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Evaluation of *Daphnia similis* as a Test Species in Ecotoxicological Assays

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The genus *Daphnia* comprises microcrustaceans extensively used in tests to evaluate acute and chronic toxicity of chemical agents and effluents, because of its great sensitivity to a large number of aquatic contaminants, its importance in freshwater food chains, its amenability for laboratory culturing and to its parthenogenetic reproduction, which allow the attainment of test organisms with constant sensitivity.

Some standard procedures utilizing this genus as test organisms for ecotoxicological assays recommend temperate species such as *Daphnia magna* and *Daphnia pulex*. The first, is indicated for waters with hardness equal or greater than 80 mg/L (expressed as $CaCO_3$) and the second, even though adapted to any hardness, is recommended when hardness is less than 80 mg/L. The utilization of other species is possible, however, its sensitivity must be greater than, or equal to, that of the indicated species (Environment Canada 1990; US EPA 2002).

At the Aquatic Ecotoxicology Laboratory of CETESB (the Environmental Agency of São Paulo State, Brazil), another daphnid species was introduced as a test organism. It was initially obtained in aquarium stores and later isolated from a sewage stabilization pond in Valinhos city (São Paulo state). Identified as *Daphnia similis* in 1982 by Dr. I. Hrbàcék, this organism is closely related to *Daphnia magna*, belonging to the same subgroup of the subgenus Ctenodaphnia (Hebert and Finston 1993).

In order to validate the use of *Daphnia similis*, already recommended as a test organism in ecotoxicological assays by standard procedures in Brazil (ABNT 1993), this paper presents the results of a comparative study using data obtained in acute toxicity tests performed with this species and *Daphnia magna.*

MATERIALS AND METHODS

Data obtained in acute toxicity tests with industrial effluents and analytical grade chemicals, run concurrently and under the same experimental conditions (dilution water, temperature, etc) with *Daphnia magna* and *Daphnia similis*, between 1979

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and 1981 (CETESB 1979; 1980; 1981), were compiled and submitted to a statistical test proposed by USEPA (1985).This statistical test permits the comparison of results for two distinct assays (expressed as 24hr EC50) and corresponds to the following calculation:

$$G = \sqrt{(\log(UL_{(1)} \div EC50_{(1)}))^2 + (\log(UL_{(2)} \div EC50_{(2)}))^2}$$

where:

$UL_{(1)}$ = Upper confidence interval for test 1
$UL_{(2)}$ = Upper confidence interval for test 2
$EC50_{(1)}$ = Effective concentration for test 1
$EC50_{(2)}$ = Effective concentration for test 2

Then, calculate:

$H = 10^G$
Z = higher EC50 ÷lower EC50
If Z > H: there is a significant difference between EC50 values.

In order to evaluate and correlate the sensitivity of both species, a regression analysis was also conducted with the logarithm of 24hr EC50 values of the different tests from Table 1, using EXCEL 1997.

RESULTS AND DISCUSSION

Based on the results of acute toxicity tests with chemical compounds and industrial effluents (Table 1), the two species exhibited similar sensitivity to most of the evaluated samples. The exceptions were ammonium chloride, herbicide (1) and effluent from galvanoplasty cyanide tank (to which *D. magna* was, respectively, 1.17, 1.2 and 1.3 times less sensitive) and effluent from galvanoplasty chromium tank, to which *D. similis* was 1.2 times less sensitive.

From an ecotoxicological point of view, even though considered statistically significant, such differences are negligible, i.e., the ratios between EC50's are less than two. According to Chapman (2000), the attainment of a factor of two is considered normal and acceptable for toxicity test variability. Even considering the same species, distinct results may be reported due to differences in individual sensitivity or between batches of organisms, due to genetic and/or phenotipic variances (Soares et al 1992).

Good correlation was demonstrated between the EC50 data for both species (Figure 1) and the results of the generated regression line are comparable with those obtained by other authors for the recommended species (Table 2). In the present study, it was also verified that the slope is 1, which means that an increase in one variable is accompanied by an equal increase in the other variable. Moreover, the intercept is very close to 0, indicating that both variables are similar. Such factors, according to Doherty (1983), point to an equivalence between the species.

Table 1: Results of acute toxicity tests and respective statistical analysis with chemical compounds and industrial effluents.

