Ecotoxic Effects of the Vehicle Solvent Dimethyl Sulfoxide on Aquatic Model Organisms

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Abstract

Dimethyl sulfoxide (DMSO) is widely used as a vehicle solvent in ecotoxicity bioassays. However, despite its frequent use, it could be toxic for organisms at some concentrations. Hence, the aim of this study was to investigate the effects of DMSO on the population growth rate of the microalgae *Raphidocelis subcapitata*, the mobility of the microcrustacean *Daphnia magna*, and the reproduction of the rotifer *Brachionus calyciflorus*. DMSO was applied to the organisms in concentrations ranging from 0.031–4%. For *R. subcapitata* significant effects in growth inhibition after 72 h of exposure was 0.125% DMSO, being the lowest observed effect concentration (LOEC). The 50% effective concentration (EC_{50}) was $2.138 \pm 0.372\%$. In *D. magna*, significant differences in the mobility after 24 h or 48 h of exposure was 1% DMSO being $1.712 \pm 0.207\%$ and $1.167 \pm 0.220\%$ DMSO the EC_{50} observed for 24 h and 48 h exposure, respectively. For *B. calyciflorus*, it was not possible to validate the tests performed, as there were insufficient animals alive in the control conditions at the end of the exposure period. Therefore, we recommended avoiding DMSO as a vehicle in assays using *B. calyciflorus*, and to use final DMSO concentrations in experimental solution not exceeding 0.125% for *R. subcapitata* and 0.5% for *D. magna*.

1. Introduction

Ecotoxicological laboratory bioassays often require solvents as vehicles to solubilize lipophilic test compounds and organic pollutants with low water solubility that need to be dissolved prior to addition to experimental systems (Jay et al. 1996, David et al. 2012). It is important to note that although in the environment these solvents are not required to dissolve the compounds in question, in laboratory conditions where pure compounds are tested in isolation is unavoidable the use of them to make experiments possible. Nonetheless, their potential effects and the stress imposed on the test organisms by their application is of great concern, and has become a crucial issue in professional papers (Jay et al. 1996, Okumura et al. 2001).

Whilst international test guidelines (e.g. the Office of Chemical Safety and Pollution Prevention [OCSPP] and the Organization for Economic Cooperation and Development [OECD]) and the US EPA (United States Environmental Protection Agency) define that solvents can be applied at their maximum acceptable concentrations, the final concentrations used in aquatic assays are often higher than the typical recommended limits of 0.1 mL/L, i.e. 0.01% (v/v) (OECD 2019, Green & Wheeler 2013). However, this recommendation is not species-specific, but a general statement and do not consider that different species respond differently to a given solvent exposure. In addition, usually there are differences also among strains of the same species. Therefore, in practice, it is not possible to be this rigorous, suggesting a review of perspective considering the work. Thus, the nature of the solvent and the exact concentration applied in a given study should be recorded and stated.

Dimethyl sulfoxide (DMSO) is the most common commonly used solvents in toxicology for the administration of chemicals (Christou et al. 2020). This sulphur-based compound is produced in nature as a result of the oxidation of biogenic dimethylsulphide (DMS) by marine algae and bacteria (Deschaseaux

et al. 2014, Kim & Lee 2021). Its popular use as a carrier vehicle is due to its low toxicity to living beings and its ability to permeate biological membranes without significant damage to their structural integrity (Kais et al. 2013). It is also an aprotic solvent with the ability to dissolve both polar and nonpolar compounds, and organic and inorganic chemicals (Huang et al. 2017).

In aquatic toxicology, the test organisms are subjected to continuous exposure equivalent to the duration of the test protocol (David et al. 2012). Therefore, there has been a steadily growing concern among ecotoxicologists over the importance of understanding the effects of DMSO *per se* on experimental outcomes, as it influences behavior, physiologic and biochemical parameters of the organism.

Thus, evaluation of the effects of DMSO itself is an important task. In this regard, the current study investigated the effects of DMSO on aquatic model organisms (green microalgae, microcrustacea, and rotifers) used for several common and standardized bioassays, at low concentrations (up to 4%) that are often employed in aquatic assays emphasizing either the differences in sensitivity among species.

2. Materials And Methods

2.1. Treatment solutions

A stock test solution containing 10% DMSO (v/v) was prepared by diluting DMSO (Sigma–Aldrich, CAS 67-68-5) in demineralized water (ELIX water). Then, fresh solutions of 0.031, 0.063, 0.125, 0.25, 0.5, 1, 2, and 4% DMSO were obtained by diluting the stock solution (10% DMSO) with the standard culture medium used for each aquatic organism tested.

Positive control solutions were prepared from commercially available salts K_2CrO_7 (prolabo, ref 26 784.231), $ZnSO_4 \cdot 7 H_2O$ (Merck, ref 1.08883.0500) and $CuSO_4 \cdot 5 H_2O$ (Merck, ref 606 A58090).

