Chemosphere 147 (2016) 114–123

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Photolytic fate and genotoxicity of benzophenone-derived compounds and their photodegradation mixtures in the aqueous environment

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HIGHLIGHTS

- Evaluation of benzophenones photostability under the influence of artificial UV light and sunlight.
- Benzophenones are not genotoxic at environmentaly relevant concentrations.
- The first evaluation of genotoxicity of 3-ethylbenzophenone and 3-acetylbenzophenone.
- Assessment of genotoxicity of photodegradation mixtures of benzophenones.

ARTICLE INFO

Article history:
Received 7 August 2015
Received in revised form
10 December 2015
Accepted 18 December 2015
Available online 5 January 2016

Handling Editor: Keith Maruya

Keywords:
Benzophenone
UV filter
Ketoprofen
Photodegradation
Genotoxicity
Environment

ABSTRACT

This study investigates the environmental fate of eight benzophenone derivatives (the pharmaceutical ketoprofen, its phototransformation products 3-ethylbenzophenone and 3-acetylbenzophenone, and five benzophenone-type UV filters) by evaluating their photolytic behaviour. In addition, the genotoxicity of these compounds and the produced photodegradation mixtures was studied. Laboratory-scale irradiation experiments using a medium pressure UV lamp revealed that photodegradation of benzophenones follows pseudo-first-order kinetics. Ketoprofen was the most photolabile ($t_{1/2} = 0.8$ min), while UV filters were more resistant to UV light with $t_{1/2}$ between 17 and 99 h. The compounds were also exposed to irradiation by natural sunlight and showed similar photostability as predicted under laboratory conditions. Solar photodegradation experiments were performed in distilled water, lake and seawater, and revealed that photosensitizers present in natural waters significantly affect the photolytic behaviour of the investigated compounds. In this case, the presence of lake water resulted in accelerated photodecomposition, while seawater showed different effects on photodegradation, depending on a compound. Further, it was shown that the transformation products of ketoprofen 3-ethylbenzophenone and 3-acetylbenzophenone were formed under environmental conditions when ketoprofen was exposed to natural sunlight. Genotoxicity testing of parent benzophenone compounds using the SOS/umuC assay revealed that UV filters exhibited weak genotoxic activity in the presence of a metabolic activation system, however the concentrations tested were much higher than found in the environment ($\geq$125 μg mL^{-1}). After irradiation of benzophenones, the produced photodegradation mixtures showed that, with the exception of benzophenone that exhibited weak genotoxic activity, all the other compounds tested did not elicit any activity when exposed to UV light.

1. Introduction

Benzophenone-derived compounds represent an important class of pharmaceuticals and personal care products (PPCPs). Their increasing use is leading to the widespread occurrence of these compounds in the environment, where actual data about their presence, effects and fate remains unclear. Benzophenone

Abbreviations: KP, ketoprofen; EtBP, 3-ethylbenzophenone; AcBP, 3-acetylbenzophenone; BP, benzophenone; H-BP, 4-hydroxybenzophenone; HM-BP, 2-hydroxy-4-methoxybenzophenone; DH-BP, 2,4-dihydroxybenzophenone; DHM-BP, 2,2'-dihydroxy-4-methoxybenzophenone; MP/UV lamp, medium pressure ultraviolet lamp; LP/UV lamp, low pressure ultraviolet lamp; LPEC, lowest positive effect concentration; IR, induction ratio; G, bacterial growth rate; DOM, dissolved organic matter.

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http://dx.doi.org/10.1016/j.chemosphere.2015.12.068
0045-6535/

derivatives all share the same chemical skeleton but they include a wide range of compounds with different properties and modes of application, examples being the pharmaceutical ketoprofen and the benzophenone-type UV filters. Ketoprofen is a nonsteroidal anti-inflammatory drug (NSAID) with analgesic, antipyretic and anti-inflammatory activity (Wang and Lin, 2014), while UV filters are common ingredients in sunscreens and other cosmetic products such as lotions, shampoos, lipsticks and fragrances. In addition, they are used as photostabilizers in fabrics, coatings, adhesives, agricultural chemicals and optical products (Jeon et al., 2006; Giokas et al., 2007; Fent et al., 2010; Pestotnik et al., 2014).

Benzophenones are known to occur in surface waters, for instance ketoprofen was found in concentrations of several hundred ng L^{-1} (Farré et al., 2001; Tixier et al., 2003; Vieno et al., 2005; Kotnik et al., 2014) and benzophenone-type UV filters in ng to μg L^{-1} levels (Lambropoulou et al., 2002; Poiger et al., 2004; Tarazona et al., 2010; Kotnik et al., 2014), but little is known about the fate of these compounds in the aquatic environment. Although photodegradation is an important abiotic elimination process determining the fate of organic compounds in the environment (Rodil et al., 2009), there is a scarcity of published data concerning photolytic behaviour of benzophenone derivatives. The studies performed show the photolabile character of ketoprofen, which rapidly transforms into benzophenone derivatives, which can cause skin photosensitivity disorders after administration (Kosjek et al., 2011). 3-ethylbenzophenone and 3-acetylbenzophenone have been identified as degradation products of ketoprofen when exposed to artificial UV and solar radiation (Kosjek et al., 2011; Szabó et al., 2011; Koumaki et al., 2015), which suggests they may be similarly formed in the environment. Kotnik et al. (2014) were the first to detect their presence in the aquatic environment, albeit their study did not investigate whether ketoprofen was their source. Further, their effects in the environment are yet to be ascertained. Until recently, the photostability of benzophenone-type UV filters was studied mainly to optimize the dosage and formulation of products to guarantee long-term protection from UV radiation (Silvia Díaz-Cruz et al., 2008; Rodil et al., 2009), but little effort has gone into examining their environmental fate. Studies report the high photostability of benzophenone-type UV filters, for instance 2-hydroxy-4-methoxybenzophenone showed almost no degradation (<10%) when irradiated with different sources of light (Rodil et al., 2009; Liu et al., 2011; Gago-Ferrero et al., 2012), which indicates that these compounds have the potential to persist in the environment. Due to high photostability of benzophenone-type UV filters, photolabile character of ketoprofen and possible toxic degradation products, further research on their fate and behaviour in the environment is needed. In addition, transformation products, formed by abiotic degradation, may exhibit different reactivity and effects compared to the parent compounds (Giokas et al., 2007; MacManus-Spencer et al., 2011), yet only a few publications address the formation of photodegradation products of benzophenones (Rodil et al., 2009; Kosjek et al., 2011; Liu et al., 2011; Szabó et al., 2011; Salgado et al., 2013).

