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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**

Julie Lucas, Valentin Logeux, Alice M S Rodrigues, Didier Stien, Philippe Lebaron

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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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7 Julie Lucas<sup>a\*</sup>, Valentin Logeux<sup>b</sup>, Alice M. S. Rodrigues<sup>a</sup>, Didier Stien<sup>a</sup>, Philippe Lebaron<sup>a</sup>

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10 <sup>a</sup> Sorbonne Université, CNRS, Laboratoire de Biodiversité et Biotechnologie Microbiennes, LBBM, Observatoire  
11 Océanologique, 66650 Banyuls-sur-Mer, France

12 <sup>b</sup> Sorbonne Université, CNRS, Fédération de Recherche, Observatoire Océanologique, 66650 Banyuls-sur-Mer,  
13 France

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27 \*Author to whom correspondence should be addressed. e-mail: julie.lucas@obs-banyuls.fr

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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 Nytex® 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:

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

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 benzocaine 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

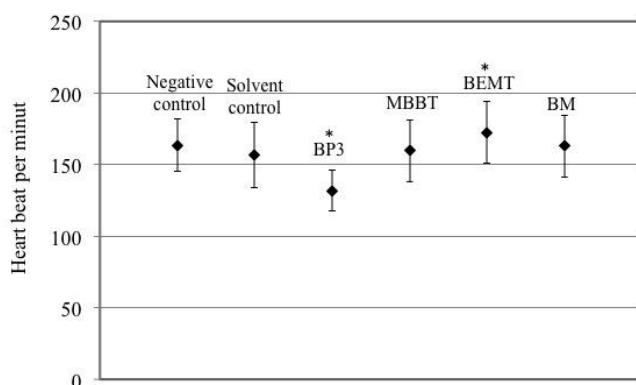


Fig 1 Cardiac frequency of 5 dpf larvae zebrafish *Danio rerio* exposed to one of the following treatment: control, exposure to BP3, MBBT, BEMT or BM. Mean values±standard deviation are plotted and \* indicates significant difference with the solvent control (t-test,  $p < 0.05$ ).

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). Additionnal 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

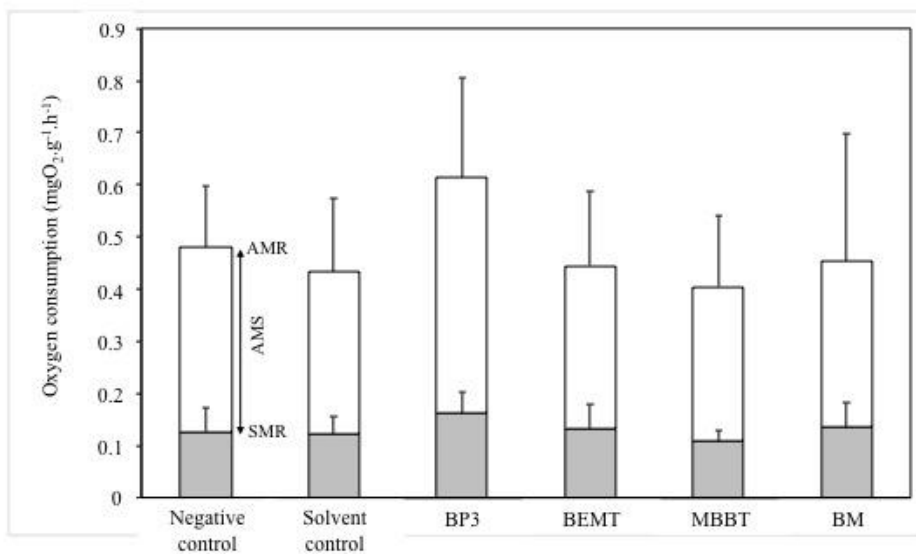


Fig 2 Active metabolic rate (AMR), standard metabolic rate (SMR) and aerobic metabolic scope (AMS) of 5 dpf larvae *Danio rerio* in control or after exposition to one of the 4 UV filters tested: BP3, BEMT, MBBT, BM. Results

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.