2.2 Microalgae: growth inhibition assay

The green microalgal species used for the test was *Raphidocelis subcapitata* (ATCC® 22662™). Briefly, three different experiments (replicates) were conducted for each concentration of DMSO, using a 96-well microplate according to ISO 8692 (2012) guideline. In each experiment, six repetitions per concentration (or control) were made. Potassium dichromate ($K_2Cr_2O_7$) (concentrations ranging from 0.2 to 3 mg L⁻¹) and zinc (prepared from $ZnSO_4$ and solutions containing 0.01 to 0.105 mg L⁻¹ of Zn²⁺) were used as two separate positive controls and distilled water as negative control. The algal culture in the exponential growth phase was diluted in ISO medium for freshwater algae (pH 8.1 ± 0.2) to obtain the inoculum for the test with a cell density of 10,000 cells mL⁻¹. The test plates were incubated in a room with continuous illumination of 70 mmol m⁻² s⁻¹ (cool-white fluorescent lamps) at 23 ± 2°C. After 72 h of incubation, the fluorescence variation was measured at wavelength of 485 nm for excitation and 685 nm for emission

with FLUOstar microplate reader (BGM Labtechnologies), to establish whether growth had been inhibited or stimulated in comparison with the control.

2.3 Microcrustacea: immobilization assay

The microcrustacean immobilization assay were performed according to ISO 6341 (2012). Briefly, three different experiments (with four replicates for each one) were performed. An assay tube with 10 mL of culture medium (pH 7.8 ± 0.5 , aerated overnight) plus the appropriate proportion of DMSO was prepared for each replicate. Five young *D. magna* (up to 24 hours of life) were then added to each tube. Potassium dichromate ($K_2Cr_2O_7$) (concentrations ranging 0.58 and 1.6 mg L⁻¹) and zinc (prepared from $ZnSO_4$ with concentrations ranging from 0.05 to 19.79 mg L⁻¹ of Zn^{2+}) were used as the positive controls and the distilled water as negative control. The test tubes were covered with aluminum foil to keep light out and were kept in an incubator at $20 \pm 2^\circ C$. Inhibition of the mobility of the individual *D. magna* was determined visually after 24 and 48 h of exposure.

2.4 Rotifers: reproduction assay

Cysts of the rotifer *Brachionus calyciflorus* were purchased from MicroBioTests Inc., Belgium. The cysts were placed in a Petri dish containing 5 mL of fresh ISO medium (ISO 20666, 2008) at pH 7.6 ± 0.3 and were incubated at $25 \pm 1^\circ C$ for 18 to 24 h under continuous illumination of 1,600 Lux. An individual rotifer that had hatched from a cyst less than 2 hours previously was placed in each well of a 24-well microplate. Three different experiments (replicates) were conducted for each concentration of DMSO (0.063% to 4%). In each experiment, eight repetitions (wells) per concentration (or control) were made. A solution containing about 10^6 cells mL⁻¹ of *Raphidocelis subcapitata* was added to each well in order to feed the individuals during the experiment. A copper solution (prepared from copper sulphate pentahydrate - $CuSO_4 \cdot 5H_2O$ containing Cu^{2+} concentrations of between 1.1 and 121.22 mg L⁻¹) was used as a positive control and distilled water as negative control. The plates were covered with aluminum foil and incubated at $25 \pm 1^\circ C$. After 48 hours, the total numbers of individuals were counted using a binocular microscope, to estimate the reproduction rate.

2.5 Statistical Analysis

The no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were determined through one-way analysis of variance (ANOVA) and followed by post-hoc comparisons using Williams's test Williams, (1972) with a significance level of 0.05. These analyses were performed with the PMCMRplus package version 1.9.0 in R 4.0.5 (Pohlert 2021). The treatment concentration that caused a 50% effect (EC_{50}) in comparison to the control treatment was derived from four-parameter log-logistic

regression models using the DRCpackage version 3.0 in R, following Ritz et al. (2015). The regression curves graphs were prepared using ggplot2 version 3.3.3 in R.

3. Results

3.1 Effects on the algae population

The half maximal effective concentration (EC_{50}) for potassium dichromate on the *R. subcapitata* strain was $0.45 \pm 0.09 \text{ mg L}^{-1}$ (Table 1). A positive control solution containing increasing concentrations of zinc (Zn^{2+}) was also tested. At LIEC (Laboratoire Interdisciplinaire de Environnements Continentaux, Université de Lorraine, France), where the experiments were conducted, $ZnSO_4$ is the preferred positive control for algal tests, as its response is reliable and a dose-response curve is well established, as demonstrated in this study (Table 1). The lower concentration (0.01 mg L^{-1} of Zn^{2+}) tested was the NOEC, while the LOEC was a concentration of 0.014 mg L^{-1} .

