Acute toxicity characterization of organic UV-filters and chronic exposure revealing multigenerational effects in *DAPHNIA MAGNA*

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Abstract

Organic ultraviolet (UV) filters have often been detected in aquatic ecosystems in concentrations ranging from ng/L to μg/L. However, both their acute and chronic effects on aquatic organisms have been insufficiently explored. This study aimed to evaluate acute toxicity of some of the main UV filters used worldwide (2-ethylhexyl,4-methoxycinnamate/EHMC, avobenzone/AVO, benzophenone-3/BP-3, and octocrylene/OC), in three aquatic organisms (*Artemia salina*, *Desmodesmus subspicatus*, and *Daphnia magna*), and to further investigate multigenerational effects in *D. magna*. After acute toxicity was confirmed, daphnids were chronically exposed to environmentally relevant concentrations of UV filters for two consecutive generations (F0 and F1), and reproductive endpoints, as well as catalase (CAT) and glutathione-S-transferase (GST) activities, were assessed. EHMC showed the most toxic potential, with the lowest EC50 values for the three organisms. On the other hand, reproductive delays and a decrease in the reproduction rate were observed in the F1 generation exposed to AVO (4.4 μg/L), BP-3 (0.17 μg/L), EHMC (0.2 μg/L), and MIX. An increase of the CAT activity in organisms exposed to BP-3 and EHMC suggested induction of the antioxidant system. Although no reproductive effect was observed in the first generation, toxic effects obtained in the F1 revealed the importance of multigenerational studies and the potential harm of UV filters to the life cycle of *D. magna*, even at environmentally relevant concentrations. This emphasizes the need for further studies considering these levels of exposure and more realistic experimental designs to better understand their potential risks.

Graphical abstract

Environmentally relevant concentrations of Organic UV filters are not lethal to aquatic organisms, however may affect reproductive parameters in *Daphnia magna* though multigenerational exposures.

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Extended author information available on the last page of the article
Keywords Sunscreen · Contaminants of emerging concern · Mixtures · Multigenerational exposure · Biomarkers

Introduction

Ultraviolet (UV) filters represent an important category of compounds that offer protection against solar radiation effects. They are classified as inorganic, which reflect UV radiation (like ZnO and TiO₂), and organic, which absorb UVA (320–400 nm) and UVB (280–320 nm) rays due to the aromatic rings in their structure (Pestotnik et al. 2014; Baki and Alexander 2015). Considering their physicochemical properties, these molecules are widely applied in the formulation of numerous personal care products (PCP), including body lotions, shampoos, and especially sunscreens (Avenel-Audran et al. 2010; Pestotnik et al. 2014; Manová et al. 2015; Baki and Alexander 2015).

To ensure broader spectrum protection against UV rays, different organic filters are combined in many formulations (Fent et al. 2008). Usually, three to eight UV filters are mixed in an organic photoprotection formulation, representing up to 15% of the final weight of the product (Fent et al. 2010). Thus, an extensive amount of these compounds is released into the environment through recreational activities, wastewater from the manufacturing process, domestic sewage, and inappropriate disposal of packaging (Pestotnik et al. 2014; Díaz-Cruz and Barceló 2015; Ramos et al. 2016).

In tropical countries, such as Brazil, the incorporation of these substances in aquatic environments is possibly higher, due to the climatic characteristics associated with higher consumption of products containing UV filters (Mizukawa et al. 2017). Labille et al. (2020) estimated that during the summer about 1.4 ton/month of UV filters are released by beachgoers into the aquatic environment in the French coast of the Mediterranean Sea. In addition, the low efficacy of domestic effluent collection and treatment systems in many countries, especially in developing countries, is responsible for the continuous introduction and accumulation of these contaminants in aquatic environments (Gago-Ferrero et al. 2013a; Mizukawa et al. 2017). Since water bodies are the main destination of UV filters and they show low biodegradability, aquatic organisms may bioaccumulate them in lipid tissues and could be subject to potential adverse effects (Blüthgen et al. 2014; Manová et al. 2015; Necasová et al. 2016).

Considering the potential risks associated with the occurrence of UV filters in aquatic ecosystems, environmental monitoring has gained focus in recent years (Ramos et al. 2015; Manová et al. 2015; Sharifan et al. 2016; Park et al. 2017). In seawater, benzophenone-3 (BP-3), 2-ethylhexyl, 4-methoxycinnamate (EHMC), and octocrylene (OC) were detected in concentrations of 33 to 118, 5 to 83, and 32 ng/L, respectively (Nguyen et al. 2011; Magi et al. 2012). Relatively higher concentrations of UV filters were found in freshwater bodies, including 170 ng/L of OC and 260 ng/L of EHMC in treated water from Spain (Díaz-Cruz et al. 2012); 112 ng/L of OC and 10 ng/L of EHMC in river water and 47.1 ng/g of EHMC of dry weight of sediment in a Colombian environment (Barón et al. 2013). In a freshwater recreational reservoir in Australia, OC was the most dominant UV filter, with concentrations ranging from 1380 to 4660 ng/L, followed by avobenzone (AVO, 4-tert-butyl-4′-methoxydibenzoylmethane) (424 to 1130 ng/L) (O’Malley et al. 2021). AVO was detected in river water in concentrations of 36 to 38 ng/L (Tsui et al. 2014), 2431 ng/L (Rodil et al. 2009a), and 20 ng/L (Balmer et al. 2005).