Compound	24hr EC50 (mg/L)		Statistical analyses (USEPA 1985)		
	D. similis	*D. magna*	H	Z	Conclusion
Potassium dichromate	0.239 (0.207-0,274)[a]	0.195 (0.147 – 0.238)	1.27	1.23	b
Mercury chloride	0.02 (0.02 – 0.03)	0.03 (0.03 – 0.04)	1.64	1.50	b
Copper sulfate pentahydrate	0.21 (0.18 – 0.24)	0.19 (0.17 – 0.21)	1.18	1.11	b
Lead acetate	237.69 (213.11-265.16)	209.26 (187.42 – 233.64)	1.17	1.14	b
Phenol	52.13 (42.93 – 63.29)	56.63 (47.48 – 67.55)	1.30	1.09	b
Ammonium chloride	234.79 (211.78-260.31)	275.54 (250.88 – 302.61)	1.15	1.17	c
Detergent (1)[d]	192.50 (162.10-222.88)	227.95 (172.23 – 289.73)	1.32	1.18	b
Detergent (2) [d]	114.42 (96.90- 138.20)	111.76 (93.67 – 133.09)	1.29	1.02	b
Herbicide (1) [d]	760.00 (681.00-874,00)	910.00 (812.08 – 1006.08)	1.19	1.20	c
Herbicide (2) [d]	56.00 (50.00 – 63.00)	50.00 (45.00 – 56.00)	1.18	1.12	b
Tannery effluent (1)	117.85 (79.20-175.36)	143.55 (127.63 – 161.46)	1.51	1.23	b
Tannery effluent (2)	217.75 (196.58-241,21)	205.22 (179.25 – 234.94)	1.19	1.06	b
Pulp and paper effluent	637.07 (451.43-899.05)	660.49 (532.73 – 818.87)	1.50	1.04	b
Galvanoplasty effluent (cyanide tank)	2.02 (1.79 – 2.29)	2.70 (2.15 – 3.40)	1.30	1.34	c
Galvanoplasty effluent (chromium tank)	5.55 (4.82 – 6.39)	4.58 (4.18 – 5.01)	1.18	1.21	c
Galvanoplasty effluent (cyanide + chromium tank)	155.15 (142.96-168.39)	141.37 (123.53 – 161.78)	1.17	1.10	b

(a) Confidence interval
(b) No significant difference
(c) Significant difference
(d) Commercial formulation

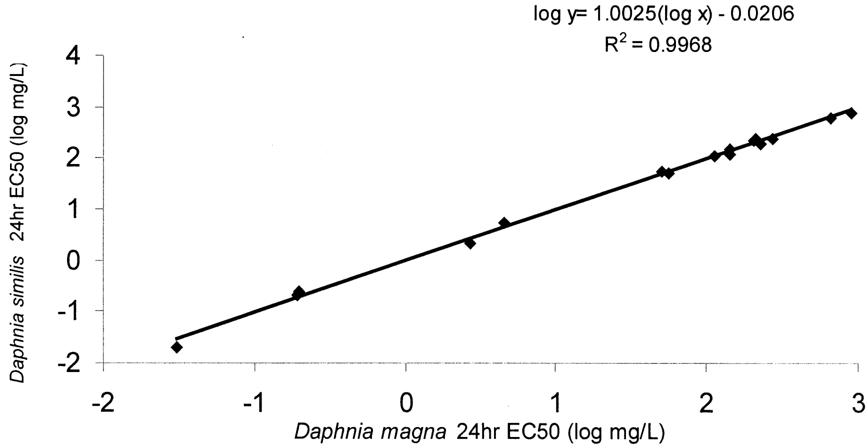

Figure 1. Correlation between *D. magna* and *D. similis* 24hr EC50 values.

Table 2. Results of regression analyses for studies with species of *Daphnia*.

Test species	r^2	Slope	Reference
D. magna/D. similis	0.99	1.00	This study
D. magna/D. pulex	0.83	0.89	Elnabarawy et al (1986)
D. magna/D. pulex	0.95	0.91	Canton and Adema (1978)
D. magna/D. pulex	0.97	1.02	Lilius et al (1995)

Canton and Adema (1978) evaluating the sensitivity of the indicated species to fifteen chemicals, found that *D. magna* was about six times less sensitive to aniline than *D. pulex.* Conversely, the latter was less sensitive to pentachlorophenol and cadmium nitrate (six and three times, respectively). On the other hand, Lewis and Weber (1985), observed that *D. magna* and *D. pulex* were equally sensitive to sodium dodecyl sulphate and pentachlorophenol, but *D. magna* was three times more sensitive to anhydrous cadmium chloride. In the study of Lilius et al (1995), that evaluated twenty five chemicals, *D. magna* was four times less sensitive to aspirin and nicotine. Conversely, *D. pulex* was three, five and seven times less sensitive to ferrous sulfate, diazepan and mercury chloride, respectively.

The results obtained in this study show that *Daphnia similis* satisfy the criteria established by standard procedures ((Environment Canada 1990; USEPA 2002) for selection of alternative species. Furthermore, although not occurring naturally in Brazil, *D. similis* is one of the six species registered in other tropical regions, mainly in Asia (Fernando et al 1987). It is easily cultured under laboratory conditions and better adapted to low hardness waters, a characteristic of São Paulo state waters (Aragão et al. 2003).

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