The concentrations of DMSO applied to the culture medium ranged from 0.031% to 4%, with the algae being subjected to 72 h of exposure. Low concentrations (0.031% and 0.63%) did not exert a significant toxic effect on the growth of the freshwater algal population (Figure 1A). However, increasing the concentration of DMSO to 0.125% and above resulted in a significantly ($p < 0.05$) growth inhibition. Maximum inhibition was observed with 4% DMSO (Figure 1A). Accordingly, the LOEC was 0.125% and the EC_{50} value from the sigmoidal regression curve was $2.138 \pm 0.372\%$ (Figure 1B).

3.2 Effects on the mobility of daphnids

For the *D. magna* immobility assay, a gradual dose-dependency inhibition of the mobility of the individuals was observed after 24 and 48 h of exposure to $K_2Cr_2O_7$ (Table 2). Accordingly, the EC_{50} values were found to be $1.11 (\pm 0.08)$ and $0.91 (\pm 0.34) \text{ mg L}^{-1}$ of $K_2Cr_2O_7$ after 24 and 48 h of exposure, respectively.

The effects of zinc were also evaluated and concentrations from 0.05 to 1.52 mg L^{-1} were not toxic to the daphnids over 24 h, but higher concentrations showed significant ($p > 0.05$) toxicity (Table 2). The observed EC_{50} values were 2.32 ± 0.58 and $1.86 \pm 0.21 \text{ mg L}^{-1}$ for 24 and 48 h of exposure respectively (Table 2).

The results for DMSO exposure demonstrated that concentrations ranging from 0.031% to 0.5% were not toxic after 24 h of exposure, while higher concentrations did exert significant ($p < 0.05$) toxicity on daphnids (Figure 2A). Observation of the behavior of the individuals after 48 h revealed that a DMSO concentration of 1% immobilized over 40% of the daphnids. The highest concentration applied (4%) completely inhibited the mobility of all individuals (Figure 2A). Hence, the LOEC after 24 and 48 h of

exposure was 1% and the EC₅₀ observed for the mobility of *D. magna* was a DMSO concentration of 1.712 ± 0.207% for 24 h exposure and 1.167 ± 0.220% for 48 h (Figure 2B).

3.3 Effects on reproduction rate of rotifers

Lower concentrations (1.1 to 11.55 mg L⁻¹) of Cu²⁺ inhibited reproduction by less than 30%, besides all concentrations tested exerted a significant effect ($p < 0.05$) on the reproduction rate of *B. calyciflorus* after 48 h of exposure (Table 3). Test concentrations above 11.55 mg L⁻¹ completely inhibited reproduction by 70% or more (Table 3), thus, the EC₅₀ value was 13.53 ± 2.55 mg L⁻¹ (Table 3).

All concentrations of DMSO tested inhibited somehow, the reproduction rate of *B. calyciflorus*. Concentrations above 1% (0.031 – 0.5%) inhibited reproduction of *B. calyciflorus* no more than 20% with reproduction being reduced by 70% with the highest concentration solution tested (4% DMSO) (Figure 3A). Significant ($p < 0.05$) inhibition on relation to control was detected only up to 2% of DMSO and the EC₅₀ was calculated as a concentration of 1.624 ± 0.988% including the LOEC (2%) and NOEC (1%).

4. Discussion

Standard guidelines available of organizations such as OECD, the American Society for Testing and Materials (ASTM) and the US EPA recognize the use of carrier solvents in situations where there is no other practical alternative to dilute hydrophobic substances in ecotoxicity tests (Hutchinson et al., 2006). Typically, a maximum concentration of a solvent accepted in those guidelines recommendations does not exceed 0.1 mL L⁻¹ or 0.01 % of the solvent in the tested medium or solution (Green & Wheeler 2013, Kais et al. 2013).

However, this recommendation is general and non-dependent of the solvent applied, neither it is specific for a given specie and do not consider the biological differences between living beings. A clear example of this different susceptibility is the result obtained with algae assays in the present study comparing the control positive substance with those found in the literature. The EC₅₀ values for potassium dichromate on the *R. subcapitata* strain was 0.45 ± 0.09 mg L⁻¹ (Table 1) which differs from the 1.19 ± 0.27 mg L⁻¹ stated in ISO 8692 (2012) for the same population of *R. subcapitata* (ATCC® 22662™).

Many bioassays employ DMSO as a carrier medium in concentrations of up to 10% (v/v) to achieve appropriate biological availability of hydrophobic toxicants (Huang et al. 2017) but effects of DMSO at this level should be discussed as it could exert toxicity. Nonetheless, the recommended concentration (0.01% in the cited guidelines) is very restrictive, and to figure the restriction of these guidelines as far as the importance to run concurrent solvent and negative controls as an experimental group to avoid misinterpretation of results and to assess the influence of the carrier in such assays (Bownik, 2019, Hu et

al., 2017), DMSO was applied in aquatic models algae, daphnia and rotifer in the present study. Recently the same approach was applied in zebrafish embryo assay (Christou et al. 2020).

