Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Acute Toxicity Characterization of Organic UV-filters and Chronic Exposure Revealing Multigenerational Effects in *Daphnia Magna*

Vinícius de C. S. de Paula
Universidade Tecnológica Federal do Paraná: Universidade Tecnologica Federal do Parana
Monike F. Gomes
Universidade Tecnológica Federal do Paraná: Universidade Tecnologica Federal do Parana
Lucia R. R. Martins
Universidade Tecnológica Federal do Paraná: Universidade Tecnologica Federal do Parana
Flávia Yamamoto
Universidade Estadual Paulista Júlio de Mesquita Filho: Universidade Estadual Paulista Julio de Mesquita Filho
Adriane Freitas (afreitas_27@yahoo.com.br)
Universidade Tecnológica Federal do Paraná https://orcid.org/0000-0002-9237-8435

Research Article

Keywords: SUNSCREEN, CONTAMINANTS OF EMERGING CONCERN, MIXTURES, MULTIGENERATIONAL EXPOSURE, BIOMARKERS

Posted Date: January 25th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1108592/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

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 firstly 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*), to further investigate multigenerational effects in *D. magna.* After confirming the acute toxicity, *D. magna* individuals 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 EC_{50} 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.

1. 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 TiO_2), and organic, which absorb UVA (320 to 400 nm) and UVB (280 to 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-Aundran 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 expressive 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); 112ng/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 oxidative stress and disturbances on endocrine regulation (Ozáez et al. 2016).

Despite the potential adverse effects caused by these substances, few studies have investigated their toxicity at environmentally relevant concentrations (Blügthgen et al. 2012; Li et al. 2018). A suitable model organism to evaluate these effects is *Daphnia magna* because of its size of body, short life cycle, high fecundity, and parthenogenetic reproduction beside its easy culture, handling and low-cost of maintenance in the laboratory (Tkaczyk et al, 2021). Reproduction bioassays, such as the protocol standardized by the OECD, are important for research in which the effects on the life cycle of organisms are evaluated (Barata et al. 2017; Castro et al. 2018; OECD, 2012). In reproduction tests with *D. magna*, multiple reproductive parameters can be evaluated, from the first period of life to the adult stage. However, these standardized tests usually do not consider multigenerational effects. Recent studies demonstrate the feasibility of extending the OECD methodology to more than one generation of

organisms exposed to xenobiotics of interest since they can show more realistic effects at populational levels (Araujo et al. 2019; Campos et al. 2016; Barata et al. 2017; Castro et al. 2018).

In this sense, the objective of this work was to initially verify and confirm 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 activities of the catalase and glutathione-*S*-transferase enzymes were evaluated for the chronic exposure experiment.

2. Material And Methods

2.1 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. Firstly, stock solutions for each of the UV filters (2g/L) were prepared in purified water (with a reverse osmose system) using dimethylsulfoxide (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 (1mg/L) were made from stock solutions in ultrapure water, followed by a second dilution to prepare the final chemical solutions tested. 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.

2.2 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 24h at 24 ºC in the dark (ABNT 2016b).

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 neonate per well, and 10 wells for each treatment concentration, or control and 0.03% DMSO, all in triplicates of microplates. The 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 48h, immobile organisms were counted to determine the percentage of immobility (ABNT 2016b). Results were expressed as a function of the effective concentration at 50% of the survived organisms

(EC_{50}).

2.3 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 25ºC), 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 72h, 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 EC_{50} values (ABNT 2018). Additionally, the no observed effect concentrations (NOEC) were determined, from EC_{10} 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.

2.4 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 2016a).

Ten neonates (6 to 24h) 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 ± 2ºC, in the dark, for 48h. 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 (EC_{50}). All tests were performed in triplicate (ABNT 2016a). 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)

2.5 Daphnia magna multigenerational exposure

2.5.1 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 2016). 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.

2.5.2 Daphnia magna multigenerational exposure

D. magna reproduction test was performed according to OECD 211 protocol (OECD, 2012). 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 by the F0 offspring that were born on the 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 2016) 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 16h light/ 8h dark. The solutions were fully renewed every 48h, 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 (Equation 1) (OECD 2012).

$$RR = (R1 + R2 + R3 + \cdots + Rn) / n \quad \text{(Equation 1)}$$

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.

2.6 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 8,000 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.

2.6.1 Total proteins determination

The procedure for the determination of total proteins followed the Bradford method (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.

2.6.2 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; H_2O_2 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.

2.6.3 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.

2.7 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 $p < 0.01$ (**).

