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Effect of food concentration on the chronic toxicity of sodium dodecyl sulphate to *Daphnia magna*

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Abstract

Food concentration supplied during subchronic and chronic toxicity tests, is one of the most important factors that might influence the response of test organisms to toxicants. The green microalga *Scenedesmus incrassatulus* was used as food for the cladoceran *Daphnia magna*, in a chronic toxicity test with the toxicant sodium dodecyl sulphate (SDS). Test concentrations were 0.625, 1.25, and 2.5 mg l^{-1} of SDS, equivalent to 1/40, 1/20, and 1/10 of the average 48-h LC50 previously determined. Food concentrations were 9.5, 19, and 38 mg l^{-1} (dry wt.). Survival and reproduction were recorded, and the data were analyzed using a life-table approach. After 55 days, the main findings were as follows:

1. Average clutch size decreased as food concentration increased and there was a negative interaction between the toxicant and the algal concentration.
2. The Net Reproductive Rate (R_0) of the controls fed the lowest food concentration was approximately 10 times higher than the controls fed the highest food concentration.
3. R_0's for the treatments with SDS fed the lowest food concentration, were 2.5 to 5 times higher than those fed the highest food concentration.
4. Reproduction in controls and treatments with the same SDS concentrations, became similar as food level increased.

According to these results, the food concentration to be supplied in chronic toxicity tests should be accurate, as an incorrect food concentration might negatively affect the survival and reproduction of test organisms, thus masking or confounding the effects of the toxicant being examined. A concentration around 10 mg l^{-1} (dry wt.) of *S. incrassatulus* seems to be appropriate for *D. magna* economic toxicity tests.

1. Introduction

The cladoceran *Daphnia* was one of the first test organisms to be used in aquatic toxicology, having been used in bioassays for more than 50 years (Baudo, 1987). Major advantages of *D. magna* for toxicological studies include its high sensitivity to a wide variety of toxicants, easy management and culture, short life-cycle, asexual reproduction by parthenogenesis, high fecundity, adequate size of the neonates, and a detailed knowledge of its ecology and life history (Ten Berge, 1978; Adema, 1978; Leeuwangh, 1978; Lewis & Weber, 1985).

D. magna is a standard ecotoxicological test organism, and several standard protocols are available for its use in assessing both acute and chronic toxicity. However, problems have been reported concerning its reliability as a test organism, with survival in control solutions characterized as "poor and usually erratic" (Lewis & Weber, 1985); this may be due to a lack of a properly standardized *Daphnia* culture method (Enserink *et al.*, 1990). Other concerns have been

reported dealing with high inter- and intra-laboratory variability in test results (Baird *et al.*, 1989). Some of these problems may be related to the "unpredictable" behavior and high sensitivity of *Daphnia* to culture and test conditions, such as unfavorable pH values, illumination, temperature, and the density and type of food (Lewis & Weber, 1985). Nevertheless, as Grothe & Kimerle (1985) have pointed out, effluent toxicity tests with *D. magna* can be highly repeatable if clearly defined test procedures are followed.

At present, most of the available toxicological information for this species is related to acute toxicity tests, as they are simple and economical procedures (Peltier & Weber, 1985). Nevertheless, acute toxicity data alone can not predict chronic effects on growth, reproduction, and survival (van Leeuwen *et al.*, 1985; Martinez-Jerónimo *et al.*, 1993). Chronic and subchronic bioassays with *D. magna* are carried out exposing the organism to sub-lethal toxicant concentrations for periods ranging from 7 days (Adams & Heidolph, 1985) to a complete life-time (over 90 days). In these tests, food supply is one of the main factors that need to be controlled in order to avoid undesirable effects on the sensitivity, due to an inadequate nutritional condition of the test organisms.

Enserink *et al.* (1990) observed that the food ration level of *Chlorella pyrenoidosa* affected the body size of the neonates and the sensitivity of the brood of *D. magna*, during acute toxicity tests. Cox *et al.* (1992) reported that both numbers and lengths of *D. magna* neonates were modified by variations in the maternal food ration, arising from variations in stocking density in cultures for neonate production. Guisande & Gliwicz (1992) fed *D. pulicaria* and *D. hyalina* with three different food concentrations of *Scenedesmus acutus*, and observed that a reduction in clutch size and a concomitant increase in egg size, were associated with a decrease in maternal food concentration; they concluded that this effect increased the probability of neonate survival. On the other hand, Martinez-Jerónimo *et al.* (1993) observed that food concentration had a striking effect on survival and reproduction of *D. magna*. They also showed that the use of different microalgae species as food, affected the capacity of *D. magna* for producing neonates as test organisms. With respect to toxicological studies, Winner *et al.* (1977) observed that food type modified the sensitivity to copper of *D. magna*, with animals fed algae being less sensitive than those fed an artificial diet.