Bioaccumulation of EHMC and OC in fish showed values of 240 and 30 ng/g dry weight, respectively (Gago-Ferrero et al. 2013). In the liver of marine mammals from Brazil, concentrations of OC ranging from 89 to 782 ng/g dry weight were detected (Gago-Ferrero et al. 2013). Besides bioaccumulation, toxic effects on exposed organisms were also reported. In the study of Kaiser et al. (2012), EHMC affected the reproduction of snails Potamopyrgus antipodarum and Melanoides tuberculata exposed to 0.4 and 10 mg/kg, respectively. Morphological changes were also observed in Danio rerio at concentrations above 100 mg/kg of EHMC, while AVO and OC did not cause significant effects. Liu et al. (2015) reported hepatic damage on fish Carassius auratus after exposure to 0.5 to 3 mg/L of BP-3. In embryos of Chironomus riparius, OC and EHMC caused stress and disturbances on endocrine regulation (Ozáez et al. 2016).

Recent works have shown that exposure to UV filters leads to increased production of reactive oxygen species (ROS) and consequently to oxidative stress in different organisms such as fish (Nataraj et al. 2020; Huang et al. 2020; Liu et al. 2015; Rodríguez-Fuentes et al. 2015); cyanobacteria (Mao et al. 2020), microalgae (Esperanza et al. 2019), bivalve mollusks (Falfushynska et al. 2021) and polychaetas (Cuccaro et al. 2022), among others. Despite the potential adverse effects caused by these substances, few studies have investigated their toxicity at environmentally relevant concentrations (Blüthgen et al. 2012; Li et al. 2018). Daphnia magna is a suitable model organism to evaluate ecotoxicological effects because of its body size, short life cycle, high fecundity, and parthenogenetic reproduction, in addition to its easy culture, handling and low-cost of maintenance in the laboratory (Tkaczyk et al. 2021). The life cycle of Daphnia may be characterized as sexual or asexual mode of reproduction. In favorable conditions, Daphnia undergo cyclic parthenogenesis (Ebert 2005; Tatarazako and Oda 2007). The main advantage of
parthenogenesis in ecotoxicological tests is the genetic uniformity of the organisms, generating clones that, in theory, will respond very similarly to the effects of exposure to the contaminant. This characteristic, together with the high fecundity of D. magna, allows for obtaining many organisms needed for tests in short time (Koivisto 1995). Also, some micropollutants can interfere with the endocrine system and cause a change in the reproductive mode from parthenogenesis to sexual reproduction, leading to the production of male neonates. (Tatarazako and Oda 2007). These findings showed usefulness of D. magna in toxicity assessment of compounds with endocrine activity. In reproduction tests with D. magna, multiple reproductive parameters can be evaluated, from the first period of life to the adult stage. However, standardized protocols generally do not consider multigenerational effects. Recent studies show that exposing organisms for several generations to xenobiotics brings more realistic and relevant results in population and ecological terms (Araújo et al. 2019; Castro et al. 2018; Barata et al. 2017; Campos et al. 2016).

In this sense, the objective of this work was to evaluate the acute toxicity of EHMC, AVO, BP-3, and OC in three different aquatic organisms (D. magna, A. salina, and D. subspicatus), followed by the investigation of the chronic effects caused by environmentally relevant concentrations of the UV-filters, both isolated and mixed, in two consecutive generations of D. magna chronically exposed. For this purpose, longevity, time to produce the first offspring, reproduction rate, and oxidative stress (activities of the catalase and glutathione-S-transferase enzymes) were evaluated for the chronic exposure experiment.

Material and methods

Chemicals

Standards of 2-ethylhexyl-4-methoxycinnamate (EHMC, CAS 5466-77-3, 98.7%), avobenzone (AVO, CAS 70356-09-1, 98%), benzophenone-3 (BP-3, CAS 131-57-7, 99.9%) and octocrylene (OC, CAS 6197-30-4, 99.8%) were purchased from Sigma-Aldrich. First, stock solutions for each of the UV filters (2 g/L) were prepared in purified water (with a reverse osmosis system) using dimethyl sulfoxide (DMSO, 99%, Synth) as solvent due to their low solubility in water. For all test solutions, the final concentration of DMSO did not exceed 0.03%. Solutions of intermediate concentration (1 mg/L) were made from stock solutions in ultrapure water, followed by a second dilution to prepare the final chemical solutions tested. Further details will be described in the methodology for each assay. Additionally, a negative control group and a control group with the solvent solution (0.03% DMSO) were also prepared in all bioassays. The pH of solutions ranged between 6.8 to 7.1. All chemicals were of analytical grade.

Artemia salina acute toxicity assay

The tests followed the methodology described in NBR 16530 (ABNT 2016b). The cysts that gave rise to the nauplii used in these tests were purchased commercially from a local producer. In a 1 L separatory funnel, 200 mg/L of cysts were added in 500 mL of reconstituted seawater, previously aerated. They were incubated for 24 h at 24 °C in the dark (ABNT 2016a).

After hatching, viable organisms were selected through a light source at the top of the separation funnel that separated unhatched shells and cysts precipitated at the bottom of the funnel. Viable organisms were individually distributed into 96-well microplates, with one nauplii per well, and 10 wells for each treatment concentration, or control and 0.03% DMSO, all in triplicates of microplates. The nominal tested concentrations ranged from: 1.25 to 3.0 mg/L for AVO; 0.25 to 3.0 mg/L for BP-3; 0.1 to 0.8 mg/L for EHMC; and 0.75 to 5.0 mg/L for OC (Detailed description available in the Supplementary Material as Table S1). These solutions were prepared in reconstituted seawater from the intermediate stock solution (1 mg/L).