## References

Amine H, Gomez E, Halwani J, Casellas C, Fenet H (2012) UV filters ethylhexyl methoxycinnamate, octocrylene and ethylhexyl dimethyl PABA from untreated wastewater in sediment from eastern Mediterranean river transition and coastal zones, Mar Pollut Bull 64, 2435–2442

Araújo MJ, Rocha RJM, Soares AMVM, Benedé JL, Chisvert A, Monteiro MS (2018) Effects of UV filter 4-methylbenzylidene camphor during early development of *Solea senegalensis* Kaup, 1858. Sci Total Environ 628–629, 1395–1404. <https://doi.org/10.1016/j.scitotenv.2018.02.112>

Barón E, Gago-Ferrero P, Gorga M, Rudolph I, Mendoza G, Zapata AM et al. (2013) Occurrence of hydrophobic organic pollutants (BFRs and UV-filters) in sediments from South America. Chemosphere, 92(3), 309–316

Barone AN, Hayes CE, Kerr JJ, Lee RC, Flaherty DB (2019) Acute toxicity testing of TiO<sub>2</sub>-based vs. oxybenzone-based sunscreens on clownfish (*Amphiprion ocellaris*). Environ Sci Pollut Res 26, 14513–14520. <https://doi.org/10.1007/s11356-019-04769-z>

Barrionuevo WR and Burggren W (1999) O<sub>2</sub> consumption and heart rate in developing zebrafish (*Danio rerio*): influence of temperature and ambient O<sub>2</sub>. Am J of Physiol 276, 505-513.

Blüthgen N, Meili N, Chew G, Odermatt A., Fent K (2014) Accumulation and effects of the UV-filter octocrylene in adult and embryonic zebrafish (*Danio rerio*). *Sci Total Environ* 476–477, 207–217.

<https://doi.org/10.1016/j.scitotenv.2014.01.015>

Burggren WW (2005) Developing animals flout prominent assumptions of ecological physiology. *Comparative Biochem Physiol, Part A* 141, 430 – 439.

Cannas M, Atzori F, Rupsard F, Bustamante P, Loizeau V, Lefrançois C (2012) PCB contamination does not alter aerobic metabolism and tolerance to hypoxia of juvenile sole (*Solea solea*). *Aquat toxicol* 127, 54–60.

Chen L, Li X, Hong H, Shi D (2018) Multigenerational effects of 4-methylbenzylidene camphor (4-MBC) on the survival, development and reproduction of the marine copepod *Tigriopus japonicus*. *Aquat Toxicol* 194, 94–102.

<https://doi.org/10.1016/j.aquatox.2017.11.008>

Claireaux G and Davoodi F (2010) Effect of exposure to petroleum hydrocarbons upon cardio-respiratory function in the common sole (*Solea solea*). *Aquat Toxicol* 98 (2), 113–119. <https://doi.org/10.1016/j.aquatox.2010.02.006>.

Clark TD, Donaldson MR, Pieperhoff S, Drenner SM, Lotto A, Cooke SJ, Hinch SG, Patterson DA and Farrell AP (2012) Physiological benefits of being small in a changing world: responses of Coho salmon (*Oncorhynchus kisutch*) to an acute thermal challenge and a simulated capture event. *PLOS ONE* 7, e39079

Coronado M, De Haro H, Deng X, Rempel MA, 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>

Danovaro R, Bongiorno L, Corinaldesi C, Giovannelli D, Damiani E, Astolfi P, Greci L, Pusceddu A (2008) Sunscreens cause coral bleaching by promoting viral infections. *Environ Health Perspect* 116:441–447.

Downs CA, Kramarsky-Winter E, Fauth JE, Segal R, Bronstein O, Jeger R, Lichtenfeld Y, Woodley CM, Pennington P, Kushmaro A, Loya Y (2014) Toxicological effects of the sunscreen UV filter, benzophenone-2, on planulae and in vitro cells of the coral, *Stylophora pistillata*. *Ecotox* 23(2):175–191.