The objective of this study was to examine the effect of food concentration on the chronic toxicity of sodium dodecyl sulphate (SDS) to *D. magna*. It should be noted that the toxicity assessment of effluents and aquatic ecosystems that receive contaminated discharges, is a matter that until this moment is beginning to be considered in Mexican laws. At his respect, the "Test procedure for the *Daphnia magna* acute toxicity determination" will be shortly published as a Mexican Official Norm (NOM), and this will be the first standard procedure available in our country for the assessment and control of aquatic pollution.

2. Materials and methods

Test organisms were obtained from a *Daphnia magna* culture that has been successfully maintained in our laboratory for five years. The toxicant tested was sodium dodecyl sulphate (SDS) (Merck®, purity > 99%). Acute toxicity (48 hours) was determined according to Peltier & Weber (1985). Two tests were conducted in 150 ml glass Pyrex® flasks, with 100 ml of volume test; ten *D. magna* neonates (less than 24 h old) were randomly distributed in each of three replicates per concentration. The effect level (LC50) was calculated using the Probit method.

Chronic bioassays were carried out using the batch-cultured green microalgae *Scenedesmus incrassatulus* as food. These were grown under aseptic conditions in Bold Basal medium, and harvested near the end of the exponential growth phase. The algal biomass was almost completely separated from the culture medium by sedimentation in darkness; it was then stored in a refrigerator for at most seven days, after which all the remainders were discarded. The experiment consisted of combinations of three food levels with three SDS concentrations. In addition, there was a control series with each of the three algal concentrations, without the toxicant, for a total of 12 treatments. SDS chronic concentrations were equivalent to 1/40, 1/20, and 1/10 of the average 48-h LC50. Food concentrations were 9.5, 19 and 38 mg l^{-1} (dry wt.), equivalent to 1, 2, and 4 E+6 cell m^{-1}. These were based on previous results, where an optimal concentration of about 1.3 E+06 cell ml^{-1}, dry wt.) was found. Higher food concentrations were included in order to determine if food availability induced changes in the reproductive strategy of *D. magna*, as was observed by Enserink *et al.* (1990, 1993), in experiments where they applied similar food concentrations of the microalga *Chlorella pyrenoidosa* (up to 1.12 E+06 cell ml^{-1}).

Chronic toxicity tests were conducted using 1000 ml of test volume in 1250 ml plexiglas containers, with reconstituted hard-water (160–180 mg l^{-1} as $CaCO_3$) as control and dilution water (Peltier & Weber, 1985); each treatment had four replicates each containing ten *D. magna* neonates (less than 24 h old). The test volume was completely renewed once a week and food concentrations were replenished as necessary, in order to maintain approximately the original concentrations. Survival and reproduction were recorded daily for 55 days. Neonates were counted and separated from the adults daily, and the data analyzed using a life-table approach. Temperature was kept at 20 ± 1 °C, and the photoperiod was 16:8 h (cool-white fluorescent light). Dissolved oxygen and pH were recorded daily.

3. Results

The 48-h LC50s for the two acute toxicity tests were 25.39 ± 2.26 mg l^{-1} and 24.25 ± 2.45 mg l^{-1} (α = 0.05). The average LC50 for SDS was considered to be 25 mg l^{-1}. The sublethal concentrations applied in the chronic test were 0.625, 1.25, and 2.5 mg l^{-1}.

For the chronic bioassays, the cumulative total offspring per replicate was calculated, and treatment values averaged (Fig. 1). Figure 1 shows the effect of food concentration on the toxic response of *D. magna*. Cumulative total offspring follows a sigmoidal trend, reaching a plateau between days 21 and 55. A two-way ANOVA indicated that food concentration had a significant effect on offspring ($p = 0.0497$), whereas SDS concentration had no significant effect ($p = 0.4605$); interaction between these factors was not significant ($p = 0.1735$). In most treatments, reproduction ceased by the 21st to the 25th day, except for the control fed 1 E+06 cell ml^{-1}. Figure 1 also shows that an increase in food concentration produced a significant reduction in total offspring, regardless of the toxicant concentration.