The results achieved in the present work, for example, demonstrated that for the algae *R. subcapitata* the first and second concentrations of DMSO tested (0.031 and 0.063%) do not exert any effects in the population growth (Figure 1). Inhibition effects on the growth were noticed in concentration 10 times more concentrated (0.125 %) than recommended in the guidelines (Figure 1).

In this sense, our result is in agreement with early experiments. Okumura et al. (2001) reported that DMSO did not affect the growth of nine species of marine microalgae at concentrations below 1%. Furthermore, Ma and Chen (2005) assessed the toxicity of seven solvents (acetone, ethanol, methanol, DMSO, N,N-dimethyl- formamide, furanidine, and acetidin) to green algae, and concluded that an adequate range of DMSO concentrations for the Chlorophyceae *Chlorella pyrenoidosa* was 0.01% to 0.50%. They found an EC₅₀ concentration of 1.49 mg L⁻¹ (or 1.49% DMSO) (Ma & Chen 2005), which is in agreement with the value we observed for *R. subcapitata* (Figure 1B) considering the biological differences discussed above.

On the other hand, Andreani et al. (2017) found that DMSO up to 1% was not in itself toxic to the bacteria *Vibrio fischeri* and the freshwater microalgae *Raphidocelis subcapitata*. This corroborates the work of Jay (1996), who tested the effects of organic solvents on the growth of two Chlorophyceae (*Chlorella vulgaris* and *Selenastrum capricornutum*), and no effect was detected in doses up to 1% DMSO. Considering that *S. capricornutum* is a synonym for *R. subcapitata*, in summary, our results is even more restrictive with this limit concentration as it should do not exceed 0.125% of DMSO, but still, more permissive than the limits recommendation in guidelines.

Daphnia a first consumer in food chain demonstrated to be less sensitive than the alga, a producer in the food chain. It was first observed comparing the positive control applied as the EC₅₀ value of K₂Cr₂O₇ found (1.11 (± 0.08) mg L⁻¹ after 24 h of exposure) is in agreement with the ISO 6341 (2012) standard, which presents 1.12 mg L⁻¹ as the EC₅₀ medium value for 24 h exposure. For DMSO the LOEC observed for *D. magna* after 24 h or 48 h of exposure was 1% DMSO. The above-mentioned LOEC concentration represent a solution 100 times more concentrated than the one established in the guidelines (Figure 2).

Similar conclusions could be made for comparisons between our findings for the acute assay in *D. magna* with the results from the previous reports available. Barbosa et al. (2003) demonstrated that the EC₅₀ for acute toxicity of DMSO to *D. magna* after 48 h of exposure was 2.23 %, while ours was 1.167%. Huang et al. (2017) observed in an immobilization assay that concentrations of DMSO ranging between 0.1% and 1% were not toxic to *Daphnia* neonates when they were exposed for 24 h, but that higher concentrations (more than 2%) were toxic over both 24 and 48 h. The EC₅₀ values observed were 2.3% for 24-h exposure and 0.5% for 48-h exposure. Comparing these results to the ones we observed in our study (Figure 2A and B), we can emphasize that concentrations of DMSO higher than 0.5% should be avoided in the classic immobilization test standardized by ISO 6341 (2012). Therefore, for acute assay,

concentrations of DMSO higher than 0.01% (recommended limits) could be used as long as 0.5% of DMSO is not exceeded.

Aside from these observations, Huang et al. (2017) also tested the effects of DMSO on behavioral traits in a time-dependent manner in chronic assays and demonstrated that DMSO can have significant consequences if it is used in concentrations ranging from 0.01 to 1% claiming cautious interpretation for lethal and behavioral parameters in these situations. According to Hutchinson et al. (2006), for acute test the tolerance of the organisms to the solvents is higher than in chronic assessments, in contrast, the effects of solvents for longer-term exposure in chronic ecotoxicity endpoints achieve sub-lethal levels (i.e. reproduction) and consequently minor doses is sufficient to provoke adverse effects. This consideration is of increasingly importance for the data obtained in the present work for *B. calyciflorus* exposed to DMSO.

However, it is important to note that according to the ISO 20666 standard (2008), the experiment could not be validated. Indeed, according to validity criteria, a reproduction should be observed in at least 7 out of the 8 replicates of the control wells by the end of the experiment, and the number of individuals per well should be three or more. In all three replicates conducted in our experiments, one or more wells had less than three *B. calyciflorus*. Yet, every well containing 1% DMSO presented only the *B. calyciflorus* specimens added at the beginning of the assay, while some of them died at 2% DMSO, and all rotifers died in wells containing 4% DMSO. Only in concentrations below 0.5% DMSO we could observe *B. calyciflorus* neonates in the wells. All in all, considering that it is a chronic assay, and that the first DMSO concentration tested (0.031 %) is above the recommended limits for acute assays (0.01 %), we could affirm that in this case DMSO exert toxicity in the tested conditions we choose. Zhan et al. (2016) found no significant difference between the DMSO treatment (0.01%) and the control group in the species *Brachionus plicatilis*. Other works dealing with reproductive factors in *B. calyciflorus* observed that 1% DMSO did not affect the population growth rate, the ratio of ovigerous females to non-ovigerous females, mitotic rate, or resting egg production (Ke et al. 2009, Snell & Carmona 1995, Snell & Janssen 1995).