Downs CA, Kramarsky-Winter E, Segal R, Fauth J, Knutson S, Bronstein O, Ciner FR, Jeger R, Lichtenfeld Y, Woodley CM, Pennington P, Cadenas K, Kushmaro A, Loya Y (2016) Toxicopathological effects of the sunscreen UV filter, oxybenzone (benzophenone-3), on coral planulae and cultured primary cells and its environmental contamination in Hawaii and the U.S. Virgin Islands. *Arch Environ Contam Toxicol* 70, 265–288.  
<https://doi.org/10.1007/s00244-015-0227-7>

Esperanza M, Seoane M, Rioboo C, Herrero C, Cid Á (2019) Differential toxicity of the UV- filters BP-3 and BP-4 in *Chlamydomonas reinhardtii*: A flow cytometric approach. *Sci Total Environ* 669, 412–420.  
<https://doi.org/10.1016/j.scitotenv.2019.03.116>

Fagervold SK, Rodrigues AMS, Rohée C, Roe R, Bourrain M, Stien D, Lebaron P (2019) Occurrence and environmental distribution of 5 UV filters during the summer season in different water bodies. *Water Air Soil Pollut* 230, 172–172.

Fent K, Zenker A and Rapp M (2010) Widespread occurrence of estrogenic UV-filters in aquatic ecosystems in Switzerland. *Environ Poll* 158(5), 1817–1824.

Gago-Ferrero P, Díaz-Cruz MS, Barceló D (2015) UV filters bioaccumulation in fish from Iberian river basins. *Sci Total Environ* 518–519, 518–525. <https://doi.org/10.1016/j.scitotenv.2015.03.026>

Gago-Ferrero P, Alonso MB, Bertozzi CP, Marigo J, Barbosa L, Cremer M et al. (2013) First determination of UV filters in marine mammals. Octocrylene levels in Franciscana dolphins. *Environ Sci Technol* 47(11), 5619–5625.

Gago-Ferrero P, Díaz-Cruz MS, Barceló D (2011) Fast pressurized liquid extraction with in-cell purification and analysis by liquid chromatography tandem mass spectrometry for the determination of UV filters and their degradation products in sediments. *Anal Bioanal Chem* 400, 2195–2204. <https://doi.org/10.1007/s00216-011-4951-1>

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>

Giokas DL, Sakkas VA, Albanis TA and Lampropoulou DA (2005) Determination of UV-filter residues in bathing waters by liquid chromatography UV-diode array and gas chromatography–mass spectrometry after micelle mediated extraction–solvent back extraction. *J Chromatogr A*, 1077(1), 19–27.

He T, Tsui MMP, Tan CJ, Ma CY, Yiu SKF, Wang LH, Chen TH, Fan TY, Lam PKS, Murphy MB (2019) Toxicological effects of two organic ultraviolet filters and a related commercial sunscreen product in adult corals. *Environ Pollut* 245, 462–471. <https://doi.org/10.1016/j.envpol.2018.11.029>

Hicken CE, Linbo TL, Baldwin DH, Willis ML, Myers MS, Holland L and Incardona JP (2011) Sub-lethal exposure to crude oil during embryonic development alters cardiac morphology and reduces aerobic capacity in adult fish. *Proc Acad sci* 108, 7086–7090.

Hill AJ, Teraoka H, Heideman W and Peterson RE (2005) Zebrafish as a model vertebrate for investigating chemical toxicity. *Toxicological sciences: an official journal of the Society of Toxicology* 86(1), 6–19.

Incardona JP, Collier TK and Scholz NL (2004) Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. *Toxicol Appl Pharm* 196, 191–205.

Incardona JP, Carls MG, Teraoka H, Sloan CA, Collier TK and Scholz NL (2005) Aryl hydrocarbon receptor-independent toxicity of weathered crude oil during fish development. *Environ Health Persp* 113, 1755–1762.

Incardona JP, Linbo TL and Scholz NL (2011) Cardiac toxicity of 5-ring polycyclic aromatic hydrocarbons is differentially dependent on the aryl hydrocarbon receptor 2 isoform during zebrafish development. *Toxicol App Pharm* 257, 242–249.