After 48 h, immobile organisms were counted to determine the percentage of immobility (ABNT 2016a). Results were expressed as a function of the effective concentration at 50% of the survived organisms (EC50).

Desmodesmus subspicatus growth inhibition test

The growth inhibition test was performed according to technical standard NBR 12648:2018 (ABNT 2018). D. subspicatus was obtained from permanent culture performed in the Laboratory of Ecotoxicology (UTFPR, Brazil).

Three days before the test initiate, the microalgae were inoculated in the liquid medium for preculture. After this period, the cell density was measured in 250 μL of sample in a 96-well microplate at a fixed wavelength of 750 nm in a Fluostar Omega spectrophotometer. Then, the corresponding volume of 10^5 cells/mL was inoculated in 125 mL Erlenmeyer containing DIN nutrient medium, water of reverse osmosis, and the test solutions of the UV filters (AVO; BP-3; EHMC; OC; DMSO 0.03% and control with osmosis water). The final volumes of the bottles were adjusted to 50 mL (ABNT 2018). The tested concentrations ranged from: 0.25 to 2.5 mg/L for AVO; 0.25 to 1.5 mg/L for BP-3; 0.05 to 1.10 mg/L for EHMC; and 0.5 to 3.0 mg/L for OC (Supplementary Material as Table S2).

All flasks (glass) and nutrient media were previously autoclaved and the procedures were performed in a laminar flow chamber. The assay was performed under controlled...
temperature (23 to 25 °C), with the flasks maintained under constant agitation (orbital shaking table, Tecnal, model TE-1400) at 145 rpm and brightness of approximately 7000 lux, measured through a luxmeter (Homis). All samples and controls were performed in triplicate (ABNT 2018).

After 72 h, cell density was measured in a Fluostar Omega microplate reader, as previously described, to determine the growth inhibition rate of each treatment concentration. Results were expressed as a function of the EC50 values (ABNT 2018). Additionally, the no observed effect concentrations (NOEC) were determined, from EC10 values (concentrations that caused 10% growth inhibition compared to the control) that were calculated from the linear relationship between the percentage of inhibition of growth and concentrations used in the exposure.

**Daphnia magna acute toxicity assay**

The organisms used in these tests were obtained through a permanent culture of *D. magna* in the Laboratory of Ecotoxicology (UTFPR, Brazil). The tests were performed according to NBR 12713: 2016 (ABNT 2016b). The sensitivity of the organisms was tested using a control chart with a reference toxicity substance (ZnSO4, 99% purity).

Ten neonates (6 to 24 h) were distributed in beakers containing 25 mL of the test solution, reconstituted water (negative control), or DMSO 0.03% (solvent control) and incubated at 20 ± 2 °C, in the dark, for 48 h. After this, the immobilized organisms were accounted for and registered for the immobility rate to calculate the effective concentration of 50% of the immobilized organisms (EC50). All tests were performed in triplicate (ABNT 2016b). The tested concentrations ranged from: 1.0 to 3.5 mg/L for AVO; 0.75 to 2.5 mg/L for BP-3; 0.1 to 1.0 mg/L for EHMC; and 0.5 to 7.0 mg/L for OC (Supplementary Material as Table S3).

**Daphnia magna multigenerational exposure**

**Environmental concentrations selection**

Environmentally relevant concentrations of EHMC, AVO, BP-3, and OC were chosen considering the available literature data until the experimental time. For all compounds, concentrations were based on data found worldwide, and for each chemical one concentration was selected: the highest concentrations reported by Brausch and Rand (2011), which were 224, 175 and 4450 ng/L for EHMC, BP-3 and OC, respectively.

To perform the chronic toxicity tests, intermediate concentration solutions (1 mg/L) were made from stock solutions in ultrapure water, followed by a second dilution to prepare the final chemical solutions in *D. magna* M4 culture medium (ABNT 2016b). The final concentrations of EHMC, AVO, BP-3, and OC were 0.2; 4.4; 0.17, and 4.4 μg/L, respectively. A mixture solution (MIX) containing all UV filters was prepared with the same concentrations of an individual compound in an M4 culture medium. The solvent control solution was prepared with DMSO in an M4 culture medium not exceeding 0.03%. All solutions were kept in amber glass bottles and refrigerated until use, and all other chemicals were of analytical grade.

**Daphnia magna multigenerational exposure**

*D. magna* reproduction test was performed according to OECD 211 protocol (OECD 2012). The experimental design of multigenerational exposure was based on the methodology described by Campos et al. (2016) and Barata et al. (2017). In addition, a negative control (M4 medium) and a solvent control (0.003% DMSO) groups were used, defined from the highest concentration of solvent present in the sample groups.

Two generations of organisms were evaluated in a 21d exposure: the first named F0 (parental generation) and the second named F1, originated from the third brood of the F0 generation (21st day of the test). The exposure and data evaluation procedures were the same for F0 and F1. Twelve neonates from *D. magna* cultures under standard conditions (ABNT 2016a) were exposed individually in beakers containing 30 mL of the test or control solutions.

The assays were performed in an incubator with a temperature of 20 ± 2 °C and a photoperiod of 16 h light/ 8 h dark. The solutions were fully renewed every 48 h, organisms were fed with the microalgae *Desmodesmus subspicatus* (10^6 cells/mL per replicate), and the offspring were counted and discarded (OECD 2012).

