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Evaluation of ecotoxicological effects of benzophenone UV filters: Luminescent bacteria toxicity, genotoxicity and hormonal activity

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ABSTRACT

The widespread use of organic ultraviolet (UV) filters in personal care products raises concerns about their potentially hazardous effects on human and ecosystem health. In this study, the toxicities of four commonly used benzophenones (BPs) UV filters including benzophenone (BP), 2-Hydroxybenzophenone (2HB), 2-Hydroxy-4-methoxybenzophenone (BP3), and 2-Hydroxy-4-methoxybenzophenone-5-sulfonicacid (BP4) in water were assayed *in vitro* using *Vibrio fischeri*, SOS/umu assay, and yeast estrogen screen (YES) assay, as well as *in vivo* using *zebrafish* larvae. The results showed that the luminescent bacteria toxicity, expressed as $\log EC_{50}$, increased with the lipophilicity ($\log Kow$) of BPs UV filters. Especially, since 2HB, BP3 and BP4 had different substituent groups, namely -OH, $-OCH_3$ and $-SO_3H$, respectively, these substituent functional groups had a major contribution to the lipophilicity and acute toxicity of these BPs. Similar tendency was observed for the genotoxicity, expressed as the value of induction ratio = 1.5. Moreover, all the target BPs UV filters showed estrogenic activity, but no significant influences of lipophilicity on the estrogenicity were observed, with BP3 having the weakest estrogenic efficiency *in vitro*. Although BP3 displayed no noticeable adverse effects in any *in vitro* assays, multiple hormonal activities were observed in *zebrafish* larvae including estrogenicity, anti-estrogenicity and anti-androgenicity by regulating the expression of target genes. The results indicated potential hazardous effects of BPs UV filters and the importance of the combination of toxicological evaluation methods including *in vitro* and *in vivo* assays.

1. Introduction

Rapid economic development and increasing demand for health protection have promoted the widespread use of personal care products worldwide. UV filters are chemical compounds extensively used in sunscreen and a variety of cosmetics, such as creams, lipsticks, and even agricultural chemicals and pharmaceuticals (Balmer et al., 2005; Roelandts et al., 1983) to protect humans and materials from the harmful effects of UV irradiation. Currently, 28 organic UV filters are registered in the European Union (Schlumpf et al., 2008) and 14 are authorized for use in the USA (Rodil et al., 2009). It is estimated that about 10,000 t of UV filters are produced annually for the global markets (Danovaro et al., 2008). Inorganic UV filters, such as titanium dioxide and/or zinc oxide, are used to scatter and/or reflect UV light, whereas organic UV filters absorb UV light. Benzophenones (BPs) are the most important members of the organic UV filters family (Suzuki et al., 2005). These aromatic UV filters are added to sunscreen products, as dominant components, in different proportions. For example, the US Food and Drugs Administration (US FDA, 2013) regulated BP3 and BP4 in sunscreen products at maximal levels of 6% and 10%, respectively.

The BPs UV filters are released into aquatic ecosystems either directly through recreational activities, such as bathing and swimming (Buser et al., 2006), or indirectly through discharges from wastewater treatment plants (WWTPs) (Balmer et al., 2005). Due to the various applications of BPs UV filters, their occurrence and contamination of the environment have been recorded in concentrations ranging from ng L^{-1} to μg L^{-1} in raw sewage, surface water, tap water and even indoor dust (Tsui et al., 2015; Díaz-Cruz et al., 2012; Wang et al., 2013). In fact, some studies have discovered the occurrence of BP3 and 4-Methylbenzylidene-camphor in samples of human urine, breast milk

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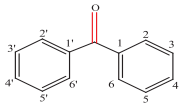
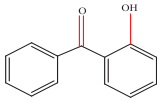
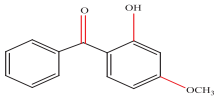
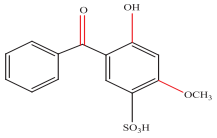
Table 1
Chemical structures, CAS numbers, purity, molecular weight and log*K*ow of target UV filters.

