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# Acute Toxicity of Organic Solvents on *Artemia salina*

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Organic solvents can make their way into the environment as industrial wastes and components of pesticide formulations. In laboratory bioassays, the use of organic solvents is often unavoidable, since many pesticides and organic pollutants have low water solubility and must be dissolved in organic solvents prior to addition into experimental systems. In the toxicant bioassays, invertebrates with special reference to aquatic arthropod species are of recent interest as test models due to the need for developing nonmammalian test systems (Baudoin and Scoppa 1974; Persoone et al. 1989; Snell and Persoone 1989). Toxic effects of organic solvents have been tested with a few aquatic species (Stratton 1987; Takahashi et al. 1987), but information on the comparative toxicity of solvents towards Artemia salina is not available. Artemia salina have, within recent years, gained popularity as test organisms for short-term toxicity testing. Because Artemia salina exhibit rapid development and growth within 48 hr after hatch, their potential as a model organism for toxicology screening has been considered. To do this, synchronous populations of Artemia salina at different development intervals must be available.

The purpose of the present study was to compare the acute toxicity of four solvents (dimethylsulfoxide, methanol, ethanol and acetonitrile) towards three ages of Artemia salina, in order to identify solvents with low toxicity for use in bioassays. One area of concern with laboratory bioassays is the stress imposed on test organism by organic solvents. On the other hand, organic solvents represent an obvious hazard for the marine environment and studies on their impact on marine organisms are necessary. The study of organic solvents within the environment takes many forms including the use of living organisms as bioindicators.

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## MATERIALS AND METHODS

A strain of Artemia salina provided in an egg shaped dry state by San Francisco Bay Brand, Inc. (Division of Metaframe Co., Menlo Park, CA, USA) was used as the test animal.

The method of Persoone et al. (1989), to obtain Artemia salina for the test, was applied and modified according to the following procedure. For this purpose, the encysted brine shrimp of species Artemia salina were obtained from 100 mg eggs. They were hydrated in distilled water at 4°C for 12 hr, followed by washing to separate the cysts that sink from those that float. The cysts that sank were collected on a Büchner funnel and washed with cold distilled water, followed by synthetic seawater. Synthetic seawater was prepared by mixing 35 ‰ of Synthetica sea salt (Waterlife Research Ltd., England), with distilled and deionized (Milli-Q) water, stirring for 24 hr, with suitable aeration and successive filtration with thick cellulose filters. They were then incubated in a graduated glass cylinder for about 24 hr at 25 °C, with continuous side illumination (15-W fluorescent lamp), in 100 mL of synthetic seawater, at pH 8.6, and with a slight aeration maintained by a small tube in contact with the botton of the cylinder.

The nauplii produced were aspired with Pasteur pipets and transferred to a glass flask containing 200 mL of the synthetic seawater. Each experiment was performed for three ages: nauplius larvae of Artemia salina aged 24-, 48- and 72-hr.

Four organic solvents were tested including dimethylsulfoxide (DMSO), methanol, ethanol and acetonitrile (ACN). All solvents were obtained from Sigma Chemical Company (St. Louis, USA), except ethanol which was obtained from Merck (Darmstadt, FRG).

For toxicity testing, samples of 10 larvae each were added to 1 mL of synthetic seawater in plastic 16-mm petri dishes containing the appropiate volume of solvent. Samples were placed at 25 °C in the dark. The acute toxicity of the four solvents to Artemia salina, acting individually, was estimated by determination of the 24-hr $LC_{50}$ (concentration of the solvent which kills 50 % of the test animals after 24-hr exposure). Larvae were considered dead if they did not exhibit any internal or external movement during 10 sec of observation. Each solvent concentration was replicated three to five times, and the range of solvent concentration tested are expressed in mg/L. Refer to the nominal concentrations at the onset of the experiments. Appropiate control systems containing no solvent were included in each experiment. All experiments

were repeated five times.

The 24-hr $LC_{50}$ values, with 95 % confidence limits, were calculated according to the method described in Litchfield and Wilcoxon (1949). Significant differences between samples or solvents were determined using an analysis of variance procedure (ANOVA), followed by post hoc contrast with Newman-Keuls Test.

**RESULTS AND DISCUSSION**

The solvents used as test toxicants in this study were chosen because of their widespread use in bioassays. The tested organism, Artemia salina, can be considered representative of saline aquatic ecosystems, in fact, they are at the base of the food chain. Artemia salina represent the food source of many aquatic organisms and, in certain ecosystems, its disappearance could lead to an alteration of existing equilibria.