In brief, DMSO did not demonstrate any significant influence on aquatic models such as microalgae and microcrustaceans, when applied according to the guidelines. In addition, the nonspecific OECD recommendation, considering specie and type of solvent, of 0.01% is overprotective for algae and microcrustacea model species. Hence, comparing our results with those from the literature, we can assume that the no-effect concentrations for the *endpoints* evaluated were up to 0.125% DMSO for algae *R. subcapitata*. This value is rather low compared to the observation reported previously by Jay et al. (1996) who demonstrated concentration up to 1% DMSO. In experiments with *D. magna*, the concentration of DMSO applied should not be above 0.5%, to avoid ecotoxic effects agreeing with Huang et al. (2018) for acute exposition.

Altogether, these results highlight the importance of considering the effect of DMSO on the experimental outcomes when designing assays and interpreting their results. If it is necessary to use solvents such as DMSO, rigorous monitoring procedures should be applied. It is also crucial to ensure that the solvent has

no toxic effects on the evaluated parameters. When preparing reports, information on the concentration of solvent should be presented, together with the use of a parallel solvent control. Therefore, reporting of the toxicity of low doses of DMSO on aquatic organisms is of high relevance, as the interference of a carrier solvent should not be a confounding factor that can potentially affect the outcome of an assay.

5. Conclusion

Regarding our results, we comfort the recommendations of the respective guidelines of the assays: for experiments with *R. subcapitata* we recommended that the final concentration of DMSO in the tested solution should not be higher than 0.125% and 0.5% in experiments with acute toxicity in *D. magna*. For *B. calyciflorus*, we were not able to identify a safety concentration value to recommend, thus, indicating to following the guidelines recommendations (0.01 % or even less).

Declarations

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Code availability: 'Not applicable'

The authors comply with **ethical standards statements, consent to participate and consent for publication**

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References

1. Abe S, Takahashi H (2007) A comparative study of the effects of dimethylsulfoxide and glycerol on the bicontinuous cubic structure of hydrated monoolein and its phase behavior. Chemistry and Physics of Lipids 147:59-68. <https://doi.org/10.1016/j.chemphyslip.2007.03.005>
2. Andreani T, Nogueira V, Pinto VV, Ferreira MJ, Rasteiro MG, Silva AM, Pereira R, Pereira CM (2017) Influence of the stabilizers on the toxicity of metallic nanomaterials in aquatic organisms and

- human cell lines. *Science of The Total Environment* 607:1264–1277.
<https://doi.org/10.1016/j.scitotenv.2017.07.098>
3. Barbosa IR, Martins, RM, Sá e Melo ML, Soares AMVM (2003) Acute and Chronic Toxicity of Dimethylsulfoxide to *Daphnia magna*. *Bulletin of Environmental Contamination and Toxicology* 70:1264–1268. <https://doi.org/10.1007/s00128-003-0119-9>
 4. Bownik A (2019) Effects of ectoine on behavioral, physiological and biochemical parameters of *Daphnia magna* exposed to dimethyl sulfoxide *Science of The Total Environment* 683:193–201. <https://doi.org/10.1016/j.scitotenv.2019.05.257>
 5. Chen X, Foote AG, Thibeault SL (2017) Cell density, dimethylsulfoxide concentration and needle gauge affect hydrogel-induced bone marrow mesenchymal stromal cell viability. *Cytotherapy* 19(12) 1522–1528. <https://doi.org/10.1016/j.jcyt.2017.08.016>
 6. Christou M, Kavaliauskis A, Ropstad E, Fraser, TWK (2020) DMSO effects larval zebrafish (*Danio rerio*) behavior, with additive and interaction effects when combined with positive controls. *Science of the Total Environment* 709: 134490. <https://doi.org/10.1016/j.scitotenv.2019.134490>
 7. David RM, Jones HS, Panter GH, Winter MJ, Hutchinson TH, Chipman JK (2012) Interference with xenobiotic metabolic activity by the commonly used vehicle solvents dimethylsulfoxide and methanol in zebrafish (*Danio rerio*) larvae but not *Daphnia magna*. *Chemosphere* 88(8) 912-917. <https://doi.org/10.1016/j.chemosphere.2012.03.018>
 8. Deschaseaux ESM, Kiene RP, Jones GB, Deseo MA, Swan HB, Oswald L, Eyre BD (2014) Dimethylsulphoxide (DMSO) in biological samples: A comparison of the TiCl₃ and NaBH₄ reduction methods using headspace analysis. *Marine Chemistry* 164:9–15. <https://doi.org/10.1016/j.marchem.2014.05.004>
 9. European Centre of the Ecotoxicology and Toxicology of Chemicals [ECETOC] (1996) Aquatic Toxicity Testing of Sparingly Soluble, Volatile and Unstable Substances. In: *ECETOC Monograph* No. 26. European Centre of the Ecotoxicology and Toxicology of Chemicals, Brussels.
 10. Galvao J, Davis B, Tilley M, Normando E, Duchon MR, Cordeiro, MF (2013) Unexpected low-dose toxicity of the universal solvent DMSO. *The FASEB Journal* 28(3) 1317– 1330. <https://doi.org/10.1096/fj.13-235440>
 11. Green J, Wheeler JR (2013) The use of carrier solvents in regulatory, aquatic toxicology testing: practical, statistical and regulatory considerations. *Aquatic Toxicology* 144-145:242-249. <https://doi.org/10.1016/j.aquatox.2013.10.004>
 12. Hallare A, Nagel K, Kohler, HR, Triebskorn R (2006) Comparative embryotoxicity and proteotoxicity of three carrier solvents to zebrafish (*Danio rerio*) embryos. *Ecotoxicology and Environmental Safety*, 63(3) 378–388. <https://doi.org/10.1016/j.ecoenv.2005.07.006>
 13. Helmstetter MF, Maccubbin AE, Alden RW (1996) The medaka embryo-larval assay: an in vivo assay for toxicity, teratogenicity, and carcinogenicity. In Ostrander, G. (Eds.), *Techniques in Aquatic Toxicology*, (CRC Press, Boca Raton USA pp. 93-124).

