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# Organic UV filters exposure induces the production of inflammatory cytokines in human macrophages

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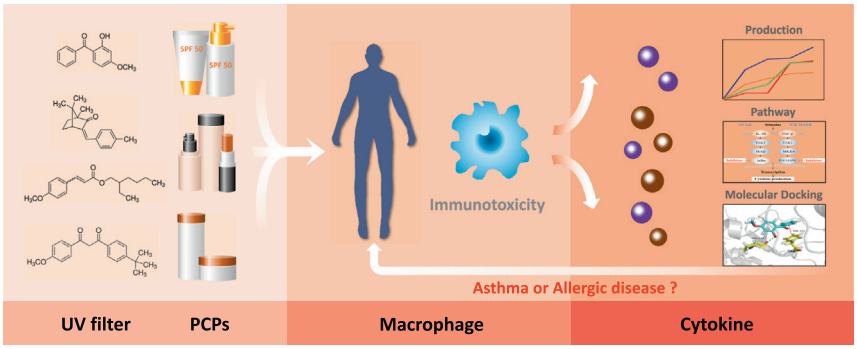
## HIGHLIGHTS

- Organic UV filters exposure increased the production of inflammatory cytokines.
- UV filters up-regulated the mRNA expression of cytokines.
- UV filters induced the phosphorylation of NF-κB and p38 MAPK signaling pathways.
- UV filter molecules would efficiently bind with TAK1.
- Exposure to UV filters may alter the immune system functions of humans.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Organic ultraviolet (UV) filters, found in many personal care products, are considered emerging contaminants due to growing concerns about potential long-term deleterious effects. We investigated the immunomodulatory effects of four commonly used organic UV filters (2-hydroxy-4-methoxybenzophenone, BP-3; 4-methylbenzylidene camphor, 4-MBC; 2-ethylhexyl 4-methoxycinnamate, EHMC; and butyl-methoxydibenzoylmethane, BDM) on human macrophages. Our results indicated that exposure to these four UV filters significantly increased the production of various inflammatory cytokines in macrophages, particular tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). After exposure to the UV filters, a significant 1.1–1.5 fold increase were found in TNF-α and IL-6 mRNA expression. In addition, both the p38 MAPK and the NF-κB signaling pathways were enhanced 2 to 10 times in terms of phosphorylation after exposure to the UV filters, suggesting that these pathways are involved in the release of TNF-α and IL-6. Molecular docking analysis predicted that all four UV filter molecules would efficiently bind transforming growth factor beta-activated kinase 1 (TAK1), which is responsible for the activation of the p38 MAPK and NF-κB pathways. Our results therefore demonstrate that exposure to the four organic UV filters investigated may alter human immune system function. It provides new clue for the development of asthma or allergic diseases in terms of the environmental pollutants.



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## 1. Introduction

Organic ultraviolet (UV) filters are active ingredients in sunscreens and other personal care products (e.g. shampoos, lip balms, soaps, and body washes) (MacManus-Spencer et al., 2011). UV filters can absorb deleterious UVA and UVB radiation and are thus generally used to protect human skin from direct sunlight (Giokas et al., 2007). Normally, in sunscreens and suncare products, 2-hydroxy-4-methoxybenzophenone (BP-3), 4-methylbenzylidene camphor (4-MBC), and 2-ethylhexyl 4-methoxycinnamate (EHMC) are widely used for UV-B protection. Also, BP-3 and butyl-methoxydibenzoylmethane (BDM) play the role of UV-A protection (Gao et al., 2013). Besides their use in personal care products, UV filters are also used to protect materials such as plastics, textiles, and rubber from sunlight (Ramos et al., 2016). Organic UV filters are listed as emerging contaminants (ECs) due to their wide distribution in the environment, and the consequent potential risks to humans and wildlife (Ramos et al., 2015). It is estimated that 10,000 tons of UV filters are produced for the global market each year (Shaath, 2005), and this figure is likely to increase because health authorities continue to recommend the use of UV filters to prevent skin cancer (Gago-Ferrero et al., 2012). Organic UV filters enter the natural environment both directly and indirectly; they have been identified in several environmental matrices, including water bodies, sewage, sludge, sediments and indoor dusts (Ao et al., 2017), with the concentrations from nanograms to micrograms per liter (Richardson, 2007; Zuloaga et al., 2012).

Organic UV filters are problematic partially because they can act as xenohormones, negatively affecting reproduction (Ramos et al., 2016). The endocrine-disrupting effects of UV filters have been studied: Ozaez et al. (2016) showed that the UV filters affected endocrine regulation during embryonic and larval development of the midge *Chironomus riparius*, while Balazs et al. (2016) found that the organic UV filter BP-3 affected multiple hormones in zebrafish embryos, leading to increased mortality and developmental disorders. In addition, our previous study showed that four organic UV filters, including BP-3 and 4-MBC, caused growth inhibition and oxidative injuries in the protozoan *Tetrahymena thermophile* at an environmentally common concentration (1.0 μg $L^{-1}$) (Gao et al., 2013).