Jourdan-Pineau H, Dupont-Prinet A, Claireaux G and McKenzie DJ (2010). An investigation of metabolic prioritization in the European Sea Bass, *Dicentrarchus labrax*. *Physiol Biochem Zool* 83, 68-77.

Kameda Y, Kimura K, Miyazaki M (2011) Occurrence and profiles of organic sun-blocking agents in surface waters and sediments in Japanese rivers and lakes. *Environ Pollut* 159, 1570-1576.  
<https://doi.org/10.1016/j.envpol.2011.02.055>

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>

Kimmel CB, Ballard WW, Kimmel SR, Ullmann B and Schilling TF (1995) Stages of Embryonic Development of the Zebrafish. *Dev Dynam* 203, 253-310.

Kinnberg KL, Petersen GI, Albrektsen M, Minghlani M, Awad S, Holbech B, Green J, Bjerregaard P, Holbech H (2015) Endocrine-disrupting effect of the ultraviolet filter benzophenone 3 in zebrafish, *Danio rerio*. *Environ Toxicol Chem* 34:2833–2840

Lammer E, Carr GJ, Wendler K, Rawlings JM, Belanger SE, Braunbeck T (2009) Is the fish embryo toxicity test (FET) with the zebrafish (*Danio rerio*) a potential alternative for the fish acute toxicity test? *Comp Biochem Physiol, Part C* 149, 196–209.

Lannig G, Flores JF and Sokolova IM (2006) Temperature-dependent stress response in oysters, *Crassostrea virginica*: Pollution reduces temperature tolerance in oysters. *Aquat Toxicol* 79, 278-287.

Lawrence C (2007) The husbandry of zebrafish (*Danio rerio*): A review. *Aquaculture* 269, 1–20.

Le Bihanic F, Morin B, Cousin X, Le Menach K, Budzinski H, Cachot J (2014a) Developmental toxicity of PAH mixtures in fish early life stages. Part I: adverse effects in rainbow trout. *Environ Sci Pollut Res Int* doi: 10.1007/s11356-014-2804-0.

Le Bihanic F, Perrichon P, Landi L, Clérandeau C, Le Menach K, Budzinski H, Cousin X, Cachot J (2014) Development of a reference artificial sediment for chemical testing adapted to the MELA sediment contact assay. *Environ Sci Pollut Res Int* (24):13689-702. doi: 10.1007/s11356-014-2607-3.

Lee J, Kim S, Park YJ, Moon HB, 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(15):8858–8865.

Lefrançois C and Claireaux G (2003) Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole *Solea solea*. *Mar Ecol Progr Series* 259, 273–284.

Li AJ, Law JCF, Chow CH, Huang Y, Li Leung KSY (2018) Joint Effects of Multiple UV Filters on Zebrafish Embryo Development. *Environ Sci Technol* 52, 9460-9467.

Lin CC, Hui MN, Cheng SH (2007). Toxicity and cardiac effects of carbaryl in early developing zebrafish (*Danio rerio*) embryos. *Toxicol Appl Pharmacol* 222(2):159-68.

Lozano C, Givens J, Stien D, Matallana-Surget S, Lebaron P (2020) Bioaccumulation and Toxicological Effects of UV-Filters on Marine Species. *The Handbook of Environmental Chemistry*. Springer, Berlin, Heidelberg. [https://doi.org/10.1007/698\\_2019\\_442](https://doi.org/10.1007/698_2019_442)

Lucas J, Schouman A, Lyphout L, Cousin X, Lefrancois C (2014a) Allometric relationship between body mass and aerobic metabolism in zebrafish *Danio rerio*. J Fish Biol 84(4):1171-8. doi: 10.1111/jfb.12306.

Lucas J, Perrichon P, Nouhaud M, Audras A, Leguen I, Lefrancois C (2014b) Aerobic metabolism and cardiac activity in the descendants of zebrafish exposed to pyrolytic polycyclic aromatic hydrocarbons. Environ Sci Pollut Res 21(24):13888-97. doi: 10.1007/s11356-014-3116-0.