As most of current methodologies for conducting chronic toxicity tests with *D. magna* require a 21-day exposure time (Adams & Heidolph, 1984), the 21-day percent survival data for controls and treatments were averaged and are shown in Fig. 2. These data were analyzed through a two-way ANOVA. As can be seen in Table 1, both food concentration and toxic concentrations had a highly significant effect on survival; interaction between these factors was also significant.

Survival and fecundity data, recorded at fixed three-day age classes, were analyzed through a life-table and fertility-table approach, following the procedure by Krebs (1985). The Reproductive Value (V_x, defined as the contribution to the future population that an individual female of age x will make), and the Net Reproductive Rate (R_0, defined as the multiplication rate per generation, is obtained by multiplying together the female proportion surviving at age x and the female offspring per adult female of age x, and summing over all age groups), for the controls and treatments with SDS were calculated. Figure 3 shows V_x values ordered according to the *S. incrassatulus* concentration. A two-way ANOVA indicated that the algal concentration produced a significant effect on V_x ($p = 0.01194$), whereas SDS concentration had no significant effect on this parameter ($p = 0.43305$); interaction food concentration × SDS concentration was not significant either ($p = 0.2183$). Figure 4 shows the estimated R_0 values.

Recorded pH during experiments ranged from 8.2 to 9.1. Average dissolved oxygen varied from 4.2 to 5.2 mg l^{-1} in all the treatments.

4. Discussion

The highest total offspring was obtained in the control fed with the lowest algal concentration. For all the SDS treatments, the highest fecundity values were also obtained with the lowest food concentration, whereas the lowest total offspring values were always associated with the highest algal concentration. SDS treatments showed a smaller difference between the highest and the lowest fecundity than the controls; although there was no significant difference in fecundity with respect to the toxicant concentration, this result emphasizes the effect produced by the SDS in the lowest food concentration (1 E+06 cell ml^{-1}).

For most treatments, reproduction stopped by the 21st day, except for the animals fed 1 E+06 cell ml^{-1}, which continued reproducing through day 55. According to these results, food concentration is a factor that can negatively influence survival and reproduction in chronic tests, as is clearly shown in treatments fed on 2 and 4 E+06 cell ml^{-1}. Under these conditions, SDS effects might be masked or even overridden by the effects of food dose. As can be seen in Fig. 1, as algal concentration increased reproduction decreased. In the treatments with the two highest food levels, no SDS effect could be detected, and reproduction for both control and SDS treatments were quite similar.