At present, studies of organic UV filter toxicity have focused mainly on invertebrates and aquatic organisms (Gao et al., 2012), less is known about the effects of chronic exposure on the human body. However, it has been shown that organic UV filters penetrate the human epidermis after dermal application and enter the circulatory system, resulting in metabolization and/or excretion (Treffel and Gabard, 1996; Kasichayanula et al., 2007; Tarazona et al., 2015). Humans are at risk of UV filter exposure via dermal absorption, dietary ingestion, and indoor air and dust inhalation (Ao et al., 2017; Wang et al., 2013). Common organic UV filters have been found in human urine (Gao et al., 2015), blood (Zhang et al., 2013), milk (Rodriguez-Gomez et al., 2015), and placental tissue (Valle-Sistac et al., 2016; Chisvert et al., 2012). It was reported that the maximum concentrations of benzophenone-type UV filters in human urinary and blood can reach 26.7 μg $mL^{-1}$ and 0.8 μg $mL^{-1}$, respectively (Wolff et al., 2007; Gonzalez et al., 2006). Human exposure to these compounds in the natural environment may result in various adverse health effects, including photoallergies or endocrine disruptions (Tarazona et al., 2015). Therefore, a reassessment of the potential health risks of organic UV filters is critical. Unfortunately, the specific effects of organic UV filters, especially for the mechanisms of immune function alteration, remain obscure.

Asthma and other allergic respiratory diseases have increased in frequency worldwide over the last 30 years (D'Amato et al., 2015). To investigate this troubling phenomenon, several studies have focused on the association between endocrine-disrupting chemicals (EDCs), such as phthalates and bisphenol-A (Liu et al., 2014; Whyatt et al., 2014; Gascon et al., 2015), and allergy risk, chronic inflammation, and immunodeficiency. Several epidemiological studies have found a relationship between the presence of phthalates in the indoor environment and airway diseases, such as asthma, in children (Bornehag et al., 2004; Whyatt et al., 2014; Robinson and Miller, 2015). In addition, our previous study firstly confirmed that the indoor dust is an important source for human exposure on organic UV filters. The estimated daily intakes (EDI) of the organic UV filters for infants and adults are from 0.85 to 6.18 ng $kg^{-1}$-bw $day^{-1}$ and 0.07 to 0.49 ng $kg^{-1}$-bw $day^{-1}$, respectively (Ao et al., 2017).

Macrophages are innate immune cells that play an important role in the inflammatory response, and participate in the pathogenesis of respiratory allergies and asthma. Macrophages release a number of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-1α, and IL-1β, to mediate inflammatory responses (Rossol et al., 2011). However, the release of pro-inflammatory mediators may contribute to the aggravation of allergic or asthmatic symptoms (Bolling et al., 2012). Some environmental pollutants have been shown to exacerbate inflammatory responses through their effects on macrophage function (Gardner, 1984). Nishioka et al. (2012) showed that the environmental contaminant di-(2-ethylhexyl) phthalate (DEHP) significantly increased the levels of TNF-α, IL-1β, IL-6, and IL-8 in human macrophages in vitro, suggesting that DEHP might be involved the onset of allergic disease. However, the mechanisms by which these contaminants degrade immune function have not yet been clarified and few studies have investigated the direct mechanistic links between contaminants and the immune system. To our knowledge, the effects of organic UV filters found in personal care products on human macrophages have not yet been investigated.

Here, we aimed to investigate the effects of four commonly used organic UV filters, BP-3, 4-MBC, EHMC, and BDM, on human macrophages in vitro. We first differentiated a human leukemia monocytic cell line (THP-1) into macrophage-like cells. The production of inflammatory cytokines, including TNF-α and IL-6, in the macrophages after exposure to the four organic UV filters at approximate environmental concentrations was analyzed. We then investigated the effects of the four UV filters on cytokine gene expression, and the phosphorylation of the p38 mitogen activated protein kinase (MAPK) and the nuclear factor kappa B (NF-κB) pathways. Furthermore, we theoretically validated our results with molecular docking tactic. This study provides evidence for the immunomodulatory effects of organic UV filters on human macrophages. It will also help to strengthen the understanding of allergy risk of these active ingredients in personal care products.

## 2. Materials and methods

### 2.1. Reagents

Standards of organic UV filters were purchased as follows: BP-3 (98% purity; Sigma-Aldrich, St. Louis, MO, USA); 4-MBC (99% purity; Alfa Aesar, Ward Hill, MA, USA); BDM (95% purity; TCI, Tokyo, Japan); EHMC (96% purity; TCI). The structures and relevant physicochemical properties of the target analytes are given in Table 1. Stock solutions of BP-3, 4-MBC, EHMC, and BDM (1 M) were prepared in dimethylsulfoxide (DMSO; J&K Scientific, Shanghai, China), and diluted with cell culture medium before further use. Stock and work solutions were stored in the dark at 4 °C for <1 month.

Additional reagents were purchased as follows: Roswell Park Memorial Institute (RPMI) 1640 medium, fetal bovine serum (FBS), penicillin-streptomycin, and phosphate buffer saline (PBS) from Gibco (Carlsbad, CA, USA); phorbol 12-myristate 13-acetate (PMA, 99% purity) from Sigma-Aldrich; p38 MAPK inhibitor (SB203580) and NF-κB inhibitor (BAY11-7082) from Meilunbio Co., Ltd. (Shanghai, China); fluorescence-conjugated monoclonal antibodies including APC mouse anti-human CD11b, FITC mouse anti-human CD14, APC mouse IgG1, and FITC mouse IgG2a from BD (San Jose, CA, USA); radioimmunoprecipitation assay (RIPA) cell lysis solution, tris-buffered