Chemicals	Structure	CAS number	Purity	M (g mol^{-1})	log *K*ow[a]	pH[d]
BP		119-61-9	≥99%	182.22	3.15[b]	6.12
2HB		117-99-7	≥99%	198.22	3.44[b]	6.50
BP3		131-57-7	≥99%	228.22	3.79[c]	7.20
BP4		4065-45-6	≥97%	308.31	0.37[c]	4.14

[a] octanol-water partition coefficient
[b] Liu et al. (2015);
[c] Molins-Delgado et al. (2016)
[d] the actual pH values of medium used for all experiments

and placental tissue (Frederiksen et al., 2013; Schlumpf et al., 2010). Although the acute and sub-chronic systemic toxicity of these UV filters after dermal application is rather low, problems caused by photoallergic reactions in patients have been reported (Schauder and Ippen, 1997). Therefore, the widespread occurrence of BPs UV filters warns us of potential harmful impacts on human and ecosystem health.

In fact, increasing evidence to support growing concerns regarding the eco-toxicity of BPs UV filters has been reported. The most impressive was that of multiple hormonal activities reported by Kunz and Fent (2006). In addition, the estrogenic activity of BPs UV filters was demonstrated with an assay using MCF-7 breast cancer cells and an immature rat uterotrophic assay (Schlumpf et al., 2001; Yamasaki et al., 2003). Recently, an acute toxicity level (50% effective concentration, EC_{50}) of BP3 on the larvae of *Mytilus galloprovincialis* of 3.42 mg L^{-1} was reported (Paredes et al., 2014). The 48 h-EC_{50} of BP4 on *Daphnia magna* was estimated to be 30.40 mg L^{-1} (Molins-Delgado et al., 2016), and the 15-min EC_{50} of BP on *Photobacterium phosphoreum* was estimated to be 34.26 mg L^{-1} (Liu et al., 2015). Nevertheless, the toxicological profile and modes of action of BPs UV filters are poorly understood. Most especially, the information on the non-specific toxicity to luminescent bacteria and the genotoxicity of UV filters remains scarce, except for a few fragmentary studies (Abramsson-Zetterberg and Svensson, 2011; Jeon et al., 2007).

On the one hand, as knowledge of the physicochemical properties, fate, and eco-toxicological effects is fundamental to the preliminary assessments, it is necessary to obtain the “basic set” of data, such as EC_{50}, from experimental tests using standardized test protocols. *Vibrio fischeri* is a luminescent marine bacterium and one of the aquatic organisms most used in non-specific toxicity assessment (ISO 11348, 2008). In addition, the SOS/umu test using *Salmonella typhimurium* (*S. typhimurium*) TA1535/pSK1002 has been used world-wide as a standard method to analyze the genotoxicity of individual compound and pollutants in water samples (ISO 13829, 2000). Moreover, the yeast estrogen screen (YES) assay is recommended for detecting estrogenic activity of chemicals, and mainly to create a useful reference for *in vivo* assay. Yeast cells have been transfected with expression plasmids carrying a reporter gene (lac-z) situated downstream from a promoter sequence, which incorporates an estrogen response element (Alvarez et al., 2013). In addition, the *in vivo assay* has the capability to metabolize chemicals resulting in the general outcome that chemicals are less active *in vitro*, but nevertheless are still more active *in vivo* than *in vitro* (Miller et al., 2001). Especially, the inactivity can translate into *in vivo* activity. *Zebrafish* has been proposed as an excellent vertebrate model for assessing the toxic effects of chemicals *in vivo*, which is especially useful for elucidating their mode of action (Hutchinson et al., 2003).

On the other hand, the toxicity of chemicals is related to their physicochemical characteristics, such as lipophilicity and substituent groups (Ranke et al., 2007; Liu et al., 2015). Zhao et al. (2013) studied the contribution of substituent group of UV filters to genetic effect by conducting the SOS/umu assay. Li et al. (2012) reported the acute toxicities of 14 BPs to a freshwater organism-*Dugesia japonica*. However, to date, little information is available on assessment of the overall biological effect of BPs from different aspects of toxicities and there is no study concerning the systematic research regarding the impacts of chemical structures on the eco-toxicity of BPs UV filters. Moreover, investigating the relationship between the substituent group of UV filters and their toxicity will provide a good chance to fill gaps of toxicity prediction for UV filters, and is in favor of the quantitative structure-activity relationship model building.

Accordingly, the aim of this study is to comprehensively analyze the biological effects of four commonly used BPs UV filters, namely BP, 2HB, BP3 and BP4 including acute toxicity, genotoxicity and endocrine disrupting effects using *in vitro* and *in vivo* bioassays. To the best of our knowledge, this is the first report to study the contribution of related substituent groups of UV filters to different aspects of toxicities. Moreover, the mechanisms of estrogenicity *in vitro* and multiple hormone activities *in vivo* of these BPs UV filters are preliminary