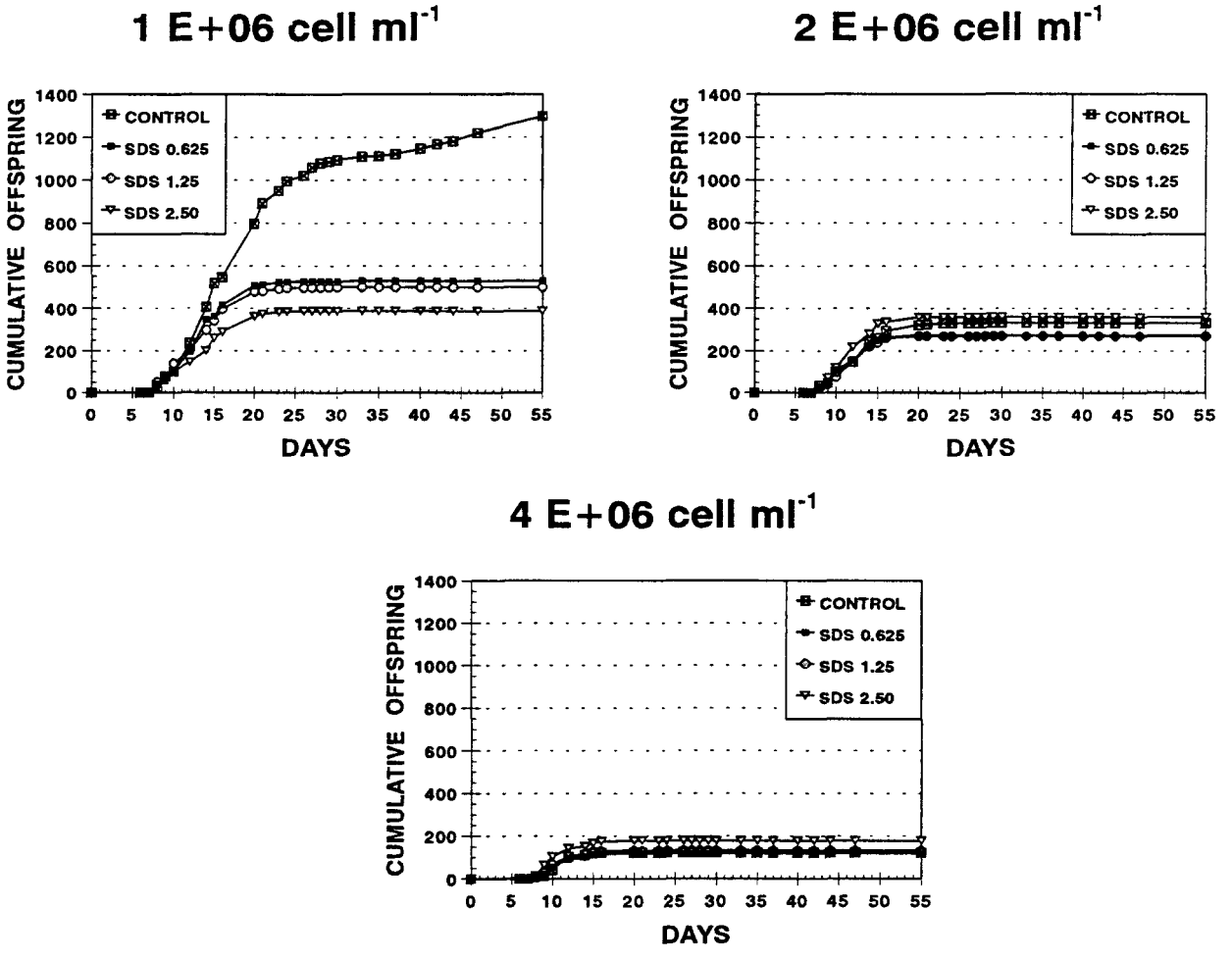

Fig. 1. Cumulative offspring for *Daphnia magna* fed with three concentrations of *Scenedesmus incrassatulus*, during a chronic bioassay with three concentrations of sodium dodecyl sulphate (SDS).

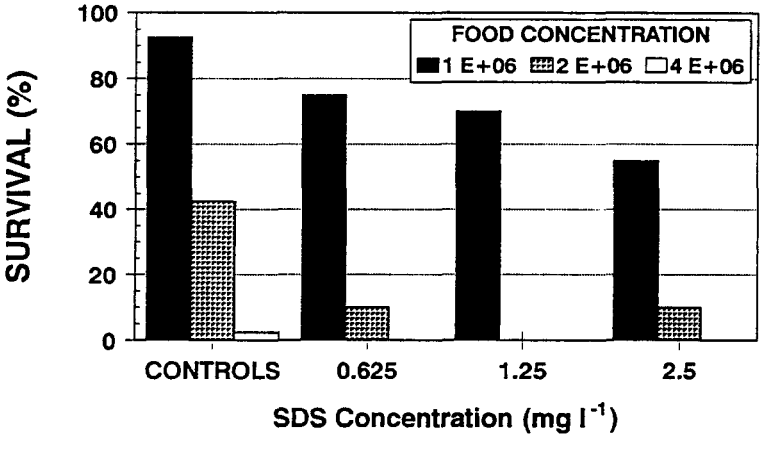

Fig. 2. Twenty one-day percentage survival of *Daphnia magna* fed with three concentrations of *Scenedesmus incrassatulus*, during a chronic bioassay with three concentrations of sodium dodecyl sulphate (SDS).