3. 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.

3.1 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 ($r^2 > 0.93$), obtained for the tested concentrations versus the responses observed for the three different organisms (Table 1). The EC_{50} 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).

Table 1
Acute toxicity of UV filters to different indicator organisms.

SUBSTANCE	$EC_{50(48h)}$ (mg L^{-1})	$EC_{50(72h)}$ (mg L^{-1})	$EC_{50(48h)}$ (mg L^{-1})
	A. salina	*D. subpicatus*	*D. magna*
EHMC	0.37	0.37	0.5
OC	2.97	1.54	2.57
AVO	2.22	1.07	1.89
BP-3	2.53	0.82	1.72

Legend: EHMC = 2-ethylhexyl, 4-methoxycinnamate; BP3 = benzophenone-3; OC = octocrylene; AVO = avobenzone.

While the EC_{50} values determined for AVO, BP-3 and OC were similar, greater toxicity was observed for *A. salina* exposed to EHMC, which showed a lower EC_{50} 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 (EC_{50} =0.6 mg/L), followed by AVO (EC_{50} =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 EC_{50} 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 EC_{50} 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. subpicatus* growth at concentrations below 0.5 mg/L. Sieratowicz et al. (2011) determined the EC_{50} for BP-3 using *D. subspicatus* and obtained similar concentrations (0.96 mg/L) from this study. They also obtained the EC_{10} 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 EC_{50} value for *Desmodesmus vacuolatus* exposed to 0.19 and 0.36 mg/L of EHMC and BP-3, respectively (Table 2).

Table 2
Comparison of acute toxicity values for *A. salina*, *D. subspicatus* and *D. magna* with the literature.

	A. salina		*D. subpicatus*		*D. magna*	
SUBSTANCE	**$EC_{50(72h)}$ (mg L^{-1})**	**Literature (mg L^{-1})**	**$EC_{50(72h)}$ (mg L^{-1})**	**Literature (mg L^{-1})**	**$EC_{50(48h)}$ (mg L^{-1})**	**Literature (mg L^{-1})**
EHMC	0.37	---	0.37/0.02	*D. subspicatus*: $EC_{10(72h)}$ = 0,07[b] *D. vacuolatus*: $EC_{50(24h)}$ = 0,19[c]	0.5	0.57[b]
OC	2.97	0.6[a]	1.54/0.46	*D. vacuolatus*: $EC_{50(24h)}$ = 0,05[c]	2.57	3.18[d]
AVO	2.22	1.8[a]	1.07/0.18	---	1.89	1.95[d]
BP-3	2.53	NT[a]	0.82/0.28	*D. subspicatus*: $EC_{50(72h)}$ = 0,96[b] *D. vacuolatus*: $EC_{50(24h)}$ = 0,36[c]	1.72	1.67[a]/1.9[e]

References: [a] = Thorel et al, 2020; [b] = Sieratowicz et al., 2011; [c] = Rodil et al., 2009b; [d] = Park et al., 2017; [e] = Fent et al., 2010

Legend: EHMC = 2-ethylhexyl, 4-methoxycinnamate; BP3 = benzophenone-3; OC = octocrylene; AVO = avobenzone.

For *Daphnia magna*, the EC_{50} 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 EC_{50} values (48h) for the three different aquatic organisms (Table 2). This effect 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-methyl-benzylidene 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 (EC_{50} = 2.57 mg/L), while EHMC (logKow= 5.43) presented the highest degree of toxicity (EC_{50} = 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, since only EHMC caused significant effects on benthic organisms exposed to the same four UV-filters from this study. The authors discussed that this could be explained by the lower bioavailability of AVO and OC,

which can interact more easily with sediments, particulate matter, and present low absorption capacity when ingested. In general, the results obtained herein corroborated with the previous descriptions of the scientific literature (Table 2), confirming the reproducibility and reliability of the experiments conducted in this study.

3.4 Daphnia magna multigenerational exposure

This is the first study evaluating *D. magna* longevity, reproductive parameters, and biochemical biomarkers in more than one generation exposed to ambient concentrations of AVO, EHMC, BP-3, and OC in the mixture. It is important to emphasize that these UV filters represent the main filters used in personal care products worldwide (Kwon and Choi 2020) (Table 3).

Table 3
Effects observed after chronic multigenerational exposure of *Daphnia magna* to AVO, BP-3, EHMC. OC and the mixture.