After 21d of exposure, organisms from each group and controls were collected in microtubes for evaluation of chronic exposure parameters and biochemical biomarkers. The following life cycle parameters were evaluated: longevity (mean percentage of living organisms up to the end of the test), time for first reproduction (average time in days needed for the first offspring), and reproduction rate (Eq. 1) (OECD 2012).

\[ RR = \frac{(R_1 + R_2 + R_3 + \ldots + R_n)}{n} \]

where RR is the reproduction rate; R is the total of neonates produced during 21d by replicate; n is the number of living replicates at the end of the test.

**Biochemical biomarkers**

Biochemical biomarkers analysis was performed only for the two generations of *D. magna*, F0 and F1, sampled after 21d of exposure. Each treatment group and control (twelve individuals each) were divided into three pooled samples (triplicates) containing four living individuals that were
placed in microtubes of 2 mL with 250 μL of phosphate buffer (0.1 M, pH 7.2). In samples containing eleven living organisms, two tubes were prepared with four individuals and one with three.

Then, the triplicates (three to four individuals per replicate) were immediately homogenized at 14,000 rpm and their tissues were mechanically disaggregated in a rotary homogenizer. After this, samples were centrifuged at 8000 G for 10 min at 4 °C in a centrifuge. The supernatant fraction was used on the same day for the determination of total proteins, the activity of the catalase (CAT) and glutathione-S-transferase (GST) enzymes, to obtain a higher activity measurement, instead of freezing and thawing samples. Negative and solvent control samples were used in both generations.

**Total proteins determination**

The procedure for the determination of total proteins followed the Bradford method (Bradford 1976), adapted for 96-well microplates. For this, 10 μL of sample or diluted bovine serum albumin (BSA, Sigma-Aldrich), used as standards for curve calibration, were used in triplicate followed by 250 μL of Bradford reagent (Sigma-Aldrich) in 96 well microplates with the absorbance measured at 595 nm in a spectrophotometer (Fluostar Omega, BMG). After determination of protein concentrations, samples were diluted and normalized to 1 mg/mL of proteins.

**Catalase (CAT) activity**

Catalase activity was measured based on the degradation of hydrogen peroxide detected with the absorbance decrease at 240 nm (Aebi 1984). In a 96-well polystyrene microplate UV-STAR® (Greiner) 30 μL of sample, followed by 70 μL of Tris-HCl buffer (20.0 mM; EDTA 1.0 mM; pH 7.6) and 150 μL of reaction medium (Tris-HCl buffer 1.0 M; EDTA 5.0 mM; pH 8.0; ultra-pure water; H₂O₂ 30 mM) were added into each well in triplicates. The plate was immediately taken to the microplate reader (Fluostar Omega, BMG) to detect the kinetic reaction, with a fixed wavelength at 240 nm and reading cycles every 20 s for 2 min.

**Glutathione-S-transferase (GST) activity**

The determination of Glutathione-S-transferase activity (GST) was performed based on the methodology described by Habig et al. (1974), where conjugation reaction between 1-chloro-2,4-dinitrobenzene (CDNB) and the enzyme glutathione-S-transferase (GST) generates thioether, which is detected by the increase of absorbance at 340 nm.

In each well of the microplate, 20 μL of sample and 180 μL of reaction media was added (1.5 mM GSH, 2.0 mM CDNB, 1.0 M potassium phosphate buffer) in triplicates. Immediately after the addition of the reaction medium, an absorbance reading was performed on a microplate reader (Fluostar Omega, BMG) with a fixed wavelength at 340 nm in 20 s cycles for 6 min.

**Statistical analysis**

Calculations were performed using the GraphPad Prism 5.0 software, in which the EC was determined, with a 95% of confidence interval (ABNT 2016b).

Results were presented as the means ± standard deviation (SD). Data were statistically analyzed, initially by analysis of normality with the Kolmogorov-Smirnov test. Data with normal distribution were evaluated with two-way ANOVA followed by Dunnett’s test, comparing treatment groups and the two generations for each group. Significant differences were set at p < 0.05 (*) and p < 0.01 (***).

**Results and discussion**

The data obtained in this study report the levels capable to induce acute toxicity of four UV-filters substances in three distinct aquatic organisms and evidenced multigenerational effects in *D. magna* exposed to environmental concentrations of these chemicals.

**Acute toxicity assays**

The toxicity tests were validated by the absence of significant effects for the control and DMSO 0.003% organisms, as well as by the high coefficients of linear regression (r² > 0.93), obtained for the tested concentrations versus the responses observed for the three different organisms (Table 1). The EC₅₀ values found for *A. salina* were: AVO = 2.22, BP-3 = 2.53, EHMC = 0.37 and OC = 2.97 mg/L (Detailed description in the Supplementary Material as Figure S1).

While the EC₅₀ values determined for AVO, BP-3 and OC were similar, greater toxicity was observed for *A. salina* exposed to EHMC, which showed a lower EC₅₀ of 0.37 mg/L. Thorel et al. (2020) evaluated the toxic effects of ten UV filters for *A. salina*. Results indicated that among the different UV filters tested, OC was the most toxic (EC₅₀ = 0.6 mg/L), followed by AVO (EC₅₀ = 1.8 mg/L). For BP-3, no toxicity was observed even at the highest concentration. It is noteworthy that this is the first study reporting the EC₅₀ concentration of EHMC in *A. salina*, highlighting the importance of investigating the potential toxicity of these chemicals in different aquatic organisms.

