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Benzophenone-3 causes oxidative stress in the brain and impairs aversive memory in adult zebrafish

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ABSTRACT

Oxybenzone (BP-3) is an ultraviolet (UV) filter widely used in industries that is directly or indirectly released into the aquatic environment. However, little is known about its effects on brain performance. Here, we investigated whether BP-3 exposure affects the redox imbalance in zebrafish and how they respond to a task that requires memory of an aversive situation. Fish were exposed to BP-3 10 and 50 μg L^{-1} for 15 days and then tested using an associative learning protocol with electric shock as a stimulus. Brains were extracted for reactive oxygen species (ROS) measurement and qPCR analysis of antioxidant enzyme genes. ROS production increased for exposed animals, and catalase (*cat*) and superoxide dismutase 2 (*sod 2*) were upregulated. Furthermore, learning and memory were reduced in zebrafish exposed to BP-3. These results suggested that BP-3 may lead to a redox status imbalance, causing impaired cognition and reinforcing the need to replace the toxic UV filters with filters that minimize environmental effects.

1. Introduction

Various organic chemicals are used in self-care and beauty products such as soaps, shampoos, moisturizers, makeups, and other items, called personal care products (PCPs) (Zhang et al., 2015; Montes-Grajales et al., 2017). These chemicals are currently classified as emerging contaminants (Sandoval-Gío et al., 2021). Their presence in the aquatic environment has been detected at low levels and associated with potential adverse effects, such as risks of ecological toxicity and human health (Mackay and Barnthouse, 2010; Buchberger, 2011).

Ultraviolet (UV) filters (including Benzophenones) are among the classes of chemical compounds widely used in PCPs, acting to reduce the harmful effects of radiation on the skin (Schneider and Lim, 2019). Benzophenone-3 (also named 2-hydroxy-4-methoxybenzophenone, oxybenzone, or BP-3) is an organic filter from the ketone class that composes several UV filters produced by industries due to its cheapness and effectiveness in protecting the skin from UVA and UVB rays. Thus, BP-3 is the most common substance in sunscreens and has been used for over 40 years (Kunisue et al., 2010). Moreover, BP-3 is added to plastic products, color agents, and textile articles to protect them against bleaching and sun-induced fragility (Wnuk et al., 2018).

In this way, BP-3 is being discarded into the environment from many sources. It enters the environment directly through aquatic recreational activities or indirectly through wastewater treatment plants (WWTPs), effluents, industrial discharges, runoff, and domestic uses (Kung et al., 2018; Cadena-Aizaga et al., 2020). BP-3 has been reported in natural waters like rivers, lakes, oceans, and reservoirs (Wang et al., 2021), and it has low degradation in the aquatic environment, with a half-life of 2.4 years (Carve et al., 2021). Its metabolites were already detected in human urine, breast milk, placental tissues, and the brain (Kunz and Fent, 2006; Zhang et al., 2013). Furthermore, BP-3 displays trophic magnification potential and is found in some species of saltwater and freshwater fish (Huang et al., 2022). Due to the widespread presence of this chemical, the concern about its toxicity and ecotoxicological effects has been increasing over the last decade. In a recent revision, Cuccaro et al. (2022) highlighted elevated levels of BP-3 in urban discharges with concentrations up to 7800 μg.L^{-1}, that even after treatment, in effluent wastewaters, reached a maximum concentration of 300 μg L^{-1}. In the

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marine environment, Cadena-Aizaga et al. (2020) observed a high concentration (1.395 mg L^{-1}) in the USA. However, the lack of studies in the current literature limits the knowledge of the global water bodies' distribution of BP-3.

In vitro and in vivo, fish and mouse experiments have pointed BP-3 as a potential endocrine disruptor (e.g. Schlumpf et al., 2001; Kunz et al., 2006; Kunz and Fent, 2006; Coronado et al., 2008; Wnuk et al., 2018; Lee et al., 2018). In addition to the estrogenic and antiandrogenic effects, exposure to different concentrations of BP-3 has been shown to induce neurotoxicity to both zebrafish and rodents neurogenesis (Skórkowska et al., 2020; Tao et al., 2020), reduction in acetylcholinesterase (AChE) activity (Sandoval-Gío et al., 2021), reduction in the number of enteric neurons (Wang et al., 2021), and disturbance in hematological parameters (Skórkowska et al., 2020). Cytotoxic and mutagenic effects of BP-3 were also demonstrated by the presence of micronucleus and altered nucleus in zebrafish erythrocytes (de Oliveira-Lima et al., 2021), while the altered activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in adult zebrafish liver (Velanganni and Miltonprabu, 2020) suggests the chemical hepatotoxicity that may compromise other hepatic functions.

Only a few studies investigate the consequences of BP-3 exposure on animals' behavior and cognition, while most only consider the effects of BP-3 on mortality, reproduction, and the endocrine system (mainly on reproductive and thyroid hormones). Cognition includes various brain processes, such as learning and memory, and comprises acquiring and processing of information to respond appropriately to environmental changes (Meshalkina et al., 2017). Impaired cognition can compromise the survival of the species by affecting its ability to explore the habitat and make decisions, for instance, the animals' access to places of refuge, feeding patches, reproduction sites, or predator areas (Strungaru et al., 2018). Given the importance of learning and memory retention for the animal's fitness, studies investigating the long-term consequences of BP-3 on brain functions are of great importance. Thus, the present research aimed to investigate the effects of different concentrations of BP-3 on the brain production of reactive oxygen species (ROS), the expression of genes related to the redox imbalance and the response to toxins, and how learning and memory could be affected in adult zebrafish. We hypothesize that BP-3 exposure leads to redox imbalance, increasing ROS and reducing defenses, ultimately leading to negative consequences to cognitive processes such as learning and memory.

2. Methods

2.1. Ethics statement

All fish maintenance and experimental protocols were approved by The Animal Use Ethics Committee of the Federal University of Rio Grande do Norte (CEUA, certificate number 211.075/2019) following the guidelines of the National Council for Animal Experimentation Control (CONCEA, Brazil) recommendations.

2.2. Animals and housing

Wild-type adult zebrafish (about 6 months old, males and females at the sex ratio of 1:1, ~0.3 g) were obtained from a local farm and kept in 50 L stock tanks (density of 2 fish/L) in a recirculation water system in the FishLab (UFRN, Natal, Brazil) for two weeks before experiments. Temperature, pH, and oxygenation were measured regularly and controlled (28ºC; pH 6.7; O_2 ~ 6 mg L^{-1}). The photoperiod adopted was 14 h light/10 h dark, with lights on at 06:00 am (190 lx). Fish were fed twice daily with commercial flakes (Alcon, 44% protein, 5% fat) and brine shrimp nauplii (*Artemia sp.*).

2.3. BP-3 exposure procedures

A stock solution of 1 mg mL^{-1} of BP-3 was prepared by dissolving BP-3 (98%purity; Sigma-Aldrich, Brazil, CAS number: 131–57–7) in 200 mL DMSO (Sigma-Aldrich, Brazil, CAS number: 67–68–5). It was stored in an amber bottle and refrigerated (~2 °C) to prevent BP-3 light degradation. The solution was dissolved into the tank water to the final concentration desired for each experimental group.