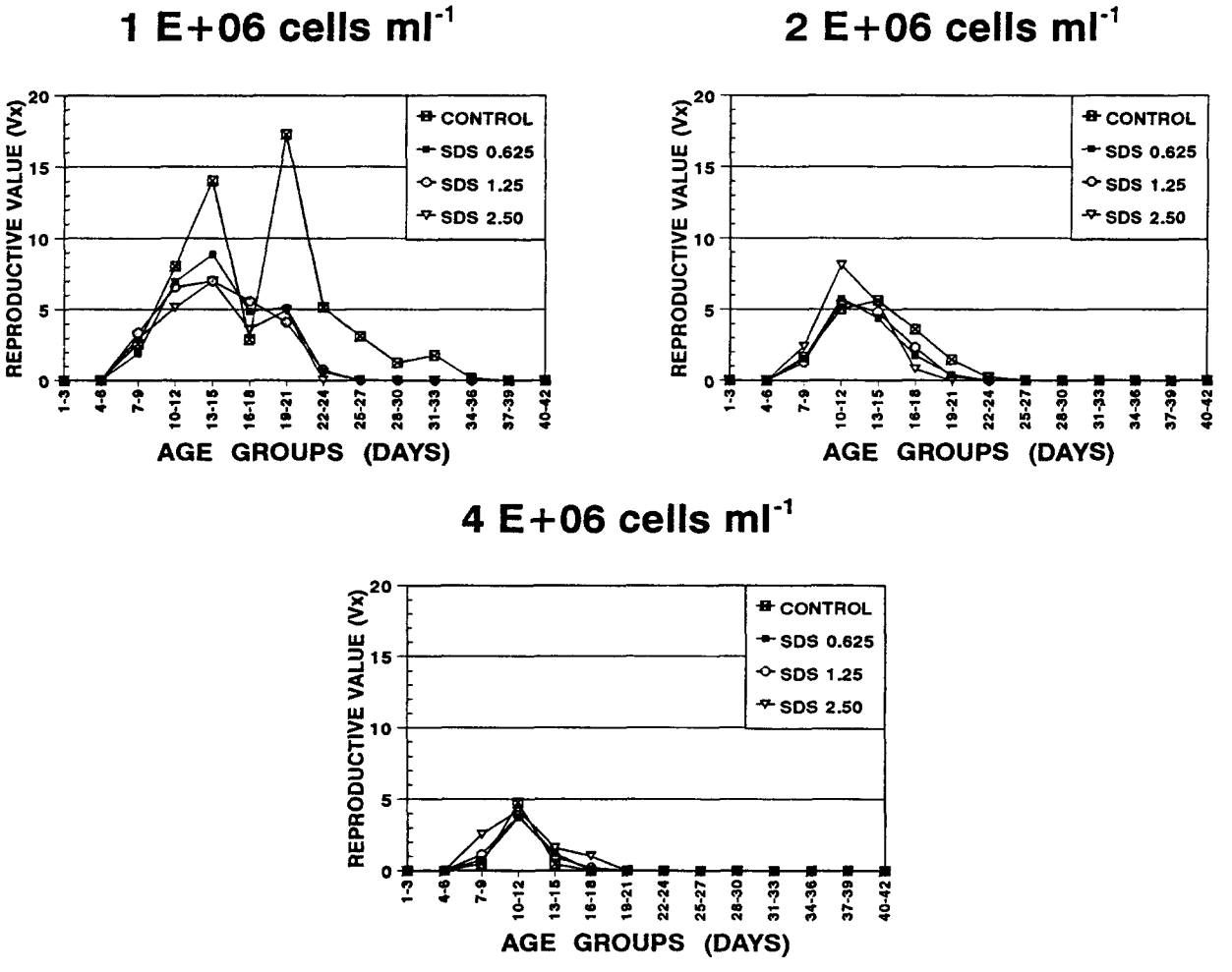

Fig. 3. Reproductive Values (V_x) for *Daphnia magna* fed with three concentrations of *Scenedesmus incrassatulus*, during a chronic bioassay with three concentrations of sodium dodecyl sulphate (SDS).

