of particles (%) of the different substances			
	Silicagel	Aluminium oxide	Dextrane	Cellulose
20 - 40	9.4	36.2	9.8	63.4
40 - 60	21.8	19.2	23.2	26.9
60 - 80	33.8	11.9	32.4	6.5
80 - 100	24.2	9.1	26.1	1.8
100 - 150	10.4	12.7	8.3	1.2
150 - 200	0.3	7.1	0.2	0.2
>200	0	3.8	0	0

Results of the reproduction tests are given in table 2. Under the assumption of normal distribution the reproduction rates of tests with substances were compared with those of the control using Student's t-test.

Table 2. Survival and reproduction rates of D. magna with different sorts of insoluble particles (33 mg/L) after 21 d.

Substance	Survival (%)	Reproduction (Young/surviving female)	n	s	Significance 1 - $\alpha$ (%)
Control	92.4	127	4	4.1	-
Silicagel	93.3	118	4	5.0	98.4
Aluminium oxide	90.0	121	4	1.5	98.3
Dextrane	92.6	116	4	5.3	99.2
Cellulose	93.3	75	4	6.0	99.9

Even with a 99 % level of significance for rejecting the zero hypothesis (equality of test and control) dextrane and cellulose in the concentration of 33 mg/L exerts an influence on the reproduction rate of D. magna. This becomes especially evident in the case of cellulose. Whereas for the tests with silicagel and aluminium oxide a direct influence of solved silicium

or aluminium either in water or in the intestinal tract cannot be excluded, a direct toxic mode of dextrane and cellulose seems to be unlikely.

As Gliwicz and Siedlar (1980) could show the carapace width of D. magna is decreased if there is a mechanical stimulus of particles bigger than 50  $\mu\text{m}$ . Although this possibility cannot be excluded in our experiments with dextrane it cannot explain the decreased production of offspring in the test with cellulose. But as this test even showed the most pronounced effect, in this case another mode of action is suggested. As could be shown (MÜLLER, in press) the quality and quantity of the food available exerts the most important influence on the production of parthenogenetic offspring of D. magna. It is rather obvious that the amount of ingested edible particles is lowered if some other particles of the same size but uneatable are also ingested. As the cellulose was the slowest substance to settle down in the test vessels and hence remained longer available for ingesting, it showed a more pronounced effect than dextrane. This could be seen on the colour of the intestinal tract of D. magna being not as dark green in tests with cellulose as in tests with dextrane. As aluminium oxide and silicagel settled down very quickly significant effects on the reproduction rate could not be observed.

On the other hand it is quite clear that the interpretation of lowered reproduction rates in toxicity tests with D. magna should be different, if there is a direct (chemical) toxic mode of action or if there is only a competition between particles and feed. At the moment there exists no test system allowing to differentiate between those two effects on D. magna's reproduction rate. Therefore it can be concluded that the test as it stands now is of only limited value for substances not soluble in water.

#### REFERENCES

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