Ninety-nine zebrafish were individually netted from the stock tanks and divided into four experimental groups arranged into two replicates: the first replicate had the control (n = 12), DMSO 0.005% as solvent control (n = 12), and BP-3 at two nominal concentrations: 10 and 50 μg L^{-1} (BP-3 10, n = 12 and BP-3 50, n = 12). Then, tests were arranged again in a second trial with the control (n = 12), DMSO (n = 12), BP-3 10 (n = 12) and BP-3 50 μg L^{-1} (n = 15). This procedure was chosen due to the time needed for recording behavior and processing biological material. The BP-3 concentration range was chosen based on our previous results (Moreira and Luchiari, 2022), which identified that concentrations of 10 and 100 μg L^{-1} could alter the behavior of adult zebrafish. The concentration range used in the present study was lower than 100 μg L^{-1}, since embryos were used rather than adults. Tao et al. (2020) also used a similar range concentration (1, 10 and 100 μg L^{-1}), which caused developmental neurotoxicity with mortality rate similar to the control group.

Fish were maintained in 12 L tanks (20 ×26×40 cm) with an air stone for 15 days before the behavioral test. During this period, fish were fed twice daily with commercial flakes (Alcon, 44% protein, 5% fat) and brine shrimp (*Artemia sp.*).

The experimental tank water was changed every 48 h and BP-3 concentrations were renewed. This procedure assured BP-3 concentration for each treatment and minimized fish handling stress. Control and DMSO groups underwent the same procedures to handle fish equally.

2.4. Aversive memory test

After a 15-days exposure period, fish were submitted to a protocol of aversive memory through an inhibitory avoidance task (described below). Behavior during trials and test phases was recorded using a Logitech HD Webcam, and videos were analyzed using ANY-maze software (Stoelting Co., USA, version 6.33). The apparatus used in the test was filled with 12 L water free of BP-3 or DMSO.

For the learning and aversive memory retention test, fish were individually tested in a 20 L shuttle box tank (40 × 25 × 20 cm), as proposed by Vianna et al. (2009) and adapted by Pinheiro-da-Silva et al. (2018). The tank was divided into two halves, one completely covered in black and the other completely covered in white. Between the two areas, an opaque partition (one side black and the other white) blocked the fish's access to both sides. Raising this partition 3 cm high allowed the fish to access both sides of the tank.

The black side of the tank received 2 copper gratings on two opposite sides connected to an electric shock delivery device (6 V, 1.0 mA, 12.6 W) (Fig. 1). Electric shock was used as an aversive stimulus. The device was turned on when the fish entered the black side, and shock was delivered for 4 s

The experiment was divided into three phases: two trials and the test, with a 24 h interval between them. On trial 1 (first day), the fish was gently positioned on the white side of the tank while the door was closed. Following the 60 s-habituation period, the door was raised, and the fish could move to the black side. Adult fish naturally prefers dark environments, representing protection from predators (Gusso et al., 2020). The door was closed as soon as the fish entered the black side, and a 4 s electric shock was delivered. The time to enter the black side was registered for each fish. The maximum time to enter the black side was 240 s. After the shock delivery, the fish was removed from the tank and kept isolated in 600 mL tank for individual identification. If any animal did not enter the black side in the trial 1 phase, it was removed from the test. Eighty fish entered the black side and were tested at all the experimental phases specified for each group: control n = 17, DMSO n = 19, BP-3 10 n = 20, and BP-3 50 n = 24.

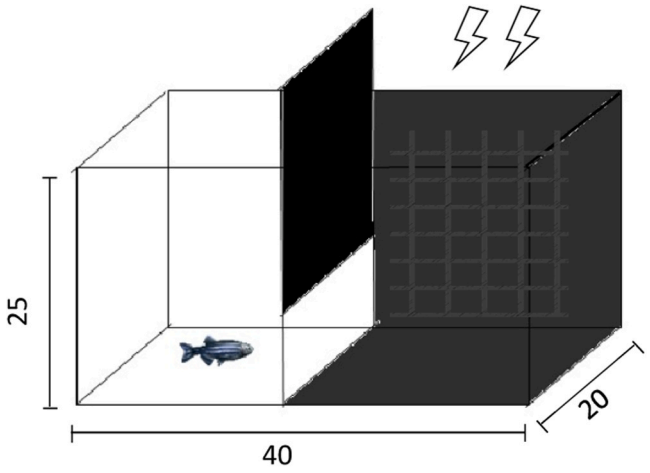

Fig. 1. Scheme of the tank used in the aversive learning and memory test, using electroshock as the stimulus. The tank was divided into two equal sides (black and white) by an opaque door and fish were trained to associate the black side with the aversive stimulus (electroshock). After 1 min habituation on the white side, the door was raised 3 cm high and fish were able to cross to the black side, where the electroshock was delivered.

After 24 h, on trial 2, the procedure was repeated. Animals that did not move to the black side within 240 s were gently guided with a net, and the electric shock was applied to guarantee that all the experimental fish received the aversive stimulus. The time to enter the black side was registered. For the fish that needed to be guided, we registered it as 240 s

On the third day, the test phase took place to assess fish memory retention capacity. Each fish was positioned on the white side of the tank and after 60 s habituation, the door was raised, and the fish could freely access the black side for up to 10 min. No electric shock was applied during the test. Fish behavior was recorded from above, and latency to enter the black side was registered. Mean speed while moving was evaluated as a locomotor parameter.

2.5. ROS detection

After the behavioral procedures, 6 fish from each experimental group were euthanized by hypothermic shock, and their brains were used for ROS (reactive oxygen species) measurement. A pool of 6 brains was used for each group.

Intracellular ROS production was measured using the fluorescence probe 2′,7′-dichlorodihydro-fluorescein (DCFH2-DA) diacetate, following the protocol described by Silva Junior et al. (2021) with some modifications. Pools of zebrafish brain (analyzed in triplicate) were transferred to 15 mL conical tubes with 5 mL of Phosphate- buffered saline (PBS), and homogenized. Subsequently, the tubes were centrifuged at 5000 rpm for 30 min at 4ºC to remove cell debris. The samples were added to a 96-well black plate, where the homogenate and the fluorescence probe at a concentration of 100 mM and PBS buffer were added to each well, then the plate was incubated for 30 min. This procedure was performed in the absence of light. ROS measurements were made in a microplate reader (GloMax®-Multi Detection System, Promega, Wisconsin, USA), with excitation at 485 nm and emission at 535 nm. ROS concentration was expressed as a percentage (%) and compared to the control.