Sample	Longevity F0 (%)	Longevity F1 (%)	First offspring F0 (days)	First offspring F1 (days)	Reproduction rate F0	Reproduction rate F1
Control	91.66	100	8.60 ± 0.84	8.25 ± 1.06	47.20 ± 6.37	46.00 ± 10.33
DMSO	100	100	8.83 ± 0.94	8.67 ± 1.78	41.08 ± 7.15	41.33 ± 11.83
AVO	91.66	100	9.17 ± 1.19	10.33 ± 0.89*****	46.18 ± 7.64	34.50 ± 8.07*****
BP-3	91.66	100	8.73 ± 1.10	10.08 ± 1.00*****	44.10 ± 5.00	31.92 ± 7.27*****
EHMC	100	100	8.50 ± 1.08	9.25 ± 1.48	47.40 ± 7.44	35.17 ± 6.63*****
OC	100	91.66	8.83 ± 094	8.73 ± 1.27	49.73 ± 8.31	39.45 ± 9.65
MIX	100	91.66	8.08 ± 0.90	10.09 ± 1.38*****	46.58 ± 6.80	35.18 ± 6.54*****

Legend: (DMSO) dimethylsulfoxide 0.003%; (AVO) avobenzone 4.4 µg L^{-1}; (BP-3) benzophenone-3 0.17 µg L^{-1}; (EHMC): 2-ethylhexyl, 4-methoxycinnamate 0.2 µg L^{-1}; (OC) octocrylene 4.4 µg L^{-1}; (MIX) mixture. (*****) Significant difference to control in ANOVA + Dunnett, with $p<0.05$.

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. 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, EC_{50} values for AVO, BP-3, EHMC, and OC in exposures of 48h have been described in mg/L range (Rodil et al. 2009b; Fent et al. 2010; 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.

Despite no significant effects were observed for the first generation exposed to the UV-filters, they were evident only 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 some cases, contaminants can affect exposed organisms and their offspring, with toxic effects being aggravated in consecutive generations (Barata et al. 2016; Campos et al. 2016).

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 is directly linked to the normal functioning of an organism. If affected, 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 may potentiate the disrupting 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 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 was exposed to a MIX sample (AVO 4.4; BP-3 0.17: EHMC 0.2; and OC 4.4 µg/L), reproductive effects were similar to those observed for the isolated compounds. According to Park et al. (2017), the interaction between AVO, EHMC, and OC is antagonistic, showing that the mixture of the three molecules is less toxic than when isolated. Despite this effect was not verified in the MIX results, it is important to consider the presence of BP-3 in the mixture, which hinders to predict their interactions (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 may potentially impact at the populational level. Thus, different levels of the biological organization can be affected and cause chain effects in future populations, as continuous exposure to contaminants directly affects the health of new individuals (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), OC, and BP-3, with particular emphasis on molting and development. They demonstrated that 4MBC, OC, and BP-3 affect development and long-term health in neonates of exposed parents at concentrations of 130, 75, and 166 µg/L, respectively. Additionally, the expression of endocrine-related genes is significantly altered by 4MBC and BP-3 exposure, which may be related to their developmental toxicity.

Boyd et al (2021) evaluated the acute and chronic effects of AVO, BP-3 and OC, isolated and mixed, in *D. magna* at environmentally realistic concentrations. The main results observed were: delayed mortality up to seven days post-exposure at 200 µg/L of AVO and OC; 21d chronic exposure to 7.5 µg/L OC yielded complete mortality within 7d, while sublethal chronic exposure to AVO increased *D. magna* reproductive output and decreased metabolic rate. BP-3 (2 µg/L) induced a 25% increase in metabolic rate of adult daphnids, and otherwise no toxic effects at this dose.

3.5 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 (Figure 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.

However, even without statistical significance, it was observed, for F1, a tendency of increased activity in the samples of AVO, BP-3 and MIX.

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 (H_2O_2) 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 (*Carssius auratus)* liver, was triggered by 0.5 mg/L of BP-3 exposure (Li et al., 2015), with the production of ROS and altered SOD, GST and CAT activities. Increase in CAT activity in *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.

In general, EHMC followed by BP-3 showed the highest toxic potential for the acute and chronic tests, respectively, among the analyzed UV-filter molecules. For the chronic exposure, both compounds were, capable to reduce reproduction rates and induce the catalase activity in F1, even at the lowest concentrations (0.17 and 0.2 µg/L). The acute toxicity responses corroborated this result, showing that EHMC followed by BP-3 induced higher toxicity at lower doses. Although NOEC levels were still above those found in aquatic environments, this study evidenced the toxic potential of EHMC and 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.