The calculated 24-hr $LC_{50}$ values of organic solvents towards different ages of Artemia salina and their 95 % confidence intervals are summarized in Table 1.

**Table 1.** 24 hr-$LC_{50}$ values (95 % CL, n=5 bioassays) for four solvents tested against Artemia salina expressed in mg/L.

| | TIME OF ARTEMIA LARVAE DEVELOPMENT | | |
|---|---|---|---|
| | 24hr | 48hr | 72hr |
| DMSO | 6825.29 (6574.4-7087.2) | 6711.92 (6341.5-7103.9) | 6539.27 (5506.7-7765.5) |
| METH | 1578.84 (1463.3-1703.5) | 1101.46 (703.7-1723.9) | 900.73 (794.8-1020.7) |
| ETH | 1833.5 (1324.6-2537.8) | 857.79 [a] (725.5-1014.1) | 695.35 [a] (588.6-821.4) |
| ACN | 640.95 (565.9-725.8) | 521.47 (437.3-621.8) | 399.65 [a] (328-486.9) |

DMSO=dimethylsulfoxide; METH=methanol; ETH=ethanol; ACN=acetonitrile.

[a] Significantly different ($p<0.05$) from $LC_{50}$ for Artemia salina 24-hr old.

As can be expected from the different modes of action of the compounds, Artemia salina displayed a range of sensitivities to the toxicants tested. Artemia salina is in some cases more sensitive and in other less sensitive, depending on the solvent and test animal life stage. A comparison of these solvents for Artemia salina 24-, 48- and 72-hr old demonstrated that there is an increase in toxicity of ethanol and acetonitrile following longer

development of Artemia salina. Artemia salina 72-hr old was more sensitive towards ethanol and acetonitrile than was Artemia salina 24-hr old. However, DMSO and methanol were about equally toxic to all age classes of Artemia salina tested.

There is no statistically significant difference between the toxicity of acetonitrile to Artemia salina 24- and 48-hr old, however, there is a statistically significant difference between the toxicity of acetonitrile to Artemia salina 24- and 72-hr old. There are statistically significant differences between the toxicity of ethanol to different ages of Artemia salina larvae. Artemia salina larvae 24-hr old are 2.13 and 2.64 times less sensitive to ethanol than Artemia salina larvae 48- and 72-hr old, respectively.

A comparison of the toxicity of DMSO, methanol, ethanol and acetonitrile to Artemia salina larvae aged 24-, 48- and 72-hr are summarized in Table 2.

**Table 2.** Comparison of solvents toxicity towards three ages of Artemia salina, by the Newman-Keuls test.

| | DMSO 24hr | METH 24hr | ETH 24hr | ACN 24hr |
|---|---|---|---|---|
| METH 24hr | 5247.32** | --- | 316.77* | --- |
| ETH 24hr | 4930.54** | --- | --- | --- |
| ACN 24hr | 6184.94** | 937.62** | 1254.4** | --- |
| | DMSO 48hr | METH 48hr | ETH 48hr | ACN 48hr |
| METH 48hr | 5542.74** | --- | --- | --- |
| ETH 48hr | 5853.3** | 310.56* | --- | --- |
| ACN 48hr | 6192.26** | 649.52* | 338.96* | --- |
| | DMSO 72hr | METH 72hr | ETH 72hr | ACN 72hr |
| METH 72hr | 5436.59** | --- | --- | --- |
| ETH 72hr | 5640.23** | 203.64* | --- | --- |
| ACN 72hr | 5937.17** | 500.58* | 296.94* | --- |

DMSO=dimethylsulfoxide; METH=methanol; ETH=ethanol; ACN=acetonitrile.
Those Newman-Keuls test values that are followed by (*) and (**) differ significantly at $p<0.05$ and $p<0.01$, respectively.

Acetonitrile was always the most toxic solvent tested. Usually, the next most toxic solvent was methanol, except to Artemia salina 24-hr old, where it was ethanol. There

are some differences among the three groups of animals in relative order of toxicitiy for solvents tested. In Artemia salina 48- and 72-hr old, ACN, ethanol, methanol are most toxic and DMSO is the least toxic. The relative toxicity of ethanol, however, is noticeably different; in Artemia salina 24-hr old, it is equivalent to DMSO whereas methanol is less toxic than ethanol.

The data presented indicate that acetonitrile would not be a suitable solvent for use in toxicity tests involving Artemia salina, because of its high toxicity.