2.6. RNA extraction and real-time qPCR for gene expression

After the behavioral analyses, 40 fish were euthanized, and the brain tissue was removed for gene expression analysis. Each experimental group contained 10 zebrafish brains in microtubes with 1 mL of RNA-Later (Sigma-Aldrich®) stored at − 20ºC. Total RNA from the sample was extracted and reverse transcribed for real-time quantitative polymerase chain reaction (qPCR). PuriLink RNA Mini Kit (Life Technologies) was used for extraction and isolation, and all instructions were followed in full as stated in the manufacturer's protocol. After the end of the extraction, the total RNA was quantified in a NanoDropTM One Microvolume UV-Vis Spectrophotometer with a purity ratio between 1.8 and 2.0. The quality was determined by electrophoresis in a 0.8% agarose gel. All groups were standardized to a concentration of 100 ng μL^{-1} and stored in a freezer at − 80ºC. The High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) was used to construct the cDNA. In this process, a mixture containing 10X RT buffer, 25X dNTP Mix, 10X RT Randon Primer, Reverse Transcriptase, and Nuclease-Free-Water was placed together with the RNA of the samples, at a concentration of 1 μg μL^{-1}.

qPCR reactions were performed with the PowerUp SYBR® Green Master Mix (Thermo Fisher Science, Waltham, MA, USA) using the Rotor-gene Q system (Qiagen, CA, USA). The qPCR thermocycler conditions were: a 10 min hold stage at 95 °C, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s, and a melt curve stage of 95 °C for 15 min, 1 h at 60 °C, and a final step of 15 min at 95 °C. It was analyzed the relative expression levels (fold change) of two genes of the nervous system (acetylcholinesterase (*ache*) and brain-derived neurotrophic factor (*bndf*) and four genes associated with oxidative stress (glutathione peroxidase 1 A (*gpx1a*), catalase (*cat*), superoxide dismutase 1 (*sod1*) and superoxide dismutase 2 (*sod2*)). The primers were selected from the literature (Capriello et al., 2021; da da da Silva Junior et al., 2021) and they are listed in Table 1. Relative mRNA expression was quantified using the $2^{-\Delta\Delta Ct}$ method with β-actin as an endogenous control (Li et al., 2019). The experiment was performed in triplicate and following the Minimum Information Guidelines for Publishing Quantitative Real-Time PCR Experiments (MIQE) (Bustin et al., 2009).

2.7. Statistical analysis

Initially, data were analyzed for normality and homoscedasticity, using Shapiro- Wilk and Levene tests, respectively. Two-way ANOVA followed by Tukey's post hoc test was conducted to evaluate the differences in latency to enter the black side on each experimental phase considering as factors the groups (control, DMSO, BP-3 10, and BP-3 50) and phases (trial 1, trial 2, and test). The Kruskal-Wallis test was used to compare mean speed while moving between groups on the test phase.

One-Way ANOVA followed by Tukey's post hoc test compared ROS production between groups. One-way ANOVA followed by Dunnett's post hoc was also used to compare each gene expression between BP-3 and the DMSO group, considered the control in this analysis. The significance level considered was $p < 0.05$. All data were analyzed using GraphPad-Prism software version 8.0.1.

3. Results

3.1. Aversive memory test

After two training trials, fish were tested for memory. In the aversive memory test, fish were expected to associate the black side with the aversive stimulus (electric shock) and avoid it. Two-way Repeated Measures ANOVA showed statistical significance for latency to enter the black side for experimental phases ($F_{(1.8,\ 120)} = 15.6$, $p < 0.0001$). However, no effect was observed for group ($F_{(3,\ 68)} = 0.8786$, $p = 0.4567$) and interaction between group and phase ($F_{(6,\ 136)} = 1.716$, $p = 0.1218$).

Tukey's multiple comparison tests showed that animals of the control group presented higher latency to enter the black side in the test phase ($p < 0.05$). The same was observed for the DMSO group, which presented higher latency to explore the black side on the test phase ($p < 0.05$). *Post hoc* test indicated that fish exposed to BP-3 10 presented no differences in latency to enter the black side on the test phase

Table 1
Details of primer sequences used for qPCR analysis.

Gene	Database Accession Number	Forward Primer Sequence (5′– 3′)	Reverse Primer Sequence (5′ – 3′)	Size (bp)	Melting Temperature (° C)
sod1	NM_131294.1	GTAATGTGACCGCTGATGCC	GAATCACCATGGTCCTCCCA	105	60
sod2	NM_199976.1	TTGGAGGCCATAAAGCGTGA	CCTTTTCAAAGCCCAGCCAG	112	60
cat	NM_130912.2	TCTCCTGATGTGGCCCGATA	GGTTTTGCACCATGCGTTTC	169	60
gpx1a	NM_001007281.2	TAAAACCTGCGTGTTGCCCT	ATAGTTTCGCGGACAGGTCG	97	60
ache	NM_131595.2	CATACGCACAATACGCTGCC	TACACAGCACCATGCGAGTT	118	60
bndf	NM_194419.1	GGCGAAGAGCGGACGAATATC	AAGGAGACCATTCAGCAGGACAG	90	60
actb	NM_181601.5	CTGTTCCAGCCATCCTTCTT	TGTTGGCATACAGGTCCTTAC	109	60

compared to training trials (trial 1 $p = 0.11$, and trial 2 $p = 0.76$). The same pattern was observed for fish exposed to BP-3 50, which did not present significance in latency to enter the black side between trial 1 and test ($p = 0.13$) or trial 2 and test ($p = 0.99$) (Fig. 2).

Regarding the mean speed while moving, the Kruskal-Wallis test showed no statistical significance between experimental groups ($p = 0.15$).

3.2. ROS detection

One-way ANOVA showed statistical significance for the brain ROS production between groups ($F_{(3, 38)} = 53.43$; $P < 0.0001$). Tukey's post hoc test showed that ROS production did not differ between the control and the DMSO ($p = 0.1870$), but it was significantly higher in fish exposed to BP-3. Fish from BP-3 10 group increased ROS production compared to the control group ($p < 0.0001$). Further increase was observed in the BP-3 50 group, in which ROS production is more than 3 times higher than the control group ($p < 0.0001$) and also significantly higher than BP3–10 ($p = 0.0004$) (Fig. 3).

3.3. Genes expression

One-Way ANOVA did not show statistical significance in mRNA levels of brain *Ache* (acetylcholinesterase) ($p = 0.3725$) and *bndf* (brain-derived neurotrophic factor) ($p = 0.1710$) between groups (Fig. 4a and b).

For the oxidative stress genes, One-Way ANOVA did not show statistical significance on levels of transcription of *gpx1a* (glutathione peroxidase 1 A) ($p = 0.1634$) and *sod1* (superoxide dismutase 1) ($p = 0.2654$) (Fig. 4c and e). However, One-Way ANOVA test showed

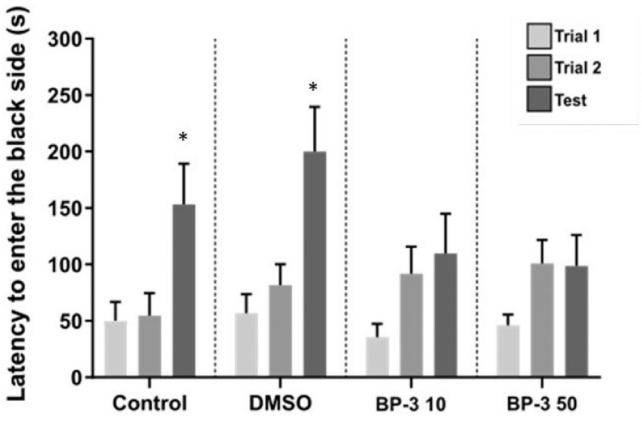

Fig. 2. Zebrafish latency to enter the black side of the tank during training trials and test phase. Control ($n = 13$), DMSO ($n = 15$) groups, and animals exposed to BP-3 10 μg L^{-1} ($n = 20$) and BP-3 50 μg L^{-1} ($n = 24$) for 15 days were trained to associate the black side of the tank (black and white shuttle box) with the aversive stimulus (electric shock) and then tested for memory retention. Data are expressed as the mean ± S.E.M., and the asterisks above bars indicate statistical significance between trials and test for each experimental group (*$p \leq 0.05$).