4. Conclusions

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.

Declarations

FUNDING

This work was supported by Federal University of Technology- Paraná (UTFPR) (Paula V.'s master scholarship) and the Fundação Araucária, Brazil (UTFPR/FA-11/2016). This study also was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil – CAPES (Gomes, M. master's scholarship). Yamamoto, F.Y. wish to thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for her Postdoctoral Research grant (Grant number 2016/15229-1)002E.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

AUTHOR CONTRIBUTIONS

Vinícius C. S. Paula: Conceptualization, Methodology, Writing, Data Interpretation, Original draft preparation, Investigation. Monike F. Gomes: Methodology, Visualization, Software, Validation. Lucia R. Rocha Martins: Validation, Experimental design, Supervision. Flávia Y. Yamamoto: curation, Methodology, Writing- Reviewing and Editing, Supervision. Adriane M. de Freitas: Experimental design, Reviewing and Editing, Supervision, funding. All authors contributed to the analysis and writing of the manuscript.

References

1. Aebi H (1984) Catalase *in vitro*. Meth Enzymol 105:121–126
2. Araujo GS, Abessa DMS, Soares AMVM, Loureiro S (2019) Multi-generational exposure to Pb in two monophyletic *Daphnia* species: Individual, functional and population related endpoints. Ecotoxicol Environ Saf 173:77–85. https://doi.org/10.1016/j.ecoenv.2019.02.001
3. Associação Brasileira de Normas Técnicas (ABNTa) (2016) NBR 12713. Ecotoxicologia aquática - Toxicidade aguda - Método de ensaio com *Daphnia* spp (*Cladocera, Crustacea*)

4. Associação Brasileira de Normas Técnicas (ABNTb) (2016) NBR 16530. Ecotoxicologia aquática - Toxicidade aguda - Método de ensaio com *Artemia sp* (*Crustacea, Brachiopoda*)
5. Associação Brasileira de Normas Técnicas (ABNT) (2018) NBR 12648:2018. Ecotoxicologia aquática - Toxicidade crônica - Método de ensaio com algas (*Clorophyceae*)
6. Avenel-Aundran M, Dutartre H, Goossens A, Jeanmougin M, Comte C, Bernier C, Benkalfate L, Michel M, Ferrier-Lebouëdec MC, Vingan M, Bourrain JL, Outtas O, Peyron JL, Martin L (2010) Octocrylene, an emerging photoallergen. Arch Dermatol 146:753–757. https://doi:10.1001/archdermatol.2010.132
7. Balmer ME, Buser H-R, Müller MD, Poiger T (2005) Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss Lakes.*Environ. Sci. Technol.*39,953–962. https://doi.org/10.1021/es040055r
8. Barata C, Campos B, Rivetti C, LeBlanc GA, Eytcheson S, McKnight S, Tobor-Kaplon M, De Vries BS, Choi J, Sarapultseva EI, Coutellec MA, Coke M, Pandard P, Chaumot A, Quéau H, Delorme N, Geffard O, Martínez-Jerónimo F, Watanabe H, Tatarazako N, Lopes I, Pestana JLT, Soares AMVM, Pereira CM, Schamphelaere K (2017) Validation of a two-generational reproduction test in *Daphnia magna*: An interlaboratory exercise. Sci Total Environ 579:1073–1083. https://doi.org/10.1016/j.scitotenv.2016.11.066
9. Barón E, Gago-Ferrero P, Gorga M, Rudolph I, Mendoza G, Zapata AM, Díaz-Cruz S, Bara R, Ocampo-Duque W, Páez M, Darbra RM, Eljarrat E, Barceló D (2013) Occurrence of hydrophobic organic pollutants (BFRs and UV-filters) in sediments from South America. Chemosphere 92:309–316. https://doi.org/10.1016/j.chemosphere.2013.03.032
10. Baki G, Alexander KS (2015) Introduction to Cosmetic Formulation and Technology. first ed. Wiley & Sons, Somerset
11. Blüthgen N, Meili M, Chew G, Odermatt A, Fent K (2014) Acumulation and effects of the UV-filter octocrylene in adult and embryonic zebrafish (*Danio rerio*). Sci Total Environ 476–475:207–217. https://doi.org/10.1016/j.scitotenv.2014.01.015
12. Boelsterli UA (2003) Mechanistic Toxicology: The molecular basis of how chemicals dirupt biological target, second edn. Taylor & Francis
13. Boyd A, Stewart CB, Philibert DA, How ZT, El-Din MG, Tierney KB, Blewett TA (2021) A burning issue: The effect of organic ultraviolet filter exposure on the behaviour and physiology of *Daphnia magna*. *Sci. Total Environ.* 750 (2021) 141707 https://doi.org/10.1016/j.scitotenv.2020.141707
14. Bradford M (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3
15. Brausch JM, Rand GM (2011) A review of personal care products in the aquatic environment: Environmental concentrations and toxicity. Chemosphere 82:1518–1532. https://doi.org/10.1016/j.chemosphere.2010.11.018