Due to the high liposolubility, these molecules tend to bioaccumulate and thus increase potential effects over time (Blüthgen et al. 2014; Manová et al. 2015; Necasová et al. 2016). The bioaccumulation and biomagnification capacity
of UV filters in brine shrimp were demonstrated by Li et al. (2018). Second-generation (F1) of Danio rerio embryos exhibited toxic effects after trophic exposure of F0 fed with contaminated nauplii with BP-3, EHMC, and OC. UV filters have a high capacity to contaminate marine environments, especially during the summer, when there is a significant consumption increase in recreational activities on the coasts (Sharifanet al. 2016).

The EC50 values found for D. subspicatus were even lower than those for A. salina: EHMC = 0.37, AVO = 1.07, BP-3 = 0.82 and OC = 1.54 mg/L (Table 1). Similarly, it was observed that EHMC demonstrated the greatest toxic potential of the analyzed UV filters, significantly inhibiting D. subspicatus growth at concentrations below 0.5 mg/L. Sieratowicz et al. (2011) determined the EC50 for BP-3 using D. subspicatus and obtained similar concentrations (0.96 mg/L) from this study. They also obtained the EC10 value of 0.61 mg/L as an inhibitory concentration of 10% for microalgae proliferation. However, for the other molecules of interest, no available studies were found for comparison, only for other species of microalgae and under different exposure conditions. Rodil et al. (2009b) reported an EC50 value for Desmodesmus vacuolatus exposed to 0.19 and 0.36 mg/L of EHMC and BP-3, respectively (Table 1).

For Daphnia magna, the EC50 values showed a similar pattern than for other organisms, that is, the EHMC was the most toxic (Table 1, Figure S2). While AVO, BP-3, and OC showed related toxicity potential, EHMC presented the lowest and very similar EC50 values for the three different aquatic organisms (Table 1). The observed toxic effects could be partially explained by the relatively high liposolubility of the four molecules, which increases the ability to penetrate cell membranes and bioaccumulate in tissues (Fent et al. 2010; Pestotnik et al. 2014; Park et al. 2017). Fent et al. (2010), reported that D. magna exposed to different organic UV-filters (BP-3, BP-4, 4-methylbenzylidene camphor, and EHMC) showed increased toxicity with greater hydrophobicity of the compound. On the other hand, for the current study, the substance with higher hydrophobicity (OC, logKow = 7.35), had the lowest toxic potential (EC50 = 2.57 mg/L), while EHMC (logKow = 5.43) presented the highest degree of toxicity (EC50 = 0.37 mg/L), even though it was less lipophilic.

According to Kaiser et al. (2012), more lipophilic molecules do not necessarily have greater toxicity. The authors evaluated the effects of AVO (butyl-methoxydibenzoylmethane, BMDM) EHMC and OC on aquatic organism (C. riparius, L. variegatus, M. tuberculata and P. antipodarum) using sediment-based bioassays. Due to their life habits, these organisms are especially affected by lipophilic substances. Additionally, two direct sediment contact assays utilizing zebra fish (D. rerio) embryos and bacteria (A. globiformis) were conducted. EHMC was the only tested UV filter that showed effects in the test organism at the applied concentration range. AVO and OC had no effect on any endpoint for all test organism. The authors discussed that this could be explained by the lower bioavailability of AVO and OC, which can interact more easily with sediments, particle matter, and present low absorption capacity when ingested. In general, the results obtained herein corroborated with the previous descriptions of the scientific literature (Table 1), confirming the reproducibility and reliability of the experiments conducted in this study.

Since all UV filters used in the present work are considered lipophilic (Gilbert et al. 2013), their toxicity will largely depend on the bioavailability of the compound to exposed organisms. An increasing number of studies have indicated that the bioavailability of organic pollutants is affected by suspended sediments, especially for substances with high logKow values, such as UV filters (Yang et al. 2021). Therefore, sediment-based bioassays or studies comparing the toxicity associated with bioavailability of a complete formulation instead of a single UV-filter are needed (Barone et al. 2019; Fastelli and Renzi 2019, Mccoshum et al. 2016).

### Daphnia magna multigenerational exposure

In longevity analysis no significant differences were found for the F0 and F1 groups, demonstrating no lethality for D. magna at the exposure conditions tested (Table 2). It is important to emphasize that these results are related to the

| Table 1 Comparison of acute toxicity values for A. salina, D. subspicatus and D. magna with the literature |
|-----------------|-----------------|-----------------|
| SUBSTANCE       | A. salina       | D. subspicatus  | D. magna       |
|                 | EC50(72h) (mg L−1) | Literature (mg L−1) | EC50(72h) (mg L−1) | Literature (mg L−1) | EC50(72h) (mg L−1) | Literature (mg L−1) |
| EHMC            | 0.37            | –               | 0.37/0.02       | D. subspicatus: EC50(72h) = 0.07b  | S. vacuolatus: EC50(72h) = 0.19b  | 0.5             |
|                 |                 |                 |                 | S. vacuolatus: EC50(72h) = 0.055  |                   | 0.57b           |
| OC              | 2.97            | 0.6e            | 1.54/0.46       | S. vacuolatus: EC50(72h) = 0.06b  | 2.57             |
|                 |                 |                 |                 | S. vacuolatus: EC50(72h) = 0.36    | 1.72             |
| AVO             | 2.22            | 1.8e            | 1.07/0.18       |                   | 1.89             |
|                 |                 |                 |                 |                   | 1.95d           |
| BP-3            | 2.53            | NT*             | 0.82/0.28       | D. subspicatus: EC50(72h) = 0.96b  | 1.07             |
|                 |                 |                 |                 | S. vacuolatus: EC50(72h) = 0.056   | 1.67*           |