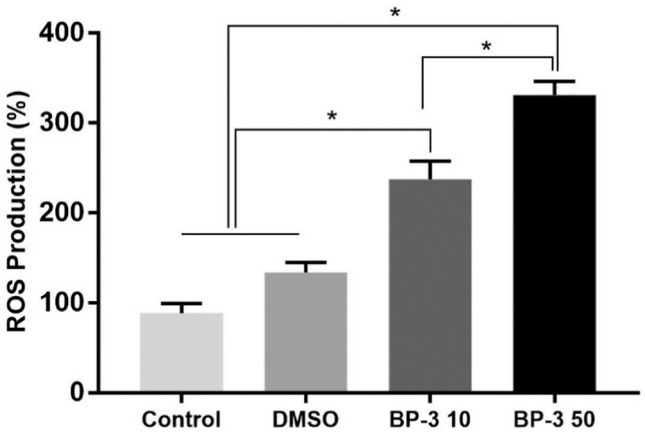

Fig. 3. ROS production in zebrafish exposed to BP-3. Fish were exposed to fresh water, DMSO 0.005%, BP-3 10 μg L^{-1} and 50 μg L^{-1} for 15 days. ROS production in the brain was measured in one pool of 6 brains per group and expressed as a percentage (%). Data are expressed as mean ± S.E.M. Asterisks above the bars indicate statistical significance between groups ($p \leq 0.0005$) (Tukey test).

statistical significance on the transcript levels of *cat* (catalase) ($F_{(2, 6)} = 35.46$; $p = 0.0005$) and *sod2* (superoxide dismutase 2) ($F_{(2, 6)} = 36.39$; $p = 0.0004$) between groups (Fig. 4d and f). Regarding *cat,* Dunnett's teste showed an up-regulation in BP-3 10 ($p = 0.0114$) and BP-3 50 ($p = 0.0003$) compared to DMSO. The same pattern was observed for *sod2*, which was also up-regulated in BP-3 10 ($p = 0.0072$) and BP-3 50 ($p = 0.0003$) compared to the control DMSO.

4. Discussion

Among the vertebrates, fish are the ones that most rapidly respond to a contaminated environment (Hong and Zha, 2019). Zebrafish is a popular, promising and reliable model that is being used to evaluate the effects of pollutants on fish community, biodiversity, and, due to its translational relevance, also the effects of pollutants exposure for humans (Strungaru et al., 2018). Specifically, zebrafish has been widely used to investigate toxicological effects on behavior and cognition (Yoo et al., 2021) since it presents well-established behaviors and advantages for learning and memory studies, e.g., it can learn a wide variety of tasks and shows memory retention that exceeds 24 h (Hong and Zha, 2019; Gusso et al., 2020). Furthermore, the zebrafish brain shows complex functioning and biochemical apparatus comparable to mammals, so the results of studies using this model can be inferred from the human brain in a translational approach (Strungaru et al., 2018).

Learning and memory are crucial for a species' survival because they favor the ability to make decisions based on previous experiences (Brown et al., 2011). Any substances that affect these abilities should be carefully considered, especially the chemical substances considered emergent pollutants widely used by the industry and constantly discarded into the environment. In this sense, our study departs from the endocrine and reproductive approach. It focuses on the impact of BP-3