16. Campos B, Jordão R, Rivetti C, Lemos MF, Soares AM, Tauler R, Barata C (2016) Two-generational effects of contaminants in *Daphnia magna*: Effects of offspring quality. Environ Toxicol Chem 35:1470–1477. https://doi.org/10.1002/etc.3290
17. Castro BB, Freches AR, Rodrigues M, Nunes B, Antunes SC Transgenerational effects of toxicants: An extension of the *Daphnia* 21-day chronic assay? 2018.*Arch. Environ. Contamin. Toxicol.*74,616–626. https://doi.org/10.1007/s00244-018-0507-0
18. Díaz-Cruz MS, Barceló D (2015) Personal care products in the aquatic environment,. The handbook of environmental chemistry. vol 36. Springer, New York
19. Díaz-Cruz MS, Gago-Ferrero P, Llorca M, Barceló D (2012) Analysis of UV filters in tap water and other clean waters in Spain. *Anal. Bioanal. Chem.* (2012) 402:2325–2333 https://doi.org/10.1007/s00216-011-5560-8
20. Fent K, Kunz PY, Zenker A, Rapp M (2010) A tentative environmental risk assessment of the UV-filters 3-(4-methylbenzylidene-camphor), 2-ethyl-hexyl-4-trimethoxycinnamate, benzophenone-3, benzophenone-4 and 3-benzylidene camphor. *Mar. Environ. Res.* 69, Supplement 1, S4-S6. https://doi.org/10.1016/j.marenvres.2009.10.010
21. Fent K, Kunzac PY, Gomezd E (2008) UV filters in the aquatic environment induce hormonal effects and affect fertility and reproduction in fish. Chimia 62:368–375. doi:10.2533/chimia.2008.368
22. Fent K, Zenker A, Rapp M (2010) Widespread occurrence of estrogenic UV filters in aquatic ecosystems in Switzerland. Environ Pollut 158:1817–1824. https://doi.org/10.1016/j.envpol.2009.11.005
23. Gago-Ferrero P, Mastroianni N, Díaz-Cruz MS, Barceló D (2013) Fully automated determination of nine ultraviolet filters and transformation products in natural waters and wastewaters by on-line solid phase extraction–liquid chromatography–tandem mass spectrometry. J Chromatogr A 1294:106–116. https://doi.org/10.1016/j.chroma.2013.04.037
24. Gago-Ferrero P, Díaz-Cruz MS, Barceló D (2013) Multi-residue method for trace level determination of UV filters in fish based on pressurized liquid extraction and liquid chromatography-quadrupole-linear ion trap-mass spectrometry. J Chromatogr A 1286:93–101. https://doi.org/10.1016/j.chroma.2013.02.056
25. Gao L, Yuan T, Zhou C, Cheng P, Bai Q, Ao J, Wang W, Zhang H (2013) Effects of four commonly used UV filters on the growth, cell viability and oxidative stress responses of the *Tetrahymena thermophila*. Chemosphere 93:2507–2513. https://doi.org/10.1016/j.chemosphere.2013.09.041
26. Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8
27. Kaiser D, Sieratowicz A, Zielke H, Oetken M, Hollert H, Oehlmann J (2012) Ecotoxicological effect characterisation of widely used organic UV filters. Environ Pollut 163:84–90. https://doi.org/10.1016/j.envpol.2011.12.014