References: aThorel et al. 2020; bSieratowicz et al. 2011; cRodil et al. 2009b; dPark et al. 2017
period of chronic exposure used at work (21 days) and that they should not be extrapolated to the complete life cycle of *D. magna*. The absence of effects at this endpoint was expected, considering the low concentrations of UV filters (ng/L) used in this study, which are lower than the concentrations capable of causing mortality, even for longer exposure tests. As confirmed in this study, EC₅₀ values for AVO, BP-3, EHMC, and OC in exposures of 48 h have been described in mg/L range (Rodil et al. 2009b; Sieratowicz et al. 2011; Kaiser et al. 2012; Park et al. 2017) and no observed effect concentrations (NOEC) in μg/L (Sieratowicz et al. 2011; Lambert et al. 2021).

Regarding reproductive endpoints, no significant delay was observed for the period to produce the first offspring in F₀, as well as no differences in reproduction rates, were found when comparing the treatment groups with the control group. On the other hand, for F₁ generation, AVO (10.33 d), BP-3 (10.08 d) and MIX (10.09 d) groups showed a longer period to produce the first offspring than F₁ control (8.2 d). Again, in the F₁ generation, an inhibitory effect after 21d of exposure was found in groups exposed to AVO (34.50 neonates/replicate), BP-3 (31.92 neonates/replicate), EHMC, and MIX (35.17 neonates/replicate) when compared to the F₀ (47.20 neonates/replicate) and F₁ controls (46.0 neonates/replicate). For all analyzed parameters, in both generations, there were no significant differences between negative control and solvent control groups, confirming the absence of effects for the DMSO solvent at the tested concentrations.

Also, possible effects related to different cultivation conditions (population density and food availability) that preceded the beginning of the F₀ and F₁ trials may have influenced the observed reproductive parameters. Barata et al. (2017) emphasize that maternal effects are an important source of unexplained variance difficult to control and highly neglected in ecotoxicological investigations. According to the authors, *D. magna* females regulate the quality and quantity of their offspring depending on the feeding conditions they encounter and require about three generations to adapt their progeny to a given new feeding condition. In this context, an adaptation of the protocols establishing density and feeding conditions in daphnid cultures before initiating multigenerational assays would be highly recommended, in order to minimize maternal effects (Barata et al. 2017).

No significant effects were observed for the first generation exposed to the UV filters, but they were evident in the F₁ generation, proving that multigenerational studies represent a more realistic approach considering longer-term effects through the life cycle and generations of *D. magna*. In most cases, contaminants can affect exposed organisms and their offspring, with toxic effects being aggravated in consecutive generations (Campos et al. 2016). Since *D. magna* has a cyclical parthenogenesis that combines sexual and asexual reproduction depending on the environmental conditions to which it is exposed, the effects of xenobiotics must be considered at all stages of the organism’s life cycle (De Meester et al. 2006). In this context, Navis et al. (2015) investigated the effects of fenoxycarb (a pesticide) exposure on dormant eggs at different embryonic developmental stages and evaluated the degree of protection against pollution provided by the ephippia case. The authors conducted a hatching experiment in which decapsulated and encapsulated dormant eggs were exposed to an insect growth regulator (fenoxycarb) at different stages during their development, both before and after activation of the eggs. The findings revealed that pesticides can bioconcentrate in and affect *D. magna* dormant eggs. The severity of the effects on developing embryos depends on the timing of pesticide exposure. The results stress the importance of considering the full life-cycle of model organisms used in ecotoxicological studies, since these are ultimately aimed at assessing risks of chemical exposure on natural aquatic ecosystems.

According to Lambert et al. (2021), significant delays in the first offspring and reduction in the total number of neonates of *D. magna* were observed after exposure to BP-3 (166 μg/L) in a traditional chronic 21d trial. The same significant effects were not observed after exposure to EHMC (75 μg/L) by these authors. These data corroborate with the results observed for...
the F1 generation of BP-3 and AVO in the current study. Even though exposed to lower concentrations, these two molecules demonstrate potential for reproductive-endocrine interference, as well as for EHMC (Morohoshi et al. 2005; Ozáez et al. 2016; Wang et al. 2016). The benzophenones group is constantly related to multiple estrogenic and androgenic changes in fish, rats, and humans. The most common effects are inhibition of estradiol, activation of estrogen receptors, induction of vitellogenin production in fish, and inhibition of the action of testosterone in rats. Endocrine regulation contributes to the normal functioning of an organism. If affected, it can trigger dysfunctions, for example, delayed reproductive development, decreased or complete inhibition of reproductive rates (Morohoshi et al. 2005; Campos et al. 2016; Ozáez et al. 2016; Wang et al. 2016). Additionally, the biotransformation of these compounds with endocrine disruption potential may increase those effects, since multiple metabolites can be generated (Morohoshi et al. 2005; Molina-Molina et al. 2008; Watanabe et al. 2015).