Benzophenone derivatives have been reported to exhibit toxic effects, for example benzophenone-type UV filters elicit oestrogenic activity both *in vitro* and *in vivo* (Schlumpf et al., 2004; Kunz and Fent, 2006; Kunz et al., 2006; Coronado et al., 2008; Gago-Ferrero et al., 2012); however, data on their genotoxic activity are inconclusive. No mutagenic activity of ketoprofen was observed in Salmonella strains TA97a, TA100 and TA102 by using the Ames mutagenicity assay, while it was weakly genotoxic in mouse bone marrow cells detected with an *in vivo* sister chromatid exchange assay (Philipose et al., 1997). UV filters 2-hydroxy-4-methoxybenzophenone, 2,4-dihydroxybenzophenone and 2,2'-dihydroxy-4-methoxybenzophenone produced a positive or pseudo-positive initiation activity in the umu assay using the *Salmonella typhimurium* TL210 strain. In addition, 2,4-dihydroxybenzophenone revealed weak tumor promotion activity in the Bhas assay (Nakajima et al., 2006). In another study, 2,2'-dihydroxy-4-methoxybenzophenone and 2,3,4-trihydroxybenzophenone increased mutation frequencies in the mouse lymphoma assay, while benzophenone and 4-hydroxybenzophenone showed no effect (Jeon et al., 2007). In a *S. typhimurium* SOS/umu assay, benzophenone showed genotoxic activity only after metabolic activation (Takemoto et al., 2002). Recently, the genotoxicity of fourteen benzophenone derivatives was studied using the SOS/umu assay and the lowest positive effect concentrations (LPEC) ranged from 82 to > 1000 mg L^{-1} (Zhao et al., 2013). Due to insufficient data further research is needed to assess the genotoxicity of benzophenone derivatives as well as of their transformation products, formed in the environment, which has not been investigated yet.

Amongst the different benzophenone-derived compounds known, this study focused on the pharmaceutical ketoprofen (KP), its phototransformation products 3-ethylbenzophenone (EtBP) and 3-acetylbenzophenone (AcBP), and the UV filters benzophenone (BP), 4-hydroxybenzophenone (H-BP), 2-hydroxy-4-methoxybenzophenone (HM-BP), 2,4-dihydroxybenzophenone (DH-BP) and 2,2'-dihydroxy-4-methoxybenzophenone (DHM-BP) (their chemical structures, abbreviations and relevant physico-chemical data are presented Electronic Supplementary Material (ESM) in Table ESM 1). Considering the insufficient data on the environmental impact of these compounds, the main objectives of this study were to (i) investigate the photostability of 8 benzophenone derivatives under the influence of artificial UV light and natural sunlight, (ii) determine their degradation kinetics and half-lives, (iii) investigate the effect of different aqueous matrices on their photolytic behaviour, (iv) evaluate the genotoxicity of the parent benzophenones and (v) to assess the genotoxicity of their photodegradation mixtures produced by UV irradiation.

2. Materials and methods

2.1. Standards, reagents and chemicals

The benzophenone derivatives BP, H-BP, DH-BP, DHM-BP and KP were supplied by Sigma–Aldrich (Steinheim, Germany), while HM-BP was purchased from Merck (Damnstadt, Germany). All were of high purity (>97%). AcBP was obtained from Synchem OHG (Felsberg-Altenburg, Germany). EtBP and 3-i-propylbenzophenone (iPrBP) were custom synthesized using Friedel–Crafts alkylation at the Faculty of Chemistry and Chemical Technology, University of Ljubljana (Ljubljana, Slovenia) (Prebil, 2010). The isotopically labelled internal standards benzophenone-2,3,4,5,6-d_5 (BP-d_5), 2-hydroxy-4-methoxybenzophenone-2′,3′,4′,5′,6′-d_5 (HM-BP-d_5) and ketoprofen-d_3 (α-methyl-d_3) (KP-d_3) were provided by CDN Isotopes (Quebec, Canada). The derivatization reagents *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) and O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) were supplied by Sigma–Aldrich (Steinheim, Germany).

Ampicillin, glucose-6-phosphate (disodium salt), D-(+)-glucose (anhydrous), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), sodium dodecyl sulphate, Na_2CO_3, $MgCl_2 \cdot H_2O$ and Na_2HPO_4 were obtained from Sigma–Aldrich (St. Louis, USA), nicotinamide adenine dinucleotide phosphate (NADP), NaCl, $Na_2HPO_4 \cdot 2H_2O$ and $NaH_2PO_4 \cdot H_2O$ from Merck (Darmstadt, Germany), while O-nitrophenyl-ß-D-galactopyranoside (ONPG), β-mercaptoetanol, KCl and $MgSO_4 \cdot 7H_2O$ were purchased from Fluka (Buchs, Switzerland). Lyophilized Aroclor 1254 induced male rat liver post-mitochondrial fraction (S9) was obtained from Moltox