Mao F, He Y, Kushmaro A, Gin KYH (2017) Effects of benzophenone-3 on the green alga *Chlamydomonas reinhardtii* and the cyanobacterium *Microcystis aeruginosa*. Aquat Toxicol 193, 1–8.  
<https://doi.org/10.1016/j.aquatox.2017.09.029>

Martins D, Monteiro MS, Soares AMVM, Quintaneiro C (2017) Effects of 4-MBC and triclosan in embryos of the frog *Pelophylax perezi*. Chemosphere, 178 325-332

McCormick MI, Nechaev IV (2002) Influence of cortisol on developmental rhythms during embryogenesis in a tropical damselfish. J Exp Zool 293(5):456-66.

Milinkovitch T, Lucas J, Le Floch S, Thomas-Guyon H and Lefrançois C (2012) Effect of dispersed crude oil exposure upon the aerobic metabolic scope in juvenile golden grey mullet (*Liza aurata*). Mar Pollut Bul 64, 1–7.

Molins-Delgado D, Máñez M, Andreu A, Hiraldo F, Eljarrat E, Barceló D and Díaz-Cruz MS (2017) A potential new threat to wild life: presence of UV filters in bird eggs from a preserved area. Environ Sci Technol 51(19), 10983–10990.

Montes-Grajales D, Fennix-Agudelo M and Miranda-Castro W (2017). Occurrence of personal care products as emerging chemicals of concern in water resources: a review. Sci Tot Environ 595, 601–614.

Nesan DM and Vijayan M (2012) Embryo exposure to elevated cortisol level leads to cardiac performance

dysfunction in zebrafish. *Mol Cell Endocrinol* 363, 85-91. <https://doi.org/10.1016/j.mce.2012.07.010>.

OCDE (1998), Test No. 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages, OECD Guidelines for the Testing of Chemicals, Section 2, Éditions OCDE, Paris, <https://doi.org/10.1787/9789264070141-en>.

OCDE (2013a) Test No. 236: Fish Embryo Acute Toxicity (FET) Test, OECD Guidelines for the Testing of Chemicals, Section 2, Éditions OCDE, Paris, <https://doi.org/10.1787/9789264203709-en>.

Perrichon P, Le Bihanic F, Bustamante P (2014) Influence of sediment composition on PAH toxicity using zebrafish (*Danio rerio*) and Japanese medaka (*Oryzias latipes*) embryo-larval assays. *Environ Sci Pollut Res* 21, 13703–13719. <https://doi.org/10.1007/s11356-014-3502-7>

Perrichon P, Le Menach K, Akcha F, Cachot J, Budzinski H, Bustamante P (2016) Toxicity assessment of water-accommodated fractions from two different oils using a zebrafish (*Danio rerio*) embryo-larval bioassay with a multilevel approach. *Sci Tot Environ* 568, 952-966. <https://doi.org/10.1016/j.scitotenv.2016.04.186>.

Pirotta G (2016) The encyclopedia of allowed sunfilters in the world. *Skin Care—Househ. Pers Care Today*, 11, 19–21.

Quintaneiro C, Teixeira B, Benedé JL, Chisvert A, Soares AMVM, Monteiro MS (2019) Toxicity effects of the organic UV-filter 4-Methylbenzylidene camphor in zebrafish embryos. *Chemosphere* 218:273-281. doi: 10.1016/j.chemosphere.2018.11.096.

Ramos S, Homem V, Alves A and Santos L (2015) Advances in analytical methods and occurrence of organic UV-filters in the environment — a review. *Sci Tot Environ* 526, 278–311.

Ramos S, Homem V, Alves A and Santos L (2016) A review of organic UV-filters in wastewater treatment plants. *Environ Internat* 86, 24–44.



Rodil R, Schrader S and Moeder M (2009) Non-porous membrane-assisted liquid–liquid extraction of UV filter compounds from water samples. *J Chromat A* 1216(24), 4887–4894.