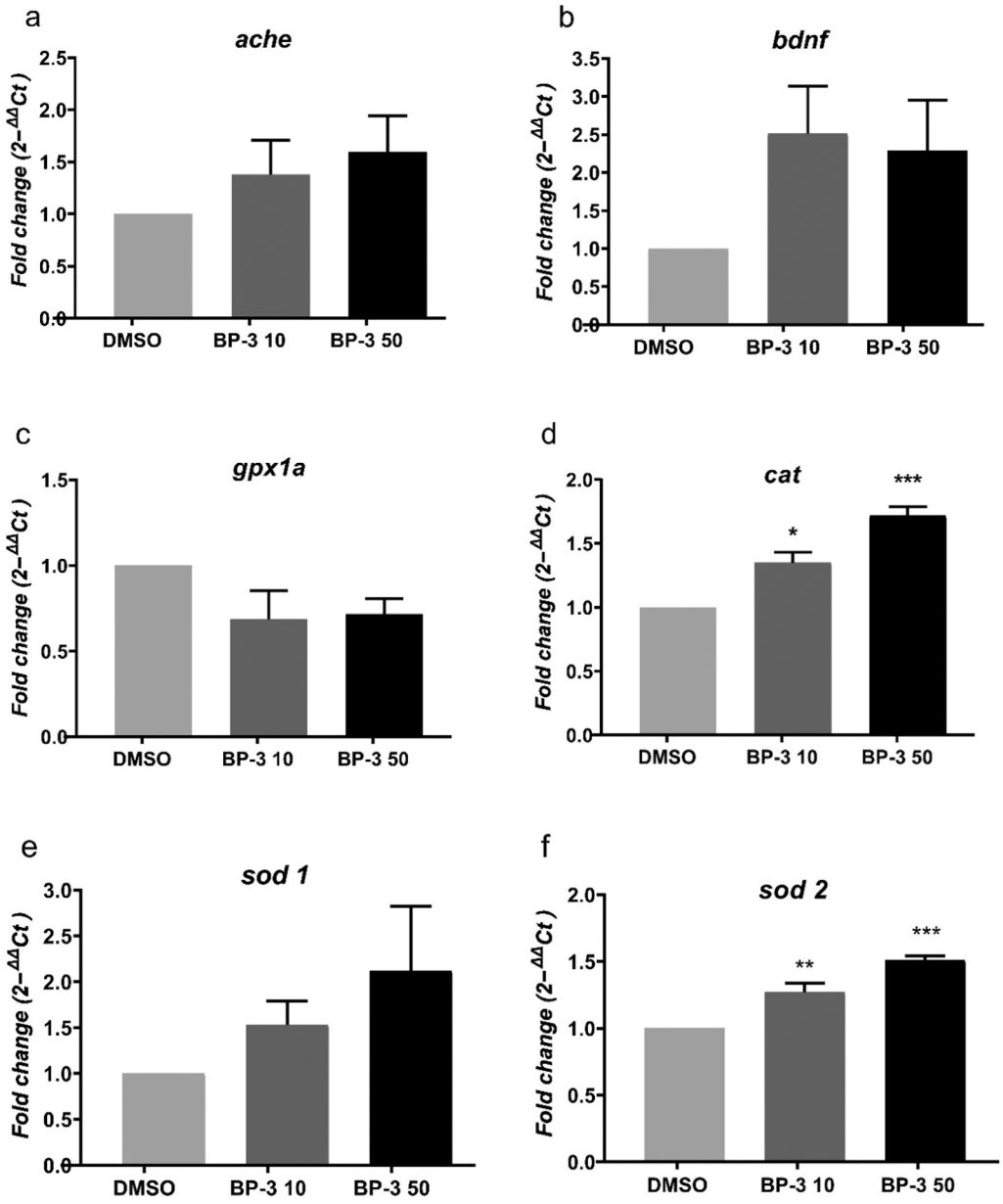

Fig. 4. Relative expression levels (fold change) of brain genes *Ache*- acetylcholinesterase (a), *bndf*- brain-derived neurotrophic factor (b), *gpx1a*- glutathione peroxidase (c), *cat*- catalase (d), *sod1*- superoxide dismutase 1 (e) and *sod2*- superoxide dismutase 2 (f). Fish were exposed to DMSO (0.005%), BP-3 10 μg L^{-1} and 50 μg L^{-1} for 15 days and gene expression was measured using a pool of 10 brains per group. One-Way ANOVA followed by Dunnett's test was used to verify the statistical significance between gene expression levels between BP-3 groups and DMSO, the control group. Data are expressed as mean ± S.E.M and the asterisks above bars indicate statistical differences to DMSO (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

on learning and long-term memory in adult zebrafish, correlating the cognitive effects to brain ROS production and expression of genes related to antioxidant defense.

Animals exposed to BP-3 for 15 days were unable to learn the association between aversive stimulus (the electric shock- unconditioned stimulus) and the black side of the tank (conditioned stimulus), i.e., BP-3 induced memory impairment, and fish could not avoid the black side, where the aversive stimulus was. (Fig. 2). Furthermore, it was noted that animals exposed to BP-3 exhibited over three times higher levels of ROS in their brain tissue compared to those who were not exposed, as depicted in Fig. 3. In addition, the transcript levels of antioxidant enzyme genes, including catalase and superoxide dismutase 2, were up-regulated in the animals exposed to BP-3.

Organic UV filters have been reported to be associated with the induction of oxidative stress in aquatic organisms (Huang et al., 2020; Sandoval-Gío et al., 2021). ROS increase was associated with changes in behavior and locomotor patterns in zebrafish exposed to pollutants (Capriello et al., 2021; da Silva Junior et al., 2021). In agreement with these effects, our results showed that increased ROS production in fish exposed to BP-3 could be associated with impaired aversive learning and memory.

ROS are considered necessary for brain development and function in moderate or low concentrations. In contrast, excessive levels are dangerous, generating oxidative stress, which can jeopardize the brain and central nervous system (CNS) functioning (Salim, 2017). Neurons are susceptible to the accumulation of oxidized macromolecules, which is associated with neurological impairments and decreased cognitive performance (Ruhl et al., 2015; Attaran et al., 2020). Zebrafish exposed to Selenium (Se), for example, present decreased social learning, which was associated with higher levels of lipid hydroperoxide (LPO), an important marker of oxidative stress (Attaran et al., 2021). *Cat* gene induction was also observed in the brain of zebrafish exposed to uranium, indicating ROS production by the metal (Lerebours et al., 2009).

Increased ROS presence and its toxic effects may be occasioned by the inefficiency in protection mechanisms, i.e. mechanisms that control oxidative stress by reducing the levels of ROS and its damaging effects. Although part of these mechanisms was increased in our study, in which the genes related to oxidative stress regulations and defenses (*cat* and

sod2) were up-regulated in animals exposed to BP-3, the antioxidant defense seems not to show adequate protection against neurotoxicity.

Thus, BP-3 causes a redox status imbalance, resulting in disruptive consequences and adverse effects on brain functioning at higher long-term levels of BP-3 exposure. Moreover, we did not observe an increment in other genes related to oxidative stress protection (e. g. *gpx1a* and *sod1*). The activities of the glutathione peroxidase families and superoxide dismutase 1 genes are closely associated with safeguarding cells against oxidative damage. Hence, it was anticipated that these genes would be up-regulated in the case of augmented ROS production. Moreover, *bdnf* and *ache* genes were not affected by 15-day exposure to BP-3. These results may be attributed to long-term exposure, which may trigger adaptation effects: some genes compensate for its expression after a period of activity. Besides, the levels and activity of the enzymes were not measured in our study, which must be considered in future research at both short- and long-term exposure. Additionally, it is important to note that BP-3 can bioaccumulate in aquatic animals, and it is partially metabolized into benzophenone-1 (BP-1), an even more estrogenic agonist agent (Molina-molina et al., 2008; Blüthgen et al., 2012; Kim et al., 2014) that may potentiate oxidative stress.

The majority of behavioral alterations induced by BP-3 exposure have been directly linked to disruption of the endocrine system, which modifies behaviors such as breeding, courtship, and agonistic interactions (Chen et al., 2016, 2018; Barone et al., 2019; Portrais et al., 2019; Carvalhais et al., 2021). In zebrafish, studies investigating the effects of BP-3 on other behaviors and cognition are emerging. Recently, we have addressed the effects of BP-3 on locomotor, anxious-like, social, aggressive, and spatial learning behaviors in zebrafish (Moreira and Luchiari, 2022). In a T-maze test, females and males exposed to BP-3 10 $\mu g.L^{-1}$ for 5 months also presented impaired learning and short-term memory (Bai et al., 2023). These results agree with the effects of BP-3 obtained herein, reinforcing the deleterious effects of BP-3 for several levels of behavior, from simple shoal association to associative learning and memory formation.

Associative learning by classical or operant conditioning is an important and highly conserved form of learning and has been demonstrated in zebrafish (Kenney et al., 2017). This type of learning can be reinforced differently by positive and negative experiences, named appetitive and aversive stimuli, respectively (Mason et al., 2021). In the case of aversive learning tasks, electric shock is widely used as an aversive stimulus to zebrafish (e. g., Amorim et al., 2017; Bridi et al., 2017; Pinheiro-da-Silva et al., 2017; Nabinger et al., 2018; Menezes et al., 2022). It is a potent stimulus that evokes behavioral and physiological changes in fish, such as altered locomotion, increased ventilatory rate, and elevated cortisol levels (Manuel et al., 2014; Amorim et al., 2017), and its responses elicit robust and stable memories (Blank et al., 2009). Importantly, aversive experiences are often associated with rapid memory acquisition and long-term memory duration (Brown et al., 2011). The negative emotional experience activates stress response and glucocorticoid release, favoring memory consolidation (Wichmann et al., 2012). However, neurotoxicity caused by BP-3 per se and the increased oxidative stress caused by BP-3 disrupt the cognitive process. Pollutants can affect the synthesis, transport, and release of several neurotransmitters, such as dopamine and glutamate. They can act as endocrine disruptors, affecting the nervous system and leading to neurotoxicity, causing behavior, cognition, learning, and memory impairment (Schug et al., 2015; Tao et al., 2022).