DMSO is the most common solvent used in toxicity testing involving Artemia salina (Kuwabara et al. 1980; Blizzard et al. 1989), and is the solvent of choice in many other bioassay sistems (Brayton 1986; Macrì et al. 1988). In the present study, DMSO had low toxicity towards the test organims. These data, together with the fact that DMSO is regarded to be an excellent solvent (Majewski et al. 1978) indicate that it would be a suitable choice for bioassays involving Artemia salina, as long as it was used at levels well below those eliciting toxic effects. These data are comparable to those found in a similar study utilizing fungi as test organisms, where the $EC_{50}$ varies from 2.0 to 12.0 % v/v (Stratton 1985).

Methanol and ethanol were also found to be of intermediate toxicity in the present study. Ethanol is sometimes used in fungal toxicity tests, but its widespread use in laboratories as a microbial biocide discourages its use in most bioassays. Ethanol is equal in toxicity to DMSO, when tested towards Chlamydomonas eugamentos and less toxic than DMSO towards Chlorella pyrenoidosa (Stratton 1987). In comparison to other test species currently used in aquatic toxicology, we found that acute toxicity of ethanol to Artemia salina, is much less than reported for Daphnia magna and Ceriodaphnia dubia. Takahashi et al. (1987), found 48-hr $LC_{50}$ values for ethanol of 12318 mg/L and 5012 mg/L, respectively. So Daphnia magna and Ceriodaphnia dubia turned out to be more tolerant to ethanol than Artemia salina. Although methanol had been used in bioassays (Calcott and Fatig 1984), we found that it would be poor choice as a solvent in bioassays, primarily due to its inferior solvent capabilities when compared with DMSO.

In summary, the present results demonstrate noticeable differences in toxicity of some organic solvents to several ages of Artemia salina. Therefore, we believe that aquatic safety data should be developed for the protection of aquatic organisms. It is very important to examine the toxicity of organic and inorganic compounds, pesticides, detergents and industrial effluents pontentially reaching

aquatic ecosystem. On the other hand, it is first necessary to choose a solvent which has low toxicity to the test organism used (Stratton 1985), in order to ensure that a given solvent does not interfere with the test toxicants effects in bioassays.

**REFERENCES**


Baudoin MF, Scoppa P (1974) Acute toxicity of various metals to freshwater zooplankton. Bull Environ Contam Toxicol 12:745-751

Blizzard TA, Ruby CL, Mrozik H, Preiser FA, Fisher MH (1989) Brine shrimp (Artemia salina) as a convenient bioassay for avermectin analogs. J Antibiot 12(8):1304-1307

Brayton CF (1986) Dimethyl sulfoxide (DMSO): A review. Cornell Vet 76:61-90

Calcott PH, Fatig RO(1984) Inhibition of chitin metabolism by avermectin in susceptible organisms. J Antibiot 37(3):253-259

Kuwabara K, Nakamura A, Kashimoto T (1980) Effect of petroleum oil, pesticides, PCBs and other environmental contaminants on the hatchability of Artemia salina dry eggs. Bull Envrion Contam Toxicol 25:69-74

Litchfield PH, Wilcoxon F (1949) A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 96:99-113

Majewski HS, Klaverkamp JF, Scott DP (1978) Acute lethal and sublethal effects of acetona, ethanol and propylene glycol on the cardiovascular and respiratory systems of rainbow trout (Salmo gairdneri) Water Res 12:217-221

Macrì A, Stazi AV, Dojmi Di Delupis (1988) Acute toxicity of furazolidone on Artemia salina, Daphnia magna and Culex pipiens molestus larvae. Ecotox Environ Safety 16:90-94

Persoone G, Van de Vel A, Van Steertegem M, De Nayer B (1989) Predictive value of laboratory tests with aquatic invertebrates: influence of experimental conditions. Aquat Toxicol 14:149-166

Snell TW, Persoone G (1989) Acute toxicity bioassays using rotifers. I. A test for brackish and marine environments with Brachionus plicatilis. Aquat Toxicol 14:65-80

Stratton GW (1985) The influence of solvent type on solvent-pesticide interactions in bioassays. Arch Environ Contam Toxicol 14:651-658

Stratton GW (1987) Toxic effects of organic solvents on the growth of blue-green algae. Bull Environ Contam Toxicol 38:1012-1019

Takahashi IT, Cowgill VM, Murphy (1987) Comparison of ethanol toxicity to Daphnia magna and Ceriodaphnia dubia tested at two different temperatures: Static acute toxicity test results. Bull Environ Contam Toxicol 39:229-236