28. Kwon B, Choi K (2021) Occurrence of major organic UV filters in aquatic environments and their endocrine disruption potentials: A mini-review. Integrat Environ Assess Manag 17:940–950. https://doi.org/10.1002/ieam.4449
29. Labille J, Slomberg D, Catalano R, Robert S, Apers-Tremelo ML, Boudenne JL, Manasfi T, Radakovitch O (2020) Assessing UV filter inputs into beach waters during recreational activity: A field study of three French Mediterranean beaches from consumer survey to water analysis. Sci Total Environ 706:136010. https://doi.org/10.1016/j.scitotenv.2019.136010
30. Lambert FN, Gracy HR, Gracy AJ, Yoon SH, Scott RW, Rincon DM, Vulpe CD (2021) Effects of ultraviolet-filters on *Daphnia magna* development and endocrine-related gene expression. *Aquat. Toxicol..* 238 (2021) 105915. https://doi.org/10.1016/j.aquatox.2021.105915
31. Li AJ, Law JC, Chow CH, Huang Y, Li K, Leung KS (2018) Joint effects of multiple UV filters on Zebrafish embryo development. Environ Sci Technol 52:9460–9467. https://doi.org/10.1021/acs.est.8b02418
32. Li W, Ma Y, Guo C, Hu W, Liu K, Wang Y, Zhu T (2007) Occurrence and behavior of four of the most used sunscreen UV filters in a wastewater reclamation plant. Water Res 41:3506–3512. https://doi.org/10.1016/j.watres.2007.05.039
33. Liu H (2015) Hepatic oxidative stress biomarker responses in freshwater fish Carssius auratus exposed to four benzophenone UV filters. Ecotoxicol Environ Saf 119:116–122. https://doi.org/10.1016/j.ecoenv.2015.05.017
34. Magi E, Carro MD, Scapolla C, Nguyen KTN (2012) Stir bar sorptive extraction and LC MS/MS for trace analysis of UV filters in different water matrices. Chromatographia 75:973–982. https://doi.org/10.1007/s10337-012-2202-z
35. Manová E, Goetz NV, Hungerbuehler K (2015) Aggregate consumer exposure to UV filter ethylhexyl methoxycinnamate via personal care products. Environ Int 74:249–257. https://doi.org/10.1016/j.envint.2014.09.008
36. Mizukawa A, Molins-Delgado D, Azevedo JCR, Fernandes CVS, Díaz-Cruz S, Barceló D (2017) Sediments as a sink for UV filters and benzotriazoles: the case study of Upper Iguaçu watershed, Curitiba (Brazil). Environ Sci Pollut Res 24:18284–18294. https://doi.org/10.1007/s11356-017-9472-9
37. Molina-Molina J, Escande A, Pillon A, Gomez E, Pakdel F, Cavaillès V, Olea N, Aït-Aïssa S, Balaguer P (2008) Profiling of benzophenone derivatives using fish and human strogen receptor-specifc in vitro bioassays. Toxicol Appl Pharmacol 232:384–395. https://doi.org/10.1016/j.taap.2008.07.017
38. Morohoshi K, Yamamoto H, Kamata R, Shiraishi F, Koda T, Morita M (2005) Estrogenic activity of 37 components of commercial sunscreen lotions evaluated by in vitro assays. Toxicol In Vitro 19:457–469. https://doi.org/10.1016/j.tiv.2005.01.004
39. Mukaka MM (2012) Statistics corner: a guide to appropriate use of correlation coefficient in medical research. Malawi Med J 24:69–71