In summary, we found that animals exposed to BP-3 presented increased ROS production, increased expression of some genes of the antioxidant machinery (*cat* and *sod2*), and impaired learning and memory. Further investigations are required to assess the impact of BP-3 and its metabolites on behavior and cognitive performance in zebrafish, encompassing diverse tasks and contexts. This is particularly important since the use of this contaminant continues, and our comprehension of its ecological repercussions remains restricted. It is crucial to investigate the impact of BP-3 exposure on zebrafish neurodevelopment, including short and long-term consequences. Additionally, it is essential to explore the substitution of benzophenone with environmentally sustainable products, such as seaweed-based sunscreens that possess UV filtering capabilities. Furthermore, eco-friendly alternatives like "beach shirts" and hats should also be considered.

5. Conclusions

To sum up, our study highlights the detrimental impact of BP-3 on the environment. It was observed that BP-3 enhances ROS production in the brain, upregulates the expression of antioxidant enzyme genes, and impairs learning and memory of aversive contexts in zebrafish. These findings emphasize the pressing requirement for ecologically sustainable alternative products, given the hazardous effects of BP-3.

CRediT authorship contribution statement

Ana Luisa Pires Moreira: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Weslley Souza Paiva**: Formal analysis, Data interpretation, Writing. **Augusto Monteiro de Souza**: Formal analysis, Data interpretation, Writing. **Maria Clara Galvão Pereira**: Methodology, Formal analysis. **Hugo Alexandre Oliveira Rocha**: Data interpretation, Writing review. **Silvia Regina Batistuzzo de Medeiros**: Data interpretation, Writing review. **Ana Carolina Luchiari**: Conceptualization, Funding acquisition, Supervision, Data interpretation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Amorim, R.R., Silva, P.F., Luchiari, A.C., 2017. Effects of alcohol on inhibitory avoidance learning in zebrafish (Danio rerio). Zebrafish 14, 430–437. https://doi.org/10.1089/zeb.2017.1438.

Attaran, A., Salahinejad, A., Naderi, M., Crane, A.L., Niyogi, S., Chivers, D.P., 2020. Effects of chronic exposure to selenomethionine on social learning outcomes in zebrafish (Danio rerio): serotonergic dysregulation and oxidative stress in the brain. Chemosphere 247. https://doi.org/10.1016/j.chemosphere.2020.125898.

Attaran, A., Salahinejad, A., Naderi, M., Crane, A.L., Chivers, D.P., Niyogi, S., 2021. Transgenerational effects of selenomethionine on behaviour, social cognition, and the expression of genes in the serotonergic pathway in zebrafish. Environ. Pollut. 286, 117289 https://doi.org/10.1016/j.envpol.2021.117289.

Bai, C., Dong, H., Tao, J., Chen, Y., Xu, H., Lin, J., Huang, C., Dong, Q., 2023. Lifetime exposure to benzophenone-3 at an environmentally relevant concentration leads to female–biased social behavior and cognition deficits in zebrafish. Sci. Total Environ. 857, 159733 https://doi.org/10.1016/j.scitotenv.2022.159733.

Barone, A.N., Hayes, C.E., Kerr, J.J., Lee, R.C., Flaherty, D.B., 2019. Acute toxicity testing of TiO 2 -based vs. oxybenzone-based sunscreens on clownfish (Amphiprion ocellaris). Environ. Sci. Pollut. Res. 14513–14520. https://doi.org/10.1007/s11356-019-04769-z.

Blank, M., Guerim, L.D., Cordeiro, R.F., Vianna, M.R.M., 2009. A one-trial inhibitory avoidance task to zebrafish: Rapid acquisition of an NMDA-dependent long-term