14. Hess FD (1980) A *Chlamydomonas* algal bioassay for detecting growth inhibitor herbicides. *Weed Science* 28:515-520.
15. Hu L-X, Tian F, Martin FL, Ying G-G (2017) Biochemical alterations in duckweed and algae induced by carrier solvents: selection of an appropriate solvent in toxicity testing. *Environmental Toxicology and Chemistry* 36:2631–2639. <https://doi.org/10.1002/etc.3804>
16. Huang Y, Cartlidge R, Walpitagama M, Kaslin J, Campana O, Wlodkowic D (2017) Unsuitable use of DMSO for assessing behavioral endpoints in aquatic model species. *Science of the Total Environment*, 615:107-114. <https://doi.org/10.1016/j.scitotenv.2017.09.260>
17. Hutchinson T, Shillabeer N, Winter M, Pickford D (2006) Acute and chronic effects of carrier solvents in aquatic organisms: a critical review. *Aquatic Toxicology* 76(1) 69–92. <https://doi.org/10.1016/j.aquatox.2005.09.008>
18. ISO 20666 (2008) Water Quality - Determination of the chronic toxicity to rotifer *Brachionus calyciflorus*. International Standard Organization, Geneva, Switzerland.
19. ISO 6341 (2012) Water Quality - Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea). International Standard Organization, Geneva, Switzerland.
20. ISO 8692 (2012) Water Quality - Freshwater algal growth inhibition test with unicellular green algae. International Standard Organization, Geneva, Switzerland.
21. Jay AE (1996) Toxic Effects of Organic Solvents on the Growth of *Chlorella vulgaris* and *Selenastrum capricornutum*. *Bulletin of Environmental Contamination and Toxicology* 57(2) 191-198. <https://doi.org/10.1007/s001289900174>.
22. Kais B, Schneider KE, Keiter, S, Henn K, Ackermann C, Braunbeck T (2013) DMSO modifies the permeability of the zebrafish (*Danio rerio*) chorion—implications for the fish embryo test (FET). *Aquatic Toxicology*, (140–141) 229–238. <https://doi.org/10.1016/j.aquatox.2013.05.022>
23. Ke LX, Xi Y-L, Zha C-W, Dong L-L (2009) Effects of three organophosphorus pesticides on population growth and sexual reproduction of rotifer *Brachionus calyciflorus* Pallas. *Acta Ecologica Sinica* 29(3) 182-185. <https://doi.org/10.1016/j.chnaes.2009.07.008>
24. Kim K., Lee, S-E (2021) Combined toxicity of dimethyl sulfoxide (DMSO) and vanadium towards zebrafish embryos (*Danio rerio*): Unexpected synergistic effect by DMSO. *Chemosphere* (270)129405. <https://doi.org/10.1016/j.chemosphere.2020.129405>
25. Kumar A, Darreh-Shori, T (2017) DMSO: A Mixed-Competitive Inhibitor of Human Acetylcholinesterase. *ACS Chemical Neuroscience* 8(12), 2618–2625. <https://doi.org/10.1021/acschemneuro.7b00344>
26. Ma J, Chen J (2005) How to accurately assay the algal toxicity of pesticides with low water solubility. *Environmental Pollution* 136(2) 267-273. <https://doi.org/10.1016/j.envpol.2005.01.005>
27. Miazek K, Kratky L, Sulc R, Jirout T, Aguedo M, Richel A, Goffin D (2017) Effect of Organic Solvents on Microalgae Growth, Metabolism and Industrial Bioproduct Extraction: A Review. *International Journal of Molecular Science* 18(7) 1429. <https://doi.org/10.3390/ijms18071429>