UV filters are molecules of low solubility in water and high lipophilicity. EHMC has a solubility of 0.156 mg/L and log Kow = 5.80, BP-3 with 68.6 mg/L and log Kow = 3.79, OC with 0.004 mg/L and log Kow = 6.88 and AVO with 2.2 mg/L, and log Kow = 6.88 (Rodil et al. 2009b; Kaiser et al. 2012). Therefore, possible bioaccumulation effects can also be suggested, since exposures over generations may cause higher levels of organic compounds in the tissues of organisms, especially in generations following the parental. This may happen because the organisms remain exposed to the contaminants from embryonic stages until adult life. In addition, when a substance is not metabolized and excreted at the same rate at which it is absorbed, bioaccumulation and biomagnification may occur, depending on the trophic level and form of exposure. Bioaccumulation of organic contaminants can compromise cell metabolism functions, generate oxidative stress, cause damage to lipid membranes, interfere with cellular permeability, and hinder the transport of nutrients (Fent et al. 2010; Pestotnik et al. 2014; Gago-Ferrero et al. 2015).

When F1 offspring were exposed to a MIX sample (AVO 4.4; BP-3 0.17; EHMC 0.2; and OC 4.4 μg/L), reproductive effects were statistically like those observed for the isolated compounds. Park et al. (2017) observed that the interaction between AVO, EHMC, and OC after acute exposure (48 h) in D. magna was antagonistic, showing that the mixture of the three molecules, in this case, was less toxic than when isolated. However, due to the presence of BP-3 in our MIX sample in addition to AVO, EHMC and OC, it was not possible to establish what type of interaction occurred, since the coexistence of different contaminants can influence the interaction, absorption and toxicity of a mixture (Pablos et al. 2015).

The reproductive effects observed for the F1 generation exposed to environmental concentrations of UV filters should be considered of ecological relevance, since a reproductive delay and a decreased reproductive rate will impact at the populational level. Under continuous exposure, the reduction in reproductive output, observed after only two successive generations, is expected to have negative consequences in terms of population size and trajectory (Campos et al. 2016; Silva et al. 2019).

Recent studies have also evaluated the chronic effects of UV filters on D. magna, but in general they differed from the filters chosen, concentrations tested, the endpoints, and the methodology, when compared to this current study. Lambert et al. (2021) investigated the sublethal effects in D. magna by chronic exposure to three organic UV filters, 4-methylbenzylidene camphor (4MBC), EHMC (OMC) and BP-3, with particular emphasis on molting and development. However, the experimental design used was significantly different from the one described in the present work. The authors performed chronic exposure of 21 days in two generations (F0 and F1), however the concentrations of the filters were different for each generation (EHMC 0.03 75 μg/L, BP-3 0.68 500 μg/L and 4MBC 0.55 400 μg/L in the F0 generation) and using acetone as a solvent. In the second generation (F1), the concentrations used were well above the environmentally relevant ones (75 μg/L EHMC, 166 μg/L BP-3 and 130 μg/L 4MBC). No significant effects were observed at the end of the first 21 days of exposure (F0 generation), only in the F1 generation. The same occurred in the present work, but the significant effects on the F1 generation were due to exposure to environmentally relevant concentrations of BP-3 and EHMC (0.17 and 0.2 μg/L, respectively). This demonstrates that even at low concentrations, continuous exposure to UV filters interferes with the life cycle and reproductive patterns of D. magna. Future studies with a greater number of exposed generations and other analyzed endpoints would bring a closer picture to what occurs in natural environments.

Boyd et al. (2021) evaluated the acute and chronic effects of AVO, BP-3 (oxybenzone) and OC, isolated and mixed, in D. magna at environmentally realistic concentrations. However, the experimental design of the chronic assay also differed from the one performed in the present work. The authors exposed two groups independently for 21 days each. In the first group the concentrations were: AVO = 150 μg/L, OC = 7.5 μg/L, BP-3 = 100 μg/L, followed by a second exposure group at concentrations approximately an order of magnitude lower (AVO = 20 μg/L, OC = 0.5 μg/L, BP-3 = 2 μg/L). Chronic exposure to 7.5 μg/L OC yielded complete mortality within 7d, while sublethal chronic exposure to AVO (20 μg/L) increased the average brood size per daphnid by nearly 1.5-fold over OECD controls to 13 ± 0.85 neonates/brood (Kruskal-
Wallis, p < 0.01). The other reproductive parameters analyzed were not affected or the effects were not statistically significant. Comparing these results with those observed in the present work, it is evident that AVO has an endocrine-disrupting effect and this affects the reproductive rate of D. magna. However, our results showed just the opposite effect, a reduction in the reproductive rate and a delay in the generation of the first offspring when compared to the control. Two relevant points in the experimental conditions may be related to the observed results: the concentration of AVO used (4.4 μg/L instead of 20 μg/L) and the multigenerational design in chronic exposure (F1 generation originated from the F0 generation). A higher concentration of AVO (20 20 μg/L) may have led to a greater reproductive effort, producing more neonates/brood. According to Chatterjee et al. (2019), this is an important phenotypic adaptation strategy, particularly in species with short generation times such as D. magna. Furthermore, in our case the individuals of the F1 generation were generated by the adults of the F0 generation submitted to chronic exposure to AVO and other UV filters. Therefore, effects inherited from the mothers may have added to the exposure to AVO and this reduced the average number of neonates/adults.

Biochemicals biomarkers

After exposure to the UV filters, the F0 and F1 generations showed different activities for the catalase (CAT) but not for the glutathione-S-transferase (GST) enzyme (Fig. 1).

With the results obtained for CAT activity, no significant differences were observed for the groups exposed in the first generation (F0). However, the F1 organisms exposed to BP-3 and EHMC had significantly increased the CAT activity, compared to F1 Control. On the other hand, activity of GST did not change significantly between the treatment groups compared to the control group, both for F0 and F1.

The increase in CAT activity in F1 may suggest an induction of the antioxidant defense system by the exposure of BP-3 and EHMC. The antioxidant system present in eukaryotic cells allows protection against reactive oxygen species (ROS) that are generated in the detoxification of xenobiotics. The enzymes CAT and GST exert important functions in the degradation of ROS. In peroxisomes, xenobiotics undergo oxidation with the generation of hydrogen peroxide (H2O2) as a by-product which is degraded by CAT (Boelsterli 2003; Oost et al. 2003). Organic molecules having aromatic rings and hydroxyls in their chemical structures, as in the case of BP-3 and EHMC, may undergo biotransformation during cellular metabolism and generate reactive intermediates increasing the production of ROS and consequently the activity of detoxifying enzymes, such as CAT, GST and SOD (Boelsterli 2003; Watanabe et al. 2015).

In fact, oxidative stress in fish (Carassius auratus) liver was triggered by 0.5 mg/L of BP-3 exposure (Liu et al. 2015), with the production of ROS and altered SOD, GST and CAT activities. Increase in CAT activity in the protozoan Tetrahymena thermophyla caused by 1 μg/L of BP-3 was also reported by Gao et al. (2013). Regarding Spearman’s correlation analysis, there was no significant correlation between any of the analyzed parameters. On the other hand, the cadisfly Sericostoma vittatum exposed to BP-3 and 4-MBC at environmental relevant concentrations showed increased levels of total glutathione, but no additional effects related to oxidative stress, biotransformation enzymes and neurotoxicity endpoints (Campos et al. 2016).

Although several studies have reported oxidative stress after exposure to UV filters, few have evaluated this effect in D. magna. Yang et al. (2021) evaluated the activities of antioxidant enzymes (CAT, SOD and GST) after acute exposure (24 h) of D. magna to AVO (100 μg/L) alone and associated with sediment. After 24 h of exposure, CAT and SOD activities increased in relation to the control. Despite the different experimental conditions (concentration, exposure time, etc.), we also observed an increase in catalase activity after 21 days of exposure to BP-3 and EHMC. Na et al. (2021) observed that microplastics (MPs) associated to...
BP-3 acute exposure (48 h) induced a synergistic increase in reactive oxygen species (ROS), total antioxidant capacity (TAC), and lipid peroxidation (LPO) in *D. magna*. These synergistic effects can be attributed to enhanced bioconcentrations of BP-3 in *D. magna* caused by MP fragments. Song et al. (2021) evaluated the chronic effects, after 7 and 17 days of exposition, of BP-3 (0.5 mg/L) isolated or associated with MPs on oxidative stress (LPO and TAC). BP-3 induced significantly (*p < 0.05*) higher LPO levels in *D. magna* at 7 d.

Among the UV filters analyzed, EHM C followed by BP-3 showed the highest toxic potential in acute tests. In chronic exposure, the effects observed in the F1 generation revealed that AVO followed by BP-3 and MIX significantly affected the first offspring while BP-3, followed by EHMC, AVO and MIX affected the reproduction rate. The acute toxicity responses corroborated this result, showing that EROs generated by the exposure to UV filters represent the main filters analyzed, EHMC followed by BP-3 associated with prolonged exposure to environmental concentrations of these chemicals. More sensitive responses, such as biochemical biomarkers, are suitable for showing earlier effects that may predict others biological responses of higher ecological relevance, such as reproductive endpoints and population impact. Therefore, additional studies considering different levels of biological responses are necessary to better understand the real impacts of environmental chemicals, such as UV filters, in aquatic organisms at concentrations of environmental relevance.

**Conclusions**

This is the first study evaluating *D. magna* longevity, reproductive parameters, and biochemical biomarkers in two generations chronically exposed to environmentally relevant concentrations of AVO, EHMC, BP-3, and OC isolated and in mixture. It is important to emphasize that these UV filters represent the main filters used in personal care products worldwide (Kwon and Choi 2021).

The results reported in this study demonstrated that environmental concentrations of sunscreens chemicals are not lethal, but capable to causing sublethal effects, interfering with reproductive parameters along the life cycle of *Daphnia magna* in a chronic multigenerational exposure. Reproductive effects were observed only for the second-generation organisms exposed to AVO, BP-3, EHMC and MIX, and evidenced higher toxic potential of BP-3 and EHMC exposure. Also, a significant increase in the CAT activity in BP-3 and EHMC was observed, indicating the mechanism of induction of the antioxidant system for the degradation of EROs generated by the exposure to UV filters.

This study highlights the novelty and importance of the results showing these effects at concentrations of environmental relevance, since they approach real conditions of exposure found in natural environments, when compared to the classic tests of ecotoxicity. The earlier effects observed in the development of the second generation of *Daphnia magna* demonstrated the toxic potential of organic UV filters and the environmental risk involved in their continuous exposure, mainly when consisting in a mixture composed by the four contaminants evaluated, simulating situations that may constantly occur in water bodies.

Therefore, the presence of sunscreens in environments, even at low concentrations, can be harmful and detrimental to the life cycle of aquatic species, with potential for interferences, over generations, resulting in impacts on reproductive and antioxidant defense systems.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare no competing interests.

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