memory. Neurobiol. Learn. Mem. 92, 529–534. https://doi.org/10.1016/j.nlm.2009.07.001.
Blüthgen, N., Zucchi, S., Fent, K., 2012. Effects of the UV filter benzophenone-3 (oxybenzone) at low concentrations in zebrafish (Danio rerio). Toxicol. Appl. Pharmacol. 263, 184–194. https://doi.org/10.1016/j.taap.2012.06.008.
Bridi, D., Altenhofen, S., Gonzalez, J.B., Reolon, G.K., Bonan, C.D., 2017. Glyphosate and Roundup® alter morphology and behavior in zebrafish. Toxicology. https://doi.org/10.1016/j.tox.2017.10.007.
Brown, C., Laland, K., Krause, J., 2011. Fish Cognition and Behaviour. Fish. Cogn. Behav. https://doi.org/10.1002/9781444342536.ch1.
Buchberger, W., 2011. Current approaches to trace analysis of pharmaceuticals and personal care products in the environment. J. Chromatogr. A 1218, 603–618. https://doi.org/10.1016/J.CHROMA.2010.10.040.
Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., Wittwer, C.T., 2009. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55, 611–622. https://doi.org/10.1373/clinchem.2008.112797.
Cadena-Aizaga, M.I., Montesdeoca-Esponda, S., Torres-Padrón, M.E., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2020. Organic UV filters in marine environments: An update of analytical methodologies, occurrence and distribution. Trends Environ. Anal. Chem. 25 https://doi.org/10.1016/j.teac.2019.e00079.
Capriello, T., Félix, L.M., Monteiro, S.M., Santos, D., Cofone, R., Ferrandino, I., 2021. Exposure to aluminium causes behavioural alterations and oxidative stress in the brain of adult zebrafish. Environ. Toxicol. Pharmacol. https://doi.org/10.1016/j.etap.2021.103636.
Carvalhais, A., Pereira, B., Sabato, M., Seixas, R., Dolbeth, M., Marques, A., Guilherme, S., Pereira, P., Pacheco, M., Mieiro, C., 2021. Mild Effects of Sunscreen Agents on a Marine Flatfish: Oxidative Stress, Energetic Profiles, Neurotoxicity and Behaviour in Response to Titanium Dioxide Nanoparticles and Oxybenzone. Int. J. Mol. Sci. Artic. https://doi.org/10.3390/ijms22041567.
Carve, M., Nugegoda, D., Allinson, G., Shimeta, J., 2021. A systematic review and ecological risk assessment for organic ultraviolet filters in aquatic environments. Environ. Pollut. 268, 115894 https://doi.org/10.1016/j.envpol.2020.115894.
Chen, T.H., Chou, S.M., Tang, C.H., Chen, C.Y., Meng, P.J., Ko, F.C., Cheng, J.O., 2016. Endocrine disrupting effects of domestic wastewater on reproduction, Sexual behavior, And gene expression in the brackish medaka Oryzias melastigma. Chemosphere 150, 566–575. https://doi.org/10.1016/j.chemosphere.2016.02.057.
Chen, T.H., Hsieh, C.Y., Ko, F.C., Cheng, J.O., 2018. Effect of the UV-filter benzophenone-3 on intra-colonial social behaviors of the false clown anemonefish (Amphiprion ocellaris). Sci. Total Environ. 644, 1625–1629. https://doi.org/10.1016/j.scitotenv.2018.07.203.
Coronado, M., De Haro, H., Deng, X., Rempel, M.A., Lavado, R., Schlenk, D., 2008. Estrogenic activity and reproductive effects of the UV-filter oxybenzone (2-hydroxy-4-methoxyphenyl-methanone) in fish. Aquat. Toxicol. 90, 182–187. https://doi.org/10.1016/j.aquatox.2008.08.018.
Cuccaro, A., Freitas, R., De Marchi, L., Oliva, M., Pretti, C., 2022. UV filters in marine environments_ a review of research trends, meta-analysis, and ecotoxicological impacts of 4-methylbenzylidene-camphor and benzophenone-3 on marine invertebrate communities. Enhanc. Read. pdf.
Gusso, D., Reolon, G.K., Gonzalez, J.B., Altenhofen, S., Kist, L.W., Bogo, M.R., Bonan, C. D., 2020. Pyriproxyfen Exposure Impairs Cognitive Parameters and Alters Cortisol Levels in Zebrafish. Front. Behav. Neurosci. 14, 1–10. https://doi.org/10.3389/fnbeh.2020.00103.
Hong, X., Zha, J., 2019. Fish behavior: A promising model for aquatic toxicology research. Sci. Total Environ. 686, 311–321. https://doi.org/10.1016/j.scitotenv.2019.06.028.
Huang, X., Li, Y., Wang, T., Liu, H., Shi, J., Zhang, X., 2020. Evaluation of the oxidative stress status in zebrafish (Danio rerio) liver induced by three typical organic uv filters (BP-4, PABA and PBSA). Int. J. Environ. Res. Public Health. https://doi.org/10.3390/ijerph17020651.
Huang, Y.F., Chang, J.P., Chen, H.C., Liu, X.R., 2022. Fish consumption is an indicator of exposure to benzophenone derivatives: A probabilistic risk assessment in Taiwanese population. Sci. Total Environ. 812, 152421 https://doi.org/10.1016/j.scitotenv.2021.152421.
Kenney, J.W., Scott, I.C., Josselyn, S.A., Frankland, P.W., 2017. Contextual fear conditioning in zebrafish. Learn. Mem. https://doi.org/10.1101/lm.045690.117.
Kim, S., Jung, D., Kho, Y., Choi, K., 2014. Effects of benzophenone-3 exposure on endocrine disruption and reproduction of japanese medaka (Oryzias latipes)-A two generation exposure study. Aquat. Toxicol. 155, 244–252. https://doi.org/10.1016/j.aquatox.2014.07.004.
Kung, T.A., Lee, S.H., Yang, T.C., Wang, W.H., 2018. Survey of selected personal care products in surface water of coral reefs in Kenting National Park, Taiwan. Sci. Total Environ. 635, 1302–1307. https://doi.org/10.1016/j.scitotenv.2018.04.115.
Kunisue, T., Wu, Q., Tanabe, S., Aldous, K.M., Kannan, K., 2010. Analysis of five benzophenone-type UV filters in human urine by liquid chromatography-tandem mass spectrometry. Anal. Methods 2, 707–713. https://doi.org/10.1039/b9ay00324j.
Kunz, P.Y., Fent, K., 2006. Estrogenic activity of UV filter mixtures. Toxicol. Appl. Pharmacol. 217, 86–99. https://doi.org/10.1016/j.taap.2006.07.014.
Kunz, P.Y., Galicia, H.F., Fent, K., 2006. Comparison of in vitro and in vivo estrogenic activity of UV filters in fish. Toxicol. Sci. 90, 349–361. https://doi.org/10.1093/toxsci/kfj082.
Lee, J., Kim, S., Park, Y.J., Moon, H.B., Choi, K., 2018. Thyroid Hormone-Disrupting Potentials of Major Benzophenones in Two Cell Lines (GH3 and FRTL-5) and Embryo-Larval Zebrafish. Environ. Sci. Technol. 52, 8858–8865. https://doi.org/10.1021/acs.est.8b01796.
Lerebours, A., Gonzalez, P., Adam, C., Camilleri, V., Bourdineaud, J.P., Garnier-Laplace, J., 2009. Comparative analysis of gene expression in brain, liver, skeletal muscles, and gills of zebrafish (Danio rerio) exposed to environmentally relevant waterborne uranium concentrations. Environ. Toxicol. Chem. 28, 1271–1278. https://doi.org/10.1897/08-357.1.
Li, P., Li, Z.H., Zhong, L., 2019. Effects of low concentrations of triphenyltin on neurobehavior and the thyroid endocrine system in zebrafish. Ecotoxicol. Environ. Saf. 186 https://doi.org/10.1016/j.ecoenv.2019.109776.
Mackay, D., Barnthouse, L., 2010. Integrated risk assessment of household chemicals and consumer products: Addressing concerns about triclosan. Integr. Environ. Assess. Manag. 6, 390–392. https://doi.org/10.1002/ieam.73.
Manuel, R., Gorissen, M., Piza Roca, C., Zethof, J., Van De Vis, H., Flik, G., Van Den Bos, R., 2014. Inhibitory avoidance learning in zebrafish (Danio rerio): Effects of shock intensity and unraveling differences in task performance. Zebrafish 11, 341–352. https://doi.org/10.1089/zeb.2013.0970.
Mason, D., Zajitschek, S., Anwer, H., O'Dea, R.E., Hesselson, D., Nakagawa, S., 2021. Low repeatability of aversive learning in zebrafish (Danio rerio). J. Exp. Biol. 224 https://doi.org/10.1242/jeb.240846.
Menezes, F.P., Amorim, R.R., Silva, P.F., Luchiari, A.C., 2022. Alcohol exposure and environmental enrichment effects on contextual fear conditioning in zebrafish. Behav. Process. 197, 104608 https://doi.org/10.1016/j.beproc.2022.104608.
Meshalkina, D.A., Kizlyk, M.N., Kysil, E.V., Collier, A.D., Echevarria, D.J., Abreu, M.S., Barcellos, L.J.G., Song, C., Kalueff, A.V., 2017. Understanding zebrafish cognition. Behav. Process. 141, 229–241. https://doi.org/10.1016/j.beproc.2016.11.020.
Molina-molina, J., Escande, A., Pillon, A., Gomez, E., Pakdel, F., 2008. Profiling of benzophenone derivatives using fi sh and human estrogen receptor-speci fi c in vitro bioassays, 232, 384–395. https://doi.org/10.1016/j.taap.2008.07.017.
Montes-Grajales, D., Fennix-Agudelo, M., Miranda-Castro, W., 2017. Occurrence of personal care products as emerging chemicals of concern in water resources: A review. Sci. Total Environ. 595, 601–614. https://doi.org/10.1016/j.scitotenv.2017.03.286.
Moreira, A.L.P., Luchiari, A.C., 2022. Effects of oxybenzone on zebra fi sh behavior and cognition. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2021.152101.
Nabinger, D.D., Altenhofen, S., Bitencourt, P.E.R., Nery, L.R., Leite, C.E., Vianna, M.R.M. R., Bonan, C.D., 2018. Nickel exposure alters behavioral parameters in larval and adult zebrafish. Sci. Total Environ. 624, 1623–1633. https://doi.org/10.1016/j.scitotenv.2017.10.057.
de Oliveira-Lima, J., Dias da Cunha, R.L., de Brito-Gitirana, L., 2021. Effect of benzophenone-3 on the blood cells of zebrafish (Danio rerio). J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes. https://doi.org/10.1080/03601234.2021.2022946.
Pinheiro-da-Silva, J., Silva, P.F., Nogueira, M.B., Luchiari, A.C., 2017. Sleep deprivation effects on object discrimination task in zebrafish (Danio rerio). Anim. Cogn. 20, 159–169. https://doi.org/10.1007/s10071-016-1034-x.
Portrais, K.B., Stevens, M.A., Trask, C.N., Mundy, S.N., Szetela, J.M., Bleakley, B.H., Dzieweczynski, T.L., 2019. Exposure to the ultraviolet filter benzophenone-3 (BP3) interferes with social behaviour in male Siamese fighting fish. Anim. Behav. 158, 175–182. https://doi.org/10.1016/j.anbehav.2019.10.014.
Ruhl, T., Jonas, A., Seidel, N.I., Prinz, N., Albayram, O., Bilkei-Gorzo, A., Von Der Emde, G., 2015. Oxidation and Cognitive Impairment in the Aging Zebrafish. Gerontology 62, 47–57. https://doi.org/10.1159/000433534.
Salim, S., 2017. Oxidative stress and the central nervous system. J. Pharmacol. Exp. Ther. 360, 201–205. https://doi.org/10.1124/jpet.116.237503.
Sandoval-Gío, J.J., Noreña-Barroso, E., Escalante-Herrera, K., Rodríguez-Fuentes, G., 2021. Effect of Benzophenone-3 to Acetylcholinesterase and Antioxidant System in Zebrafish (Danio rerio) Embryos. Bull. Environ. Contam. Toxicol. https://doi.org/10.1007/s00128-021-03277-6.
Schlumpf, M., Cotton, B., Conscience, M., Haller, V., Steinmann, B., Lichtensteiger, W., 2001. In Vitro and in Vivo Estrogenicity of UV Screens. Environ. Health Perspect. • VOLUME.
Schneider, S.L., Lim, H.W., 2019. Review of environmental effects of oxybenzone and other sunscreen active ingredients. J. Am. Acad. Dermatol. 80, 266–271. https://doi.org/10.1016/j.jaad.2018.06.033.
Schug, T.T., Blawas, A.M., Gray, K., Heindel, J.J., Lawler, C.P., 2015. Elucidating the links between endocrine disruptors and neurodevelopment. Endocrinology.
da Silva Junior, F.C., Agues-Barbosa, T., Luchiari, A.C., Batistuzzo de Medeiros, S.R., 2021. Genotoxicity and behavioral alterations induced by retene in adult zebrafish. J. Environ. Chem. Eng. https://doi.org/10.1016/j.jece.2021.106518.
Skórkowska, A., Maciejska, A., Pomierny, B., Krzyżanowska, W., Starek-Świechowicz, B., Bystrowska, B., Broniowska, Ż., Kazek, G., Budziszewska, B., 2020. Effect of Combined Prenatal and Adult Benzophenone-3 Dermal Exposure on Factors Regulating Neurodegenerative Processes, Blood Hormone Levels, and Hematological Parameters in Female Rats. Neurotox. Res. https://doi.org/10.1007/s12640-020-00163-7.
Strungaru, S.A., Robea, M.A., Plavan, G., Todirascu-Ciornea, E., Ciobica, A., Nicoara, M., 2018. Acute exposure to methylmercury chloride induces fast changes in swimming performance, cognitive processes and oxidative stress of zebrafish (Danio rerio) as reference model for fish community. J. Trace Elem. Med. Biol. 47, 115–123. https://doi.org/10.1016/j.jtemb.2018.01.019.
Tao, J., Bai, C., Chen, Y., Zhou, H., Liu, Y., Shi, Q., Pan, W., Dong, H., Li, L., Xu, H., Tanguay, R., Huang, C., Dong, Q., 2020. Environmental relevant concentrations of benzophenone-3 induced developmental neurotoxicity in zebrafish. Sci. Total Environ. 721, 137686 https://doi.org/10.1016/j.scitotenv.2020.137686.

Tao, Y., Li, Z., Yang, Y., Jiao, Y., Qu, J., Wang, Y., Zhang, Y., 2022. Effects of common environmental endocrine-disrupting chemicals on zebrafish behavior. Water Res 208. https://doi.org/10.1016/j.watres.2021.117826.

Velanganni, S., Miltonprabu, S., 2020. Effect of benzophenone-3 at the environmentally relevant concentration on the liver of Zebra fish (Danio rerio (HAmilton). Int. J. Ecol. Environ. Sci. 2, 640–646.

Wang, J., Meng, X., Feng, C., Xiao, J., Zhao, X., Xiong, B., Feng, J., 2021. Benzophenone-3 induced abnormal development of enteric nervous system in zebrafish through MAPK/ERK signaling pathway. Chemosphere 280. https://doi.org/10.1016/j.chemosphere.2021.130670.

Wichmann, R., Fornari, R.V., Roozendaal, B., 2012. Glucocorticoids interact with the noradrenergic arousal system in the nucleus accumbens shell to enhance memory consolidation of both appetitive and aversive taste learning. Neurobiol. Learn. Mem. 98, 197–205. https://doi.org/10.1016/j.nlm.2012.06.004.

Wnuk, A., Rzemieniec, J., Litwa, E., Lasoń, W., Kajta, M., 2018. Prenatal exposure to benzophenone-3 (BP-3) induces apoptosis, disrupts estrogen receptor expression and alters the epigenetic status of mouse neurons. J. Steroid Biochem. Mol. Biol. 182, 106–118. https://doi.org/10.1016/j.jsbmb.2018.04.016.

Yoo, J.W., Sohn, S.H., Kim, Y.H., Min, T.J., 2021. The Effect of Ulinastatin to the Learning and Memory in Zebrafish. NeuroMolecular Med. https://doi.org/10.1007/s12017-021-08653-3.

Zhang, N.S., Liu, Y. sheng, Van den Brink, P.J., Price, O.R., Ying, G.G., 2015. Ecological risks of home and personal care products in the riverine environment of a rural region in South China without domestic wastewater treatment facilities. Ecotoxicol. Environ. Saf. 122, 417–425. https://doi.org/10.1016/j.ecoenv.2015.09.004.

Zhang, T., Sun, H., Qin, X., Wu, Q., Zhang, Y., Ma, J., Kannan, K., 2013. Benzophenone-type UV filters in urine and blood from children, adults, and pregnant women in China: Partitioning between blood and urine as well as maternal and fetal cord blood. Sci. Total Environ. 461–462, 49–55. https://doi.org/10.1016/j.scitotenv.2013.04.074.