Sánchez-Quiles D, Tovar-Sánchez A (2015) Are sunscreens a new environmental risk associated with coastal tourism? *Environ Int* 83, 158–170. <https://doi.org/10.1016/j.envint.2015.06.007>

Schreurs R, Lanser P, Seinen W, Van der burg B (2002) Estrogenic activity of UV filters determined by an in vitro reporter gene assay and an in vivo transgenic zebrafish assay. *Arch Toxicol* 76:257–261

Schurmann H and Steffensen JF (1997) Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. *J Fish Biol* 50, 1166–1180.

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 Part A* 46, 1311–1319. <https://doi.org/10.1080/10934529.2011.602936>

Schlenk D, Sapozhnikova Y, Irwin MA, Xie L, Hwang W, Reddy S, Brownawell BJ, Armstrong J, Kelly M, Montagne DE, Kolodziej EP, Sedlak D and Snyder S (2005) In vivo bioassay-guided fractionation of marine sediment extracts from the Southern California Bight, USA, for estrogenic activity. *Environ Toxicol Chem* 24: 2820-2826. doi:10.1897/05-116R.1

Spitsbergen JM and Kent ML (2003) The State of the Art of the Zebrafish Model for Toxicology and Toxicologic Pathology Research : Advantages and Current Limitations. *Toxicol Pathol* 31,62–87. DOI: 10.1080/01926230390174959

Steffensen JF, Bushnell PG and Schurmann H (1994) Oxygen consumption in four species of teleosts from Greenland: no evidence of metabolic cold adaptation. *Polar Biol* 14, 49-54.

Stien D, Clergeaud F, Rodrigues AMS, Lebaron K, Pillot R, Romans P, Fagervold S, Lebaron P (2019) Metabolomics reveal that octocrylene accumulates in *Pocillopora damicornis* tissues as fatty acid conjugates and triggers coral cell mitochondrial dysfunction. *Anal Chem* 91, 990–995.  
<https://doi.org/10.1021/acs.analchem.8b04187>

Thorel E, Clergeaud F, Jaugeon L, Rodrigues A, 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; doi:10.3390/toxics8020029

Torres T, Cunha I, Martins R, Santos MM (2016) Screening the toxicity of selected personal care products using embryo bioassays: 4-MBC, propylparaben and triclocarban. *Int J Mol Sci* 17, 1762.  
<https://doi.org/10.3390/ijms17101762>

Tovar-Sanchez A, Sanchez-Quiles D, Basterretxea G, Benede JL, Chisvert A, Salvador A et al. (2013) Sunscreen products as emerging pollutants to coastal waters. *PLoS One*, 8(6), e65451.

Tsui MMP, Leung HW, Wai TC, Yamashita N, Taniyasu S, Liu W, Lam PKS, Murphy MB (2014) Occurrence, distribution and ecological risk assessment of multiple classes of UV filters in surface waters from different countries. *Water Res* 67, 55–65. <https://doi.org/10.1016/j.watres.2014.09.013>

Tsui MMP, Leung HW, Lam PKS and Murphy MB (2014) Seasonal occurrence, removal efficiencies and preliminary risk assessment of multiple classes of organic UV filters in wastewater treatment plants. *Water Research*, 53, 58–67.

Tsui MMP, Leung HW, Kwan BKY, Ng KY, Yamashita N, Taniyasu S et al. (2015) Occurrence, distribution and ecological risk assessment of multiple classes of UV filters in marine sediments in Hong Kong and Japan. *J Hazard Mater* 292, 180–187.

Zhang QY, Ma XY, Wang XC, Ngo HH (2016) Assessment of multiple hormone activities of a UV-filter (octocrylene) in zebrafish (*Danio rerio*). *Chemosphere* 159, 433-441

Zucchi S, Oggier DM, Fent K (2011) Global gene expression profile induced by the UV-filter 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC) in zebrafish (*Danio rerio*). *Environ Pollut* 159, 3086–3096.

<https://doi.org/10.1016/j.envpol.2011.04.013>