40. Necasová A, Bányiová K, Literák J, Cupr P (2016) New probabilistic risk assessment of ethylhexyl methoxycinnamate: comparing the genotoxic effects of trans and cis EHMC. Environ Toxicol 32:569–580. https://doi.org/10.1002/tox.22260
41. Nguyen KTN, Scapolla C, Carro MD, Magi E (2011) Rapid and selective determination of UV filters in seawater by liquid chromatography-tandem mass spectrometry combined with stir bar sorptive extraction. Talanta 85:2375–2384. https://doi.org/10.1016/j.talanta.2011.07.085
42. O'Malley E, McLachlan MSS, O'Brien JW, Verhagen R, Mueller JF (2021) The presence of selected UV filters in a freshwater recreational reservoir and fate in controlled experiments. *Sci. Total Environ.* 754 (2021) 142373. https://doi.org/10.1016/j.scitotenv.2020.142373
43. Oost RVD, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13:57–149. https://doi.org/10.1016/S1382-6689(02)00126-6
44. Organisation For Economic Co-operation And Development (OECD/OCD) (2012) Test-211 - Guidelines for the testing of chemicals -. Daphnia magna Reproduction
45. Ozáez I, Morcillo G, Martínez-Guitarte JL (2016) Ultraviolet filters differentially impact the expression of key endocrine and stress genes in embryos and larvae of *Chironomus riparius*. Sci Total Environ 557–558:240–247. https://doi.org/10.1016/j.scitotenv.2016.03.078
46. Pablos MV, García-Hortigüela P, Fernández C (2015) Acute and chronic toxicity of emerging contaminants, alone or in combination, in *Chlorella vulgaris* and *Daphnia magna*. *Environ Sci. Pollut. Res.* 22, 5417-5424. https://doi.org/10.1007/s11356-015-4119-1
47. Park CB, Jang J, Kim S, Kim YJ (2017) Single and mixture toxicity of three organic UV-filters, ethylhexyl methoxycinnamate, octocrylene, and avobenzone on *Daphnia magna*. Ecotoxicol Environ Saf 137:57–63. https://doi.org/10.1007/s11356-015-4119-1
48. Pestotnik K, Kosjek T, Heath E (2014) Transformation Products of Personal Care Products: UV Filters Case Studies.. In: In: Lambropoulou DA, Nollet LML (eds) Transformation Products of Emerging Contaminants in the Environment: Analysis, Processes, Occurrence, Effects and Risks. John Wiley & Sons, Somerset, GB
49. Ramos S, Homem V, Alves V, Santos L (2015) Advances in analytical methods and occurrence of organic UV-filters in the environment — A review. Sci Total Environ 526:278–311. http://dx.doi.org/10.1016/j.scitotenv.2015.04.055
50. Ramos S, Homem V, Alves V, Santos L (2016) A review of organic UV-filters in wastewater treatment plants. Environ Int 86:24–44. https://doi.org/10.1016/j.envint.2015.10.004
51. Rodil R, Schrader S, Moeder M (2009a) Non-porous membrane-assisted liquid–liquid extraction of UV filter compounds from water samples. J Chromatogr A 1216:4887–4894. https://doi.org/10.1016/j.chroma.2009.04.042
52. Rodil R, Moeder M, Altenburguer R, Schmitt-Jansen M (2009b) Photostability and phytotoxicity of selected sunscreen agents and their degradation mixtures in water. Anal Bioanal Chem Biochem 395:1513–1524. https://doi.org/10.1007/s00216-009-3113-1

53. Sharifan H, Klein D, Morse AN (2016) UV filters interaction in the chlorinated swimming pool, a new challenge for urbanization, a need for community scale investigations. Environ Res 148:273–276. https://doi.org/10.1016/j.envres.2016.04.002
54. Sieratowicz A, Kaiser D, Behr M, Oetken M, Oehlmann J (2011) Acute and chronic toxicity of four frequently used UV filter substances for *Desmodesmus subspicatus* and *Daphnia magna*. J Environ Sci Health - Toxic/Hazard 46:1311–1319. https://doi.org/10.1080/10934529.2011.602936
55. Silva ARR, Santos CSA, Ferreira NGC, Morgado R, Cardoso DN, Cruz A, Mendo S, Soares AMVM, Loureiro S (2019) Multigenerational effects of carbendazim in *Daphnia magna*: From a subcellular to a population level. Environ Toxicol Chem 38:412–422. https://doi.org/10.1002/etc.4307
56. Angelika Tkaczyk A, Bownik A, Dudka J, Kowal K, Ślaska B (2021) Daphnia magna model in the toxicity assessment of pharmaceuticals: A review. Sci Total Environ 763:143038. https://doi.org/10.1016/j.scitotenv.2020.143038
57. Thorel E, Clergeaud F, Jaugeon L, Rodrigues AMS, Lucas J, Stien D, Lebaron P (2020) Effect of 10 UV Filters on the Brine Shrimp *Artemia salina* and the Marine Microalga *Tetraselmis* sp. Toxics 8:29. https://doi.org/10.3390/toxics8020029
58. Tsui MM, Leung HW, Wai TC, Yamashita N, Taniyasu S, Liu W, Lam PK, Murphy MB (2014) Occurrence, distribution and ecological risk assessment of multiple classes of UV filters in surface waters from different countries. Water Res 67c:55–65. https://doi.org/10.1016/j.watres.2014.09.013
59. Wang J, Pan W, Wu S, Lu L, Xu Y, Zhu Y, Guo M, Zhuang S (2016) Recent advances on endocrine disrupting effects of UV filters. Int J Environ Res Public Health 13:782. https://doi.org/10.3390/ijerph13080782
60. Watanabe Y, Kojima H, Takeuchi S, Uramaru N, Sanoh S, Sugihara K, Kitamura S, Ohta S (2015) Metabolism of UV-filter benzophenone-3 by rat and human liver microsomes and its effect on endocrine-disrupting activity. Toxicol Appl Pharmacol 282:119–128. https://doi.org/10.1016/j.taap.2014.12.002

Supplemental Data

The Graphical Abstract is not available with this version.

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.