28. Obregon ADC, Schetinger MRC, Correa MM, Morsch VM, da Silva JEP, Martins MAP, Bonacorso HG, Zanatta N (2005) Effects *per se* of organic solvents in the cerebral acetylcholinesterase of rats. *Neurochemical Research* 30 :379-384. <https://doi.org/10.1007/s11064-005-2612-5>
29. Okumura Y, Koyama J, Takaku H, Satoh H (2001) Influence of Organic Solvents on the Growth of Marine Microalgae. *Archives of Environmental Contamination and Toxicology* 41:123–128. <https://doi.org/10.1007/s002440010229>
30. Organization for Economic Cooperation and Development [OECD]. (2019). Guidance Document on Aqueous-phase Aquatic Toxicity Testing of Difficult Test Chemicals. Series on Testing and Assessment No. 23 (second ed.) (2019) ENV/JM/MONO(2000)6/REV. Paris, France. 81 pp.
31. Pagán OR, Rowlands AL, Urban KR (2006) Toxicity and behavioral effects of dimethylsulfoxide in planaria. *Neuroscience Letters* 407(3) 274–278. <https://doi.org/10.1016/j.neulet.2006.08.073>
32. Plummer JM, Greenberg MJ, Lehman HK, Watts JA (1983) Competitive inhibition by dimethylsulfoxide of molluscan and vertebrate acetylcholinesterase. *Biochemical Pharmacology* 32(1) 151–158. [https://doi.org/10.1016/0006-2952\(83\)90668-8](https://doi.org/10.1016/0006-2952(83)90668-8)
33. Pohler T (2021) *Calculate Pairwise Multiple Comparisons of Mean Rank SumsExtended*. Package 'PMCMRplus', CRAN. Retrieved April 15, 2021 from <https://cran.r-project.org/web/packages/PMCMRplus/PMCMRplus.pdf>
34. Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-Response Analysis Using R. *PLoS ONE*, 10(12), e0146021. <https://doi.org/10.1371/journal.pone.0146021>
35. Sha J, Wang Y, Lv J, Wang H, Chen H, Qi L, Tang X (2015) Effects of two polybrominated diphenyl ethers (BDE-47, BDE-209) on the swimming behavior, population growth and reproduction of the rotifer *Brachionus plicatilis*. *Journal of Environmental Sciences* 28: 54–63. <https://doi.org/10.1016/j.jes.2014.07.020>
36. Snell TW, Carmona M J (1995) Comparative toxicity sensitivity of sexual and asexual reproduction in the rotifer *Brachionus calyciflorus*. *Environmental Toxicology and Chemistry* 14:415– 420. <https://doi.org/10.1002/etc.5620140310>
37. Snell T, Janssen C (1995) Rotifers in ecotoxicology: a review. *Hydrobiologia* 313-314(1) 231-247. <https://doi.org/10.1007/BF00025956>
38. Williams DA (1972) The comparison of several dose levels with a zero dose control. *Biometrics* 28(2) 519-531. <https://doi.org/10.2307/2556164>
39. Zhang J, Wang Y, Sun K-M, Fang K, Tang X (2016) A study of oxidative stress induced by two polybrominated diphenyl ethers in the rotifer *Brachionus plicatilis*. *Marine Pollution Bulletin* 113(1-2) 408-413. <https://doi.org/10.1016/j.marpolbul.2016.10.032>

Tables

Table 1 - *Raphidocelis subcapitata* growth inhibition assay after 72 h exposure to different concentrations of potassium dichromate (K₂Cr₂O₇) and of zinc (Zn²⁺).

Dichromate concentration (mg L ⁻¹)	Inhibition of growth (%) after 72 h
0.2	22.35 ± 17.00
0.6	83.20 ± 17.67*
1.0	99.83 ± 14.83
1.4	97.47 ± 15.61
1.8	96.47 ± 17.43
2.2	96.98 ± 17.40
2.6	96.96 ± 18.50
3.0	95.99 ± 17.81
<i>EC₅₀ calculated = 0.45 ± 0.09 mg L⁻¹</i>	
<i>EC₅₀ ISO 8692 (2012) = 1.19 ± 0.27 mg L⁻¹</i>	
Zinc concentration (mg L ⁻¹)	Inhibition of growth (%) after 72 h
0.010	16.02 ± 5.43
0.014	36.17 ± 6.83*
0.020	58.79 ± 6.62
0.027	66.50 ± 10.91
0.038	74.36 ± 11.24
0.054	85.87 ± 9.88
0.075	95.18 ± 6.33
0.105	97.78 ± 15.36
<i>EC₅₀ calculated = 0.019 ± 0.004 mg L⁻¹</i>	

Data presents the mean ± Standard Deviation. Asterisk representing the Low Observed Effectiveness Concentration (LOEC) and the previous treatment concentration is the No Observed Effectiveness Concentration (NOEC).

