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## **Exposure to four chemical UV filters through contaminated sediment: impact on survival, hatching success, cardiac frequency and aerobic metabolic scope in embryo-larval stage of zebrafish**

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1 **Exposure to four chemical UV filters through contaminated sediment: impact on survival,**  
2 **hatching success, cardiac frequency and aerobic metabolic scope in embryo-larval stage of**  
3 **zebrafish**

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56 **Abstract**

57 UV-filters are widely used in many pharmaceutical and personal care products such as sunscreen and cosmetics to  
58 protect from UV irradiation. Due to their hydrophobic properties and relative stability, they have a high capacity to  
59 accumulate in sediment. Little information is available on their ecotoxicity on fish. In aquatic ecosystems, fish eggs  
60 could be directly affected by UV-filters through contact with contaminated sediment. The aim of this study was to  
61 investigate the individual toxicity of four UV-filters: benzophenone-3 (BP3), butyl methoxydibenzoylmethane (BM),  
62 bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT) and methylene bis-benzotriazolyl tetramethylbutylphenol  
63 (MBBT), in embryo-larval stages of zebrafish *Danio rerio*. Fish eggs were exposed to single UV-filters by contact  
64 with spiked sediment during 96 h at a concentration of  $10 \mu\text{g.g}^{-1}$ . Among the four UV-filters tested, BP3 was the  
65 more toxic, reducing cardiac frequency and increasing standard metabolic rate of larvae.

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67 **Keyword:** *Danio rerio*, artificial sediment, metabolic rate, heart rate, sub-lethal concentration, sunscreen

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## 84 **Introduction**

85  
86 The increase of anthropogenic activities has a continuous impact on aquatic ecosystems by the input of many  
87 chemicals and the resulting degradation of habitat quality. They also represent a significant threat for aquatic life.  
88 Among them, UV filters are emerging potential pollutants that have received public interest since the last decade  
89 (Tovar-Sánchez et al., 2013; Tsui et al., 2014). These chemicals can be found in many personal care products  
90 (sunscreen, cosmetics) and as additive in plastics or clothes to protect from UV radiation damage (Fent et al., 2010;  
91 Waldman and Grant-Kels, 2019). UV filters enter the aquatic ecosystem directly by washing off from the skin  
92 especially during summer related activities or indirectly through wastewater (Gioka et al. 2007; Fent et al., 2010;  
93 Ramos et al. 2016). Sixty different UV filters are currently found in the global market (Pirota, 2016) and it is  
94 estimated that tourism in the coastal areas introduces around 14,000 tons of sunscreen in the oceans every year  
95 (Downs et al. 2014, 2016). Among them, four UV filters commonly found in sunscreens and in environment were  
96 selected in this study: benzophenone-3 (BP3), butyl methoxydibenzoylmethane (BM), bis-ethylhexyloxyphenol  
97 methoxyphenyl triazine (BEMT) and methylene bis-benzotriazolyl tetramethylbutylphenol (MBBT). Environmental  
98 concentrations of these compounds are found in numerous reports, with recent reviews summarizing their  
99 concentrations in different matrices/compartments including rivers, lakes, coastal ocean, surface water or sediments  
100 (Giokas et al. 2007; Ramos et al. 2015; Tsui et al. 2015; Molins-Delgado et al. 2016; Montes-Grajales et al. 2017).  
101 This study focused on sediment compartment. Due to their lipophilic properties, organic UV filters were found to  
102 accumulate in sediments that is considered to be the major way of contamination (Fagervold et al., 2019). Of the four  
103 UV filters studied, most occurrence data are available for BP3 and BM. The average concentration of 49.4 ng/g for  
104 BP3 measured in Banyuls bay sediments during summer by Fagervold et al. (2019) is in the range of concentrations  
105 found by Sanchez-Quiles et al. (2015) and Mao et al. (2018). Lower concentrations of 1.3 and 3.6 ng/g were also  
106 found in river sediment (Kameda et al. 2011) and in a German lake (Kaiser et al. 2012a), respectively. An extreme  
107 BP3 concentration of 1600 ng/g was measured in marine sediments in California (Schlenk et al., 2005) and a  
108 concentration of 1735 ng/g was found in a Japanese river sediment (Kameda et al. 2011). BM was detected at  
109 concentrations between 2.5 to 64.4 ng/g (Kaiser et al. 2012a, b; Sanchez-Quiles et al. 2015, Tsui et al. 2015,  
110 Fagervold et al. 2019). Only few studies measured MBBT and BEMT concentrations. Fagervold et al. (2019)  
111 detected maximum concentrations of 115.0 ng/g BEMT, and 75.2 ng/g MBBT (dry weight sediment). More data are  
112 needed, in particular for these last two UV filters. A summary of the UV-filters concentrations measured in