Figures

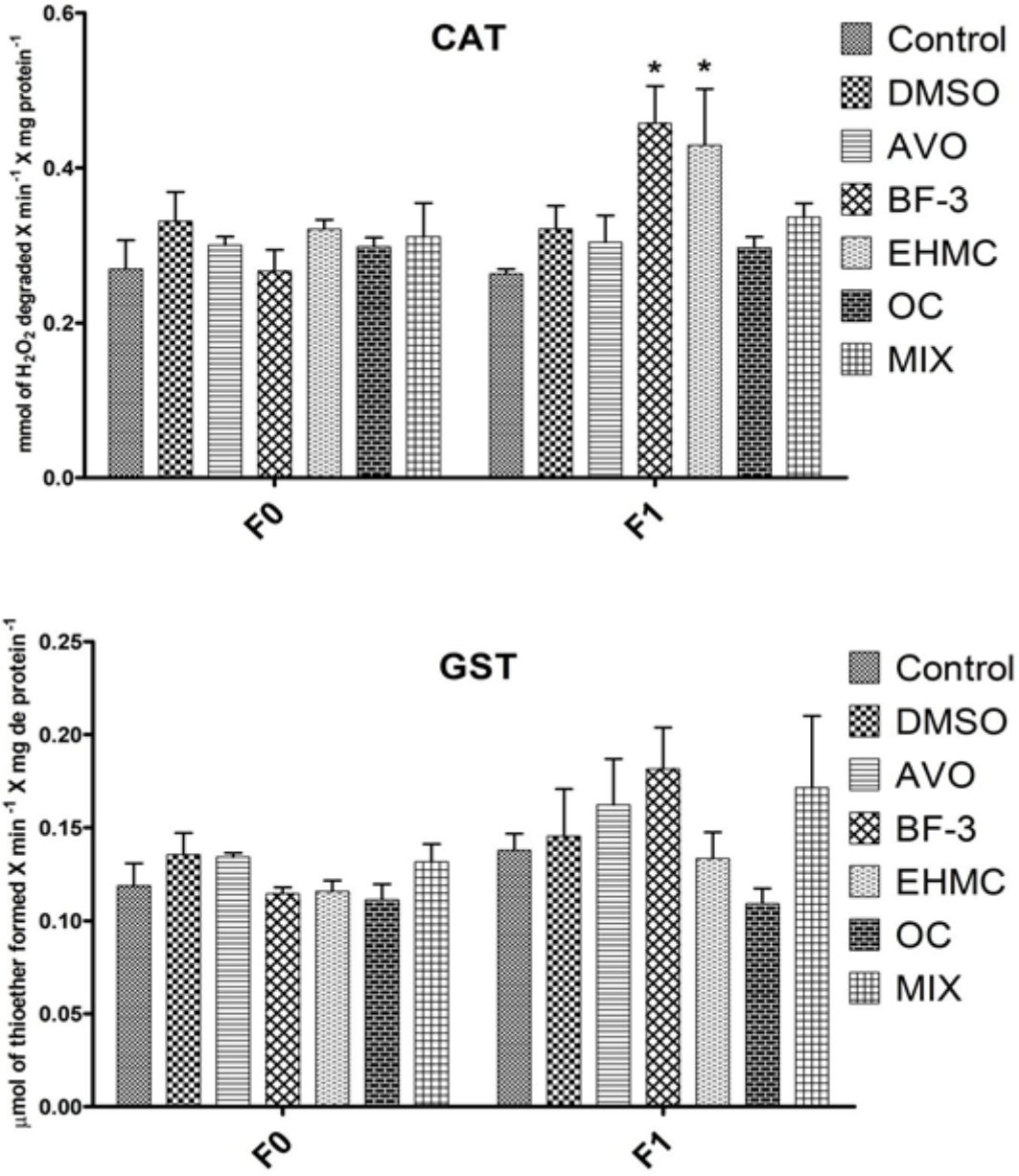

Figure 1

Activity of catalase (CAT) and glutathione-S-transferase (GST) enzymes in F0 and F1 generations of *D. magna*.

(CAT) Catalase; (GST) Glutathione-S-Transferase; (DMSO) dimethylsulfoxide 0.003%; (AVO) avobenzone 4.4 µg/L; (BP-3) benzophenone-3 0.17 µg/L; (EHMC): 2-ethylhexyl, 4-methoxycinnamate

0.2 µg/L; (OC) octocrylene 4.4 µg/L; (MIX) mixture. (*****) Significant difference between Control in ANOVA + Dunnett, with $p<0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx