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Acute and Chronic Toxicity of Dimethylsulfoxide to *Daphnia magna*

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The organic solvents acetone, ethanol, methanol, dimethylformamide, and triethylene glycol are commonly used to facilitate the solubilization or introduction of lipophilic compounds into the media during aquatic toxicity tests. Standardised procedures in aquatic toxicity testing generally recommend that solvents should not be used in excess of 500 μL/L during acute short-term toxicity tests (USEPA 1975) and 100 μL/L during longer-term toxicity tests (OECD 1998). Acute and chronic toxicity data exists for acetone, dimethylformamide and triethylene glycol, for both fish and *Daphnia magna* (LeBlanc and Surprenant 1983).

Dimethylsulfoxide (DMSO) is a dipolar aprotic compound that has a hydrophilic sulfoxide group, hydrophobic methyl groups and is a highly hygroscopic solvent. The dipolar nucleophilic character of the molecule is due to the available free electron pairs at the S and O terminals. This is the reason why DMSO is readily soluble in both polar and non-polar solvents, and can thus solubilize a wide range of compounds (Kligman 1965). This property is largely responsible for the ability of DMSO to cross biological membranes (Jacob et al. 1964). Since its introduction to the market, DMSO has been mildly used as solvent, and also in medicine, for human and veterinary therapeutics due to its relatively low toxicity and to the absence of carcinogenic effects. Thus, animals can tolerate it at rather high doses (Ali 2001).

The purpose of this study was to determine the acute and chronic toxicity of DMSO to the aquatic invertebrate *Daphnia magna* and to investigate the possibility of using this solvent as a carrier for hydrophobic drugs.

Daphnia magna was selected as the test organism because it is commonly used in acute and chronic toxicity tests and due to the relative dearth of toxicity data on organic solvents to this aquatic invertebrate (Soares 1989). The toxicity of DMSO was also evaluated using the Microtox®assay.

MATERIALS AND METHODS

Dimethylsulfoxide (DMSO) Merck® was of analytical grade (>99% purity).

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The medium was ASTM hard water (ASTM 1994) enriched with the organic additive Marinure '25' (Pann Britannica Industries Ltd. Waltham Abbey, U.K.), an extract from the algae *Ascophyllum nodosum* (Baird et al. 1989). ASTM hard water has a total hardness of 160-180 mg/L $CaCO_3$, a pH range of 7.5-8.0 and a conductivity of 580 μScm^{-1} (ASTM 1994).

Daphnids were obtained from laboratory stock cultures maintained in our laboratory for ca. 10 years, fed with *Selenastrum capricornutum* ($3x10^5$ cells/mL /daphnia), and kept at 20 ± 1°C with a 16:8-hr light:dark period. All experiments were initiated with 3rd to 5th brood neonates (≤ 24-hr old) from a single clone derived from a healthy parent stock. Test conditions were similar to culture conditions. During the experiments the temperature, dissolved oxygen, pH, total hardness and conductivity were monitored weekly. Endpoints included: adult survival; first day of reproduction; number of moults; total number of live and dead neonates produced per female; total number of broods, and growth measured as body length. One-way analysis of variance (ANOVA) was used to compare survival and reproduction between exposure groups, at a significance level of $p<0.05$.

DMSO test concentrations of 16.5, 19.8, 23.7, 28.6 and 34.1 g/L plus a control were used. Two hundred mL of each test solution and control were divided into four beakers to provide replicate exposure treatments. For each test concentration and control, 20 neonates were introduced into 175 mL beakers containing 50 mL of test medium. During the tests, daphnids were not fed and the medium was not renewed. Mortality (toxicological endpoint: immobilisation) and behavioural abnormalities were recorded at 0, 24 and 48 hours. The 48-hr LC_{50}, with the 95% confidence interval, was calculated by probit analysis (Finney 1971).

Daphnids used in this test were transferred to newly prepared solutions every other day and fed with the algae *Selenastrum capricornutum*. Ten animals were used per treatment and control. Oxygen concentrations and pH levels in test vessels were measured weekly. Other experimental conditions were similar to those described for the stock cultures. DMSO concentrations tested were 6.8×10^{-3}, 1.4×10^{-2}, 2.8×10^{-2}, 5.5×10^{-2} and 1.1×10^{-2} g/L. At the start of the test, neonates were randomly assigned to the test vessels and placed individually in 100 mL of test medium. Adult survival and offspring production was checked daily during 21 days of exposure. When present, newborn neonates were counted and discarded immediately. Growth was evaluated at the time of the first moult, at the first reproductive event and at the end of the test, by measuring the length of the first exopodite of the second antennae (Soares 1989), a character that can be easily checked at the moult.

Microtox® test was performed according to the Microbiotics Corporation detailed protocol for the basic test and was conducted in a Microtox Model 500 Analyser (Microbics Corporation 1992).

RESULTS AND DISCUSSION

The results of the acute toxicity test for DMSO shows a 48-hr LC_{50} value of 24.6 ± 19.1-31.7 g/L (95% C.I.). Control survival was always 100%.

For the Microtox® assay the following EC_{50} values were obtained: 66.1 ± 58.2 - 87.4 (95% C.I.), 71.8 ± 58.6-88.2 (95% C.I.) and 71.4 ± 52.2-83.9 g/L (95% C.I.) at 5, 15 and 30 min, respectively. This data when compared with the data reported in the literature (see table 1 and table 2) clearly indicated that DMSO has a lower toxicity than most of the other solvents listed, with the potential exception of methanol (EC_{50} = 90.2 g/L, but with a SD of ± 110.8).

Table 1. Acute toxicity of solvents tested with *Daphnia magna.*

Solvents	Average 48-hr LC_{50} or 48-hr EC_{50} values (g/L)	Reported 48-hr LC_{50} or 48-hr EC_{50} values (g/L)		
Acetone	-	6.1[b]	30.7[c]	9.2[d]
Ethanol	10.5[a]	5.4[b]	-	9.2[d]
Methanol	10.0[a]	13.2[b]	-	-
Phenol	3.6×10^{-2} [a]	1.0×10^{-2} [b]	-	1.3×10^{-2} [d]
2-propanol	7.8[a]	13.3[b]	-	-
Dimethylsulfoxide	-	24.6[e]	-	-

[a]Genoni 1997, [b]Vaishnav and Korthals 1990, [c]LeBlanc and Surprenant 1983, [d]Cowgill and Milazo 1991, [e]Data reported in this work

The exposure of *Daphnia magna* to sub-lethal concentrations of DMSO had no effect on reproduction, at either the first brood release or after 21 days (F = 1.19; d.f. = 5.54; p = 0.32). Growth was also unaffected (F = 0.05; d.f = 1.53; p = 0.83), thus, the NOEC value for DMSO is lower than 6.8×10^{-3} g/L.

In all test vessels oxygen concentration was always higher than 6.5 mg/L and the pH variation between medium renewals was always less than 1 pH unit.

The results of this study indicate that DMSO is not toxic to *Daphnia magna* in the range of concentrations tested. In fact, it is possible to use more concentrated solutions of DMSO for chronic tests than when using other solvents. Thus, DMSO may be the first choice when selecting a carrier solvent for tests with *Daphnia magna.* According to the data of this study the recommended usage limits adequate for the prevention of the solvent toxicity to *Daphnia magna* is between 6.8×10^{-3} and 1.1×10^{-2} g/L.

Table 2. Toxicity of solvents assessed by the Microtox® test.

	EC_{50} (g/L) obtained from literature expressed by mean ± SD and min-max values [a]		
	5 min	15 min	30 min
Acetone	16.9 ± 3.6 (n=8) 12.4-22.1	17.6 ± 5.3 (n=8) 12.7-29.1	14.9 ± 2.0 (n=3) 13.3-17.1
Acetonitrile	18.2-24.2	18.2	24.2
Chloroform	$6.7 \times 10^{-1} \pm 3.3 \times 10^{-1}$ (n=3) 4.3×10^{-1}-1.0	$1.5 \pm 9.3 \times 10^{-1}$ (n=3) 6.7×10^{-1} -2.5	6.7×10^{-1}
Dichloromethane	- 3.6	2.7 ± 1.3 (n=4) 1.0-4.1	- -
Dimethylformamide	9.2-20.1	9.1	-
Dimethylsulfoxide	9.1 ± 10.7 (n=3) 82.5-103.0 66.1 [b]	81.8 ± 17.5 (n=3) 63.5-98.4 71.8 [b]	- 98.4 71.4 [b]
Ethanol	41.7 ± 8.9 (n=6) 31.2-55.4	30.4 ± 6.5(n=3) 23.1-35.4	-
Ethyleneglycol	6.5×10^{-1} - 58.7	6.5×10^{-1} - 55.6	6.2×10^{-1}
Methanol	85.1 ± 44.4 (n=6) 54.4-156.9	90.2 ± 110.8 (n=5) 11.4-285.6	- 50.8-320.4
2-propanol	33.8 ± 8.7 (n=3) 24.3-41.6	22.4	-

[a]Values reported by Kaiser and Palabrica 1991, Layton et al. 1999, Cassells et al. 2000, [b]Data reported in this work

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