113 environmental sediment is provided in table 1. In the present study, the concentration tested of 10  $\mu$  g/g is around  
114 100 times higher (but only ~6 to 10 times for BP3) than highest concentrations of UV filters measured in aquatic  
115 sediment. This factor corresponds to the secure ratio generally accepted for regulations.

116 The toxicity of UV filters has been documented in diverse organisms such as algae (Gao et al. 2013; Esperaza et al.  
117 2019, Mao et al. 2019, Thorel et al, 2020), coral (Downs et al. 2014, 2016; He et al. 2019, Stien et al, 2019),  
118 crustacean (Sieratowicz et al. 2011; Chen et al. 2018, Thorel et al. 2020) or amphibians (Martins et al, 2017). On  
119 fish, previous studies have demonstrated that some compounds can act as endocrine disruptors leading to adverse  
120 reproductive effects on zebrafish, Japanese medaka, rainbow trout or clownfish (Schreurs et al. 2002; Coronado et  
121 al. 2008; Kinnberg et al. 2015; Lee et al 2018, Barone et al. 2019). Moreover, UV filters can impair gene expression  
122 (Zucchi et al., 2011; Blüthgen et al 2014), development (Torres et al. 2016) or fish behavior (Araújo et al. 2018;  
123 Barone et al. 2019). For a recent review see Lozano et al (2020).

124 In the present study, zebrafish *Danio rerio* was selected as experimental model that have been recommended for  
125 chemical risk assessment (Lammer et al. 2009; OECD 1998; 2013a). This species is well described in litterature  
126 (Kimmel et al. 1995; Lawrence et al. 2007) and offers multiple advantages for ecotoxicity testing (Hill et al. 2005;  
127 Spitsbergen and Kent 2003). As there is a need for a better understanding of the toxicity of UV filters, the aim of this  
128 study was to address the effect of UV filters-contaminated sediment on zebrafish early life stages. Toxic responses of  
129 exposed embryos and larvae were examined through physiological endpoints such as survival, hatching success,  
130 cardiac frequency and aerobic metabolism.

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## 132 **Material and methods**

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### 134 Chemicals and preparation of spiked artificial sediment

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136 The UV filters used in this study are listed in Table 1. Analytical standards of BEMT and BM were provided by  
137 Pierre Fabre Laboratories whereas BP3, MBBT and dichloromethane were purchased from Sigma Aldrich (Saint-  
138 Quentin-Fallavier, France).

Abbr.	Name	Alternative names	CAS #	Formula	Maximum concentration in final product <sup>a</sup>			Environmental concentration measured in sediment (in ng/g)	References
					USA	EU	Aus.		
MBBT	Methylene bis-benzotriazolyl tetramethylbutylphenol	Bisocetrizole, Tinosorb M, Milestab 360	103597-45-1	C <sub>41</sub> H <sub>50</sub> N <sub>6</sub> O <sub>2</sub>	n.a.	10 %	10 %	48 to 75.2	Fagervold et al 2019 Kaiser et al 2012a
BP3	Benzophenone-3	Oxybenzone	131-57-7	C <sub>14</sub> H <sub>12</sub> O <sub>3</sub>	6 %	6 %	10 %	1.3 to 1735	Fagervold et al 2019 Kameda et al 2011 Kaiser et al 2012a Mao et al 2018 Sanchez-Quiles et al 2015 Schlenk et al 2009
BM	Butyl methoxydibenzoyl methane	Avobenzene	70356-09-1	C <sub>20</sub> H <sub>22</sub> O <sub>3</sub>	3 %	5 %	5 %	2.5 to 64.4	Fagervold et al 2019 Kaiser et al 2012a Sanchez-Quiles et al 2015 Tsui et al. 2015
BEMT	bis-Ethylhexyloxyphenol methoxyphenyl triazine	Bemotrizinol, Tinosorb S, Escalol S	187393-00-6	C <sub>38</sub> H <sub>49</sub> N <sub>3</sub> O <sub>5</sub>	n.a.	10 %	10 %	48 to 111.5	Fagervold et al 2019 Kaiser et al 2012a, b

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Table 1 UV filters tested. <sup>a</sup> USA: United States of America, EU: European Union, Aus.: Australia. n.a.: not approved

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Artificial sediment was prepared according to Le Bihanic et al. (2014a, b). The reconstituted sediment was composed of sand measuring 0.2–0.5 mm (sable de Loire SCALARE, Aquastore, France), 5 % kaolin clay (Merck, Darmstadt, Germany), and Sphagnum blond peat (Florentaise, Saint-Mars-du-Désert, France). Briefly, peat was dried for 48 h and sieved (0.5 mm). Milli-Q water was added to the peat (12:1, v/v) in glass bottles and shaken for 48 h at 180 rpm at room temperature. Sand and 5 % kaolin clay were then mixed to humid peat and shaken for 24 h. pH was adjusted to 6.5 with 10 % CaCO<sub>3</sub> solution. After 7 days of stabilization at room temperature, 1:4 v/v of milli-Q water was added to the sediment. After a 24 h equilibration period, supernatant water was removed and the sediment was dried at 105 °C for 14 h. After cooling, 4 g artificial sediment added in glass Petri dishes were spiked with one of the UV filters at 10 μg.g<sup>-1</sup> dry weight sediment each as follows: a solution of the UV filter in dichloromethane (10 μl, 4 mg.mL<sup>-1</sup>) was mixed to sediment and then solvent was left to evaporated overnight in the dark at room temperature under a fume hood. This ensures complete residual solvent removal. A control with solvent (dichloromethane without UV filters) and sediment was prepared in the same manner. A negative control was tested too (no solvent, only artificial water E3 and sediment).

#### Zebrafish embryo-larval assay

##### *Maintenance of zebrafish and egg production*

Adults Wild-type AB-TU strain (Tübingen, Germany) zebrafish (*Danio rerio*) came from the Institute of Functional Genomics of Lyon (IGFL). Fish were maintained in groups of 10 individuals in 10 L tanks. Eggs were obtained from this culture by random pairwise mating of zebrafish. One adult male and one female were placed together in spawning boxes (AquaSchwarz, Germany) the evening before eggs were required. Spawning and fertilization took place within 30 min after the onset of light in the morning. Aquaria were filled with a mix of 1/3 reverse osmosis-treated water and 2/3 tap water without chlorine, both being filtered beforehand with dust and charcoal filters and using a flow-through system to obtain a pH of 7.8±0.5 and a conductivity of 450 ± 50 μS.cm<sup>-1</sup>. Rack aeration allowed an oxygen level ≥80 %. Water and room temperature were maintained at 28 ± 1 °C with a 14-h light/10-h dark photoperiod. Ammonia, nitrite, and nitrate were monitored weekly and remained within recommended ranges (Lawrence 2007). The fish were fed twice a day with commercial granulates (Ocean nutrition Europe, Belgique) and



an additional distribution of *Artemia* sp. nauplii (*Artemia* EG>225000 nauplii/g) occasionally supplemented with red sludge worms (Boschetto-Frozen fish food).

#### *Zebrafish exposure*

Experiments were done for each of the 4 individual UV filters. Fertilized and normally developed eggs were selected at eight-cell stage (1 h 15 post-fertilization) using a binocular loupe. The egg fertilization rate had to be  $\geq 70\%$ , and a mix of three spawns was used to avoid a potential spawn effect. Artificial water (E3) according to ISO 7346/3 (1996) was used as test medium (0.33 mM CaCl<sub>2</sub>, 0.33 mM MgSO<sub>4</sub>, 5 mM NaCl, and 0.17 mM KCl). Exposure tests were carried out in glass Petri dishes (35-mm diameter) with 4 g dw artificial sediment and 8 mL E3. Thirty embryos were placed randomly on a 1-mm thermoformed Nyltex® grid to avoid being buried in the sediment and exposed for 96 h in an incubator at 28±0.5 °C, with the same photoperiod as the rearing room. At the end of exposure, larvae were transferred to new Petri dishes containing only freshly prepared E3 medium until 10 days post fertilization (dpf) for analysis. Larvae were not fed during the experiment.

Exposures were performed using a procedure adapted from Le Bihanic et al. (2014a, b) for the 10-day embryo-larval toxicity assay (including 96 h of exposure, embryos, and pro-larvae) with zebrafish (OECD 1998, 2013b).

#### *Survival and hatching success*

Embryonic and larvae survival was recorded daily until 10 dpf. For each condition tested, four replicates of 30 individuals were observed. Dead individuals were removed. The survival rate (SR, %) was estimated as:

$$SR = 100 \times (SN_{t_x} / SN_{t_0}) \quad \text{equation (1)}$$

where SN<sub>t<sub>x</sub></sub> and SN<sub>t<sub>0</sub></sub> are the number of live individuals at time t<sub>x</sub> (time of the measure, x hours post fertilization – hpf) and t<sub>0</sub> (beginning of the experiment, 0 hpf).

Similarly, hatched individuals were counted between 48 and 72 hpf. At the end of exposure (96 hpf), hatching success (HS, %) was calculated as:

$$HS = 100 \times (HI_{96h} / SN_{96h}) \quad \text{equation (2)}$$

where  $HI_{96h}$  is the number of hatched and living larvae.

### *Cardiac frequency*

In order to assess cardiac frequency  $f_H$ , a total of 183 larvae were tested individually ( $n_{control}=33$ ,  $n_{BP3}=39$ ,  $n_{BEMT}=40$ ,  $n_{MBBT}=39$ ,  $n_{BM}=32$ ). The low pigmentation and transparency of larvae at 5 dpf permitted to visually measure  $f_H$  through the body wall. In order to do so, individuals were placed in lateral position into 3 % methylcellulose on Petri dishes (i.e., 6–7 ml). Two hours-acclimation were necessary to distress from the transfer and reach basal heart rate (Lucas et al. 2014b). Three consecutive videos of 20 s were recorded to observe *in vivo* beating heart of each larva. The use of a microscope (Olympus DP71), above which was placed a color camera, permitted to record videos on “Cell” software. Estimation of  $f_H$  was based on the principle that each passage of blood from the atrium to the ventricle corresponds to a cardiac contraction. In transparent larvae, blood circulation between these two cardiac chambers induces a difference in contrast on which the video analysis is based.  $f_H$  was manually counted for each video.

### *Respirometry*

#### Experimental set-up

The set-up consisted of eight independent glass micro respirometer chambers (diameter  $d = 9$  mm, volume  $V = 0.6$  mL; Loligo systems, Denmark). Respirometers were submerged into buffer tanks (depth×length×height 14×35×13 cm) and filled with oxygenated E3 solution at a constant temperature of 28 °C. Peristaltic pumps controlled the water supply from the buffer tank to each respirometer. Each pump was controlled by a timer, allowing intermittent flow respirometry (Steffensen 1994), where phases of oxygen renewal alternated with phases of oxygen consumption measurements with a period of 30:15 min. Each respirometer was equipped with an optic fiber sensor (PreSens) connected to a multichannel oxygen measuring system (Witrox 4, PreSens) to record dissolved oxygen levels. Optic fibers were calibrated at 0 and 100 % of air saturation at a temperature of 28 °C. A conversion factor based on oxygen solubility into water was used to convert oxygen data from percentage saturation to  $mgO_2.L^{-1}$  (i.e., 100 % was equivalent to  $7.94 mgO_2.L^{-1}$  for a 28 °C temperature and a 0 salinity). Oxygen saturation was recorded every 5 s

with the program Witroxview (PreSens).

### Experimental protocol

Each experimental trial consisted in testing simultaneously four groups of 5 larvae exposed to one of the 4 UV filters tested. Ten groups per filter were tested. Each experimental trial involved two steps. First, to assess active metabolic rate (AMR), metabolism of larvae was increased through chasing with a stick into Petri dishes (Schurmann and Steffensen 1997; Lefrançois and Claireaux 2003; Jourdan-Pineau et al. 2010; Cannas et al. 2013; Clark et al. 2012). When exhausted (*i.e.*, when fish did not respond to stimulation anymore), each group of five larvae was transferred into one of the four respirometers to assess AMR through a first oxygen consumption over the next 15 min. Second, to estimate standard metabolic rate (SMR), larvae were left undisturbed in the respirometer for 48 h, during which oxygen consumption was regularly and automatically measured. After this period of measurements, larvae were removed from the respirometers and slightly anesthetized with benzocaïne at a concentration of 50 mg.L<sup>-1</sup>. The body mass of each individual was determined using a microbalance (Sartorius Secura 26-1S). Before and after each trial, a blank measurement was performed to quantify microbial oxygen consumption in the respirometer. The average of these two values was subtracted from the measured oxygen consumption. Each group of larvae was tested once.

### Calculations

Oxygen consumption MO<sub>2</sub> is expressed in mgO<sub>2</sub>.g<sup>-1</sup>.h<sup>-1</sup> and calculated using the following formula:

$$MO_{2\text{meas}} = \Delta [O_2] \cdot \Delta t^{-1} \cdot V \cdot M_{\text{meas}}^{-1} \quad \text{Equation (3)}$$

where  $\Delta [O_2]$  (in mgO<sub>2</sub>.L<sup>-1</sup>) is the variation in oxygen concentration during the measurement period  $\Delta t$  (in h),  $V$  (in L) is the volume of the respirometer minus the volume of the fish and  $M_{\text{meas}}$  (in g) is the mass of the corresponding group of 5 larvae.

An allometric relationship exists between oxygen consumption and body mass, which encourages correction of MO<sub>2meas</sub> using the following formula:

$$MO_{2\text{cor}} = MO_{2\text{meas}} \cdot (M_{\text{meas}} \cdot M_{\text{cor}}^{-1})^{1-b} \quad \text{Equation (4)}$$

where  $MO_{2cor}$  (in  $mg\ O_2 \cdot g^{-1} \cdot h^{-1}$ ) is the oxygen consumption related to a standard fish of 1 g ( $M_{cor}$ ),  $MO_{2meas}$  (in  $mg\ O_2 \cdot g^{-1} \cdot h^{-1}$ ) is the oxygen consumption estimated with eq. 3 for experimental fish whose mass was  $M_{meas}$  (in g) and  $b$  is the allometric scaling exponent describing the relationship between oxygen consumption and mass of fish. In the case of this study, we used  $b_{AMR} = 0.926$  for the correction of active metabolic rate and  $b_{SMR} = 0.965$  for the correction of standard metabolic rate (Lucas et al. 2014a).

AMR was assessed as the maximal  $MO_2$  obtained after chasing the larvae. SMR was estimated according to the method described by Steffensen et al. (1994) and Lucas et al. (2014a). Aerobic metabolic scope (AMS) was calculated as the difference between AMR and SMR. AMR, SMR, and AMS were assessed for each group.

#### Statistical analysis

The statistical analysis was carried out using GraphPad Prism software. For all analyses the normality and homoscedasticity were checked through the Shapiro and Barlett tests, respectively. An unpaired t-test was then used to determine which chemical treatment differed significantly from the solvent control. The results were considered to be significantly different when  $p < 0.05$ .

## Results and discussion

In last decades, several different ecotoxicity tests have been established demonstrating the robustness of fish embryo larval assays to reflect the toxicity of organic pollutants. Our study investigated for the first time the toxicity of BP3, BMDBM, BEMT and MBBT through sediment exposure on embryo larval stages of the zebrafish and through different physiological endpoints.

Sediment contact assays on fish embryos stages have been proposed as an alternative to classic ecotoxicity assays for chemical testing (Barjoux et al. 2012, Le bihannic et al 2014, Viquelin et al. 2011). This kind of assay better reproduces the multiple sources of exposure to benthic organisms via sediment, particle and dissolved phase.

For all physiological endpoints, a negative control (sediment) and solvent control (solvent and sediment) were tested in order to estimate the impact of solvent. No difference was observed between these two controls whatever the parameters measured ( $p > 0.05$ ).

#### 4.1 Impact on survival and hatching

Treatment	Number of replicate	Survival rate (%)		Hatching success (%)
		96 hpf	10 dpf	96 hpf
Negative control	3	86.67±6.7	85.58±5.1	95.75±5.68
Solvent control	4	84.2±7.4	84.2±7.4	92.5±11.2
BP3	4	82.2±6.9	82.2±6.9	72.6±23.1
MBBT	4	70.8±11.2	69.2±7.4	85.4±17.7
BEMT	4	76.7±3.3	74.4±1.9	97.1±2.5
BM	4	87.8±1.9	86.6±0.0	92.5±6.5

Table 2 Survival and hatching success on zebrafish assessing UV filters toxicity. Mean values±standard deviation. All results are not significantly different from solvent control experiment (t-test,  $p < 0.05$ ).

Survival and hatching success measured following UV filters exposure are shown in Table 2. At  $10 \mu\text{g}\cdot\text{g}^{-1}$ , none of the four UV filters tested induced significant mortality, hatching failure, or delayed hatching of embryos and larvae compared to the solvent control (t-test,  $p > 0.05$  : BP3  $p_{4j}=0.7396$ ,  $p_{10j}=1.000$ ; BEMT  $p_{4j}=0.0075$ ,  $p_{10j}=0.0721$ ; MBBT  $p_{4j}=0.01683$ ,  $p_{10j}=0.1649$ ; BM  $p_{4j}=0.4588$ ,  $p_{10j}=0.3739$ ). This means that sediment exposure to BP3, BEMT, MBBT or BM did not display any lethal effect in zebrafish embryo larval stage in our conditions of exposure. It also indicates that direct embryonic exposure to these compounds may not impact the fish recruitment in environmental conditions. This is the first report on the effect of BP3, MBBT, BEMT or BM in zebrafish embryo larval stages.

#### 4.2 Cardiotoxicity

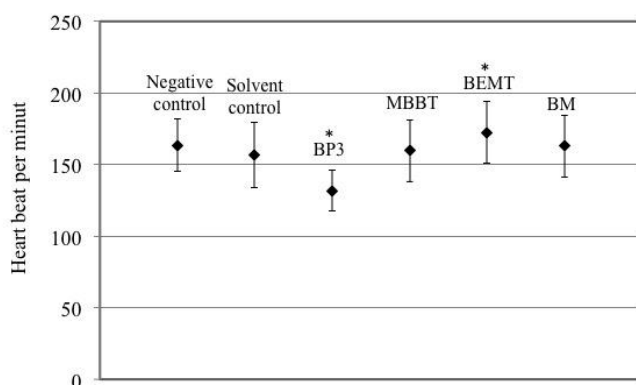


Fig 1 Cardiac frequency of 5 dpf larvae zebrafish *Danio rerio* exposed to one of the following treatment: control, exposure to BP3, MBBT, BEMT or BM. Mean values±standard deviation are plotted and \* indicates significant difference with the solvent control (t-test,  $p < 0.05$ ).

Negative control and solvent control presented heartbeat rate of  $163.42 \pm 18.44 \text{ bt. min}^{-1}$  and  $156.44 \pm 22.81 \text{ bt. min}^{-1}$  respectively which is in accordance with normal zebrafish heartbeat rate measured in others studies (Barrionuevo and Burggren, 1999; Burggren, 2005; Lin et al. 2007; Lucas et al., 2014, Quintaneiro et al., 2019). No significant effect was observed on MBBT (t-test,  $p=0.5376$ ) and BM (t-test,  $p=0.2435$ ) treatments compared to the solvent control. BP3 exposure induced a significantly 15.8 % decreased heart rate (Fig 1; t-test,  $p < 0.0001$ ). Bradychardia of this kind was also observed on zebrafish for 4-methylbenzylidene camphor (4-MBC) exposures at higher concentration using exposure through water (Torres et al., 2016; Quintaneiro et al., 2019). Similar pattern was observed in studies investigating the cardiotoxicity of polyaromatic hydrocarbons (PAHs) in early life stages of zebrafish directly exposed to contaminated sediment or oil (Incardona 2004, 2005, 2011; Hicken et al. 2011; Perrichon et al. 2014, 2016). Li et al. (2018) and Quintaneiro et al. (2019) suggested a direct association between heart rate decrease and heart deformities. In our study, no heart deformities were observed during embryo development (pericardial oedema for example). Additional histological analysis of fish heart could show internal lesions characteristics of cardiac insufficiency. Furthermore, cardiac frequency might be related with the functioning of cardiac muscle, which should be further investigated in future studies as well as cardiac gene expression. In contrast to BP3, we observed

that BEMT induced a significant 10.2 % increase of cardiac frequency (Fig 1, t-test,  $p = 0.003$ ). This kind of increase was also observed by Lucas et al. (2014) in zebrafish larvae born from progenitors exposed to polycyclic aromatic hydrocarbons PAHs at environmental concentration (i.e. 12.5 % increased cardiac frequency). This increase was not lethal and maybe only due to a stress response but require more investigations to be interpreted. One hypothesis to explain this tachycardia pattern is the production of stress hormones such as cortisol. Pollutant exposure could induce an elevated production of this hormone which could modulate cardiac frequency (McCormick and Nechaev, 2002; Nesan and Vijayan, 2012). Another hypothesis is the stress related to the UV filter. It is known that UV-filters can lead to oxidative stress by increasing production of reactive oxygen species (ROS). This can result in activation of antioxidant defences and/or detoxification system in order to prevent oxidative damage (Liu et al., 2015), therefore increasing heartbeat rate. These processes are energy costly which could have negative impact on aerobic metabolic scope, increasing SMR (Lannig et al., 2006, Lucas et al. 2014b).

#### 4.3. Aerobic metabolic scope

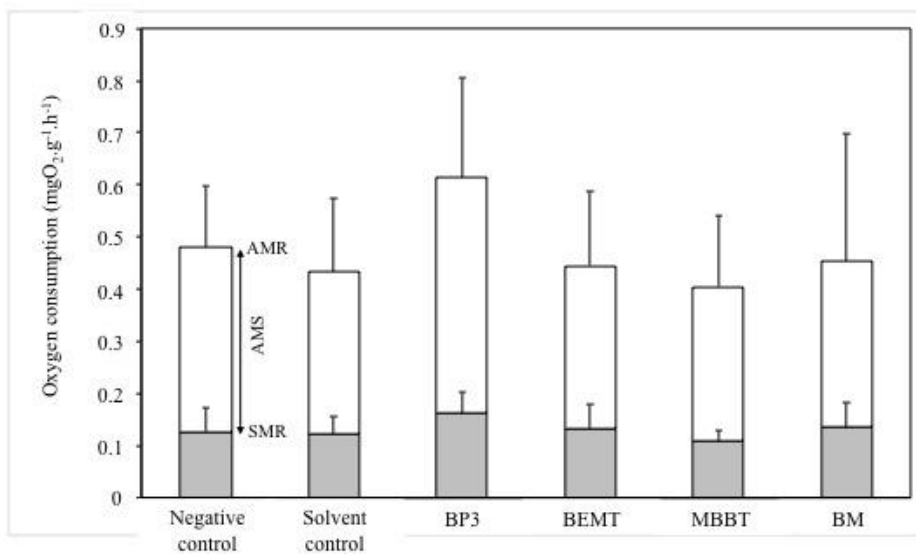


Fig 2 Active metabolic rate (AMR), standard metabolic rate (SMR) and aerobic metabolic scope (AMS) of 5 dpf larvae *Danio rerio* in control or after exposition to one of the 4 UV filters tested: BP3, BEMT, MBBT, BM. Results

are expressed as mean values  $\pm$  standard deviation. Among all the metabolic parameters measured and UV-filters tested, only SMR for BP3 differed significantly from the solvent control (t-test,  $p < 0.05$ ).

This is the first work on the impact of UV filters on fish metabolism. Therefore, comparison with other studies are still limited. No effect was observed on SMR, AMR or AMS measured in 5 dpf embryo larvae for exposure to BEMT (Fig 2, t-test,  $p_{SMR}=0.2578$ ,  $p_{AMR}=0.9471$ ,  $p_{AMS}=0.9103$ ), MBBT (Fig 2, t-test,  $p_{SMR}=0.08426$ ,  $p_{AMR}=0.6822$ ,  $p_{AMS}=0.9103$ ) and BM (Fig 2, t-test,  $p_{SMR}=0.2185$ ,  $p_{AMR}=0.8715$ ,  $p_{AMS}=0.949$ ) compared to Solvent control. For BP3 exposure, no effect was observed on AMR (Fig 2, t-test,  $p = 0.0969$ ) and AMS (Fig 2, t-test,  $p = 0.2113$ ) and thus the decreased cardiac frequency observed for BP3 cannot be associated with lower AMR and AMS as observed for other organic pollutants (Claireaux and Davoodi 2010, Hiken et al. 2011). SMR was significantly higher than the control (Fig 2, t-test,  $p=0.0195$ ). It is known that exposure to pollutant can modulate cardio-respiratory performance and aerobic capacity of fish. Pollutant exposure can induce detoxification processes that are energy costly. This supplementary energetic demand would increase SMR (Lannig et al., 2006, Lucas et al. 2014b) as observed for BP3. Even if SMR increased AMS was not significantly different from the solvent control. Fishes seem to maintain their aerobic metabolic scope despite UV filter exposure. Furthermore, although alteration of the cardiovascular system can have harmful consequences on energetic metabolism, the aerobic metabolic rate did not correlate to the effect on cardiac frequency observed with BP3 and BEMT. We hypothesized that larvae may offset by modulating other mechanisms implied in blood circulation and oxygen transport. Moreover, a part of respiration is cutaneous in early life stage, hereby possibly modulating oxygen transport capacity. Overall, the compounds tested in our study did not induce impairment in the mechanisms involved in metabolic regulation at the tested concentration. These results suggest that *Danio rerio* larvae would not have a reduced capacity to sustain oxygen-demanding activities such as locomotion, digestion or growth (Claireaux & Lefrançois, 2007).

## **Conclusion**

In sum, in the tested conditions, BP3 and BEMT significantly altered *D. rerio* larvae heartbeat rate after exposure of fish eggs to contaminated sediments. BP3 also increased SMR. BP3 seems to be the more toxic UV-filter of the four



tested. This is consistent with numerous reports on BP3 toxicity on aquatic species (review of Lozano et al. 2020). This work is the very first report on the effect of BEMT and MBBT on fish development and larval metabolism. BEMT and BM had no effect on the different parameters tested. In our conditions, these compounds were not toxic for zebrafish embryo larvae. Since environmental toxicity data remain scarce for many UV filters, and considering the intense use of personal care products resulting in notable concentrations in the aquatic environment, more data on ecotoxicological effects of UV filters on fish are urgently needed (review of Lozano et al. 2020), and it is necessary to continue to perform ecotoxicological study and to develop new models.

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