Table 2 - *Daphnia magna* immobilization assay after 24 h and 48 h exposure to different concentrations of potassium dichromate (K₂Cr₂O₇) and of zinc (Zn²⁺).

Dichromate concentration (mg L ⁻¹)	Inhibition of immobilization (%) after 24h	Inhibition of immobilization (%) after 48h
0.58	0	10.17 ± 6.54
0.67	3.34 ± 2.97	10.17 ± 6.54
0.78	13.34 ± 0.67*	33.90 ± 1.54*
0.9	16.67 ± 0.92	45.76 ± 3.01
1.04	36.67 ± 2.28	66.10 ± 1.73
1.2	40.00 ± 2.89	79.67 ± 7.5
1.39	53.34 ± 1.64	96.61 ± 8.66
1.6	75.00 ± 8.00	100
<i>EC₅₀ ISO 6341 (2012) = 1.12 mg L⁻¹ (24h)</i>		
<i>EC₅₀ calculated = 1.11 ± 0.08 mg L⁻¹ (24h) and 0.91 ± 0.34 mg L⁻¹ (48h)</i>		
Zinc concentration (mg L ⁻¹)	Inhibition of immobilization (%) after 24h	Immobilization (%) after 48h
0.05	0	05 ± 10.53
0.12	0	0
0.28	05 ± 1.05	05 ± 6.05
0.65	05 ± 1.05	10 ± 10.28
1.52	10 ± 4.28	30 ± 1.65*
3.58	55 ± 4.26*	95 ± 20.00
8.42	65 ± 5.47	100
19.79	55 ± 4.26	100
<i>EC₅₀ calculated = 2.32 ± 0.58 mg L⁻¹ (24h) and 1.86 ± 0.21 mg L⁻¹ (48h)</i>		

Data presents the mean ± Standard Deviation. Asterisk representing the Low Observed Effectiveness Concentration (LOEC) and the previous treatment concentration is the No Observed Effectiveness Concentration (NOEC).

Table 3 - *Brachionus calyciflorus* reproduction assay after 48 h exposure to different concentrations of Copper (CuSO₄ 5H₂O).

Copper concentration (mg L ⁻¹)	Inhibition of reproduction (%) after 48h
01.10	22.39 ± 4.80 ^a
01.98	17.91 ± 3.30
03.56	13.43 ± 2.30
06.42	15.67 ± 1.31
11.55	30.60 ± 1.16
20.79	84.33 ± 12.70
37.41	100
67.34	100
121.22	100
<i>EC₅₀ ISO 20666 (2008) = 53.50 ± 17.70 mg L⁻¹</i>	
<i>EC₅₀ calculated = 13.53 ± 2.55 mg L⁻¹</i>	

Data presents the mean ± Standard Deviation. ^aAll concentrations tested presented effects in comparison to control.

Graphical Abstract

Graphical abstract is not provided with this version.

Graphical Abstract - The effects of the vehicle solvent dimethyl sulfoxide (DMSO) was investigated in three aquatic model organisms, the microalgae *Raphidocelis subcapitata*, the microcrustacean *Daphnia magna*, and the rotifer *Brachionus calyciflorus*. As a result, negative effects on growth rate for the algae population, immobilization of daphnids and inhibition of rotifer reproduction was observed obtaining EC50 close to 1% of DMSO for all these cases.

Figures

Figure 1

Raphidocelis subcapitata growth inhibition assay after 72 h exposure to different concentrations of DMSO. A) Frequency bar representing the variations of inhibition in population growth in relation to control. Bar with asterisk presents inhibition statistically different from do control group according to William's test ($p < 0.05$). Vertical red dotted line represents the OECD recommended limits for DMSO (0.01%) in experimental medium. B) Growth curves observed after 72 h exposure. Points represent experimental data (means $\pm$ SE).

Figure 2

Daphnia magna immobilization assay after 24 h and 48 h exposure to different concentrations of DMSO. A) Frequency bar representing the variations of inhibition in mobility in relation to control. Bar with asterisk presents inhibition statistically different from do control group according to William's test

($p < 0.05$). Vertical red dotted line represent the OECD recommended limits for DMSO (0.01%) in experimental medium. B) Growth curves observed after 24 (ligh blue) or 48 h (dark blue) exposure. Points represent experimental data (means $\pm$ SE).

Figure 3

Brachionus calyciflorus reproduction assay after 48 h exposure to different concentrations of DMSO. A) Frequency bar representing the variations of inhibition in reproduction in relation to control. Bar with asterisk presents inhibition statistically different from do control group according to William’s test ($p < 0.05$). Vertical blue dotted line represent the OECD recommended limits for DMSO (0.01%) in experimental medium. B) Growth curves observed after 72 h exposure. Points represent experimental data (means $\pm$ SE).

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