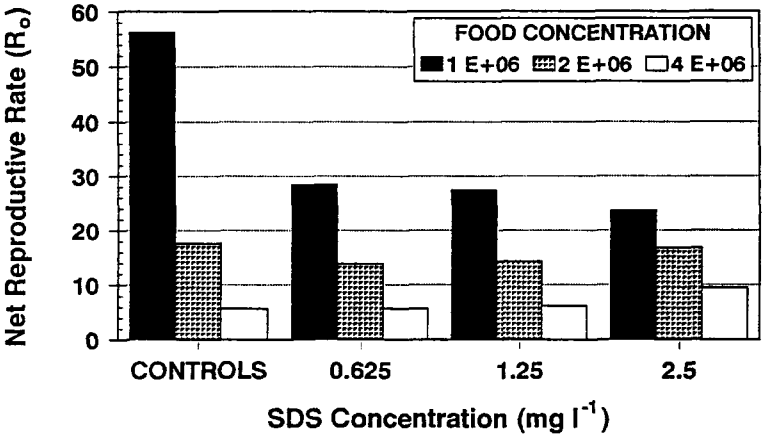

Fig. 4. Net Reproductive Rate (R_0) for *Daphnia magna* fed with three concentrations of *Scenedesmus incrassatulus*, during a chronic bioassay with three concentrations of sodium dodecyl sulphate (SDS).

Table 1. Two-way ANOVA results for the 21-day survival of *Daphnia magna* fed on three concentrations of *Scenedesmus incrassatulus*, in a chronic test with sodium dodecyl sulphate (SDS)

Source of variation	SS	df	MS	F	P-value	F critical
Food concentration	468.667	2	234.333	519.139	2.84E-27	3.259
SDS concentration	44.062	3	14.687	32.538	2.36E-10	2.866
Interaction	26	6	4.333	9.6	2.61E-06	2.364
Within	16.25	36	0.451			
Total	554.980	47				

Results on survival data at the 21st day (Fig. 2) clearly show the deleterious effect of both applied treatments on this parameter. The statistical significance determined for the interaction between food concentration and SDS concentration revealed the importance of food factor during chronic toxicity tests, as this influenced and modified the toxic response. As can be seen in Fig. 2, 21-day survival for controls were clearly higher than those of the treatments of the corresponding food concentration. In the same way, survival in controls was negatively influenced by food concentration, and at the 21st day, the control fed 4 E+06 cells ml^{-1} had a survival of 2.5%, whereas all the SDS treatments with the same food concentration had no survivors at this time.

Reproductive Values (V_x's) followed a trend similar to a normal distribution curve, with a peak by the middle of the observation time; the highest V_x value was obtained in the control fed on the lowest food concentration (Fig. 3).

The highest Net Reproductive Rates (R_0) were obtained in the control and treatments fed the lowest algal concentration (Fig. 4). In the highest food concentration, R_0 for the control was lower than those of all the SDS treatments, which suggests that the toxic effects of the SDS were masked by the negative effect of the high food concentration on the performance of test organisms. Analyzing the results as a whole, it can be concluded that survival at the 21 day was the best criterion for assess the toxicity of SDS and for discriminate collateral effects due to food supply during the chronic tests carried out.

Although the two highest food concentration applied in our experiments could be considered as excessively high, Enserink *et al.* (1990, 1993) determined that in the range of 1.4 E+05 to 1.12 E+06 cell ml^{-1} of *Ch. pyrenoidosa*, low food levels were associated with small broods of large neonates, whereas an increase in food ration produces large broods of smaller neonates; similar results were observed with *D. hyalina* and *D. pulicaria* fed on *S. acutus* (Guisande & Gliwicz, 1992). *Ch. pyrenoidosa* and *S. incrassatulus* are different in size, which may account for the different conclusions.

No significant toxic effects of SDS on total offspring neither on Reproductive Value (V_x) were detected in this study. Nevertheless, 21-day survival showed a negative significant effect of SDS concentration, and indicate a significant interaction between the two factors applied (Table 1). Apparently exposure times longer than 21 days mask toxic effects of SDS, and can lead to different conclusions. It was clear that in the test organisms fed with the lowest food concentration, SDS intoxication could be more apparent, but this effect was only revealed by the analysis of 21-day survival data through a two-way ANOVA. Even though the food supply during acute tests may reduce the toxicity of chemicals (Adams & Heidolph, 1985), an excessively high food concentration may also reduce survival and reproduction. This effect may be due to improper food usage and consumption by *D. magna*, leading to a mechanical occlusion of the filtering structures or adverse effects on respiration (Kersting & Leeuw-Leegwater, 1976). With high food concentrations, *D. magna* shows a constant ingestion rate, coupled to an over-collection of particles and a high elimination of unassimilated food and, as a consequence, respiration rate increases, and food assimilation rate is low (Porter *et al.*, 1982). According to these results, and considering our own previous experience in culturing *D. magna*, the lowest *S. incrassatulus* concentration applied in this study is an appropriate food ration for chronic bioassays.

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