Environment International 70 (2014) 143–157

Contents lists available at ScienceDirect

Environment International

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

Review

Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: A mini-review

Sujin Kim, Kyungho Choi *

School of Public Health, Seoul National University, Seoul 151-742, Republic of Korea

ARTICLE INFO

Article history:
Received 13 December 2013
Accepted 21 May 2014
Available online 14 June 2014

Keywords:
UV filter
Benzophenone-1
Endocrine disruption
Surface water

ABSTRACT

Benzophenone-3 (BP-3) has been widely used in sunscreens and many other consumer products, including cosmetics. The widespread use of BP-3 has resulted in its release into the water environment, and hence its potential impact on aquatic ecosystem is of concern. To better understand the risk associated with BP-3 in aquatic ecosystems, we conducted a thorough review of available articles regarding the physicochemical properties, toxicokinetics, environmental occurrence, and toxic effects of BP-3 and its suspected metabolites. BP-3 is lipophilic, photostable, and bioaccumulative, and can be rapidly absorbed via oral and dermal routes. BP-3 is reported to be transformed into three major metabolites in vivo, i.e., benzophenone-1 (BP-1), benzophenone-8 (BP-8), and 2,3,4-trihydroxybenzophenone (THB). BP-1 has a longer biological half-life than its parent compound and exhibits greater estrogenic potency in vitro. BP-3 has been detected in water, soil, sediments, sludge, and biota. The maximum detected level in ambient freshwater and seawater is 125 ng/L and 577.5 ng/L, respectively, and in wastewater influent is 10,400 ng/L. The major sources of BP-3 are reported to be human recreational activities and wastewater treatment plant (WWTP) effluents. BP-3 and its derivatives have been also detected in fish lipid. In humans, BP-3 has been detected in urine, serum, and breast milk samples worldwide. BP-1 has also been detected in placental tissues of delivering women. While sunscreens and cosmetics are known to be major sources of exposure, the fact that BP-3 has been detected frequently among young children and men suggests other sources. An increasing number of in vitro studies have indicated the endocrine disrupting capacity of BP-3. Based on a receptor binding assay, BP-3 has shown strong anti-androgenic and weak estrogenic activities but at the same time BP-3 displays anti-estrogenic activity as well. Predicted no effect concentration (PNEC) for BP-3 was derived at 1.32 μg/L. The levels observed in ambient water are generally an order of magnitude lower than the PNEC, but in wastewater influents, hazard quotients (HQs) greater than 1 were noted. Considering limited ecotoxicological information and significant seasonal and spatial variations of BP-3 in water, further studies on environmental monitoring and potential consequences of long-term exposure in aquatic ecosystem are warranted.

Contents

* Corresponding author at: Department of Environmental Health, School of Public Health, Seoul National University, 1, Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea. Tel.: +82 2 880 2738.
E-mail address: kyungho@snu.ac.kr (K. Choi).

http://dx.doi.org/10.1016/j.envint.2014.05.015
0160-4120/

1. Introduction

Exposure to ultraviolet (UV) radiation may pose a public health threat, including the risk of various skin diseases such as sunburn, photo-aging and skin cancers (Pathak, 1987). While solar UVC (wavelength range 200–280 nm) is absorbed by ozone in the stratosphere, UVA (320–400 nm) and UVB (290–320 nm) can reach the earth surface and therefore may influence humans and ecosystems (Clydesdale et al., 2001; de Gruijl, 2002). Long-wave UVA comprises more than 90% of solar light, and can penetrate deep into both the epidermis and dermis of the skin eventually causing premature photo-aging. UVB is a minor component in proportion, but is more active and more capable of causing sunburn than UVA. UVB is considered to be responsible for inducing DNA damage and ultimately skin cancer (Svobodová et al., 2003). The concern over the deleterious effects of solar UV light has increased the demand for sunscreen products. Consequently, sunscreen products, commonly referred to as UV filters, have been widely used to reduce sunlight exposure and to protect human skin.

Based on composition, UV filters can be classified roughly into two groups, i.e., organic (or chemical) and inorganic (or physical). Inorganic filters include mineral particles such as TiO_2 and ZnO, and function by reflecting and scattering UV light from the skin. On the other hand, organic filters usually possess aromatic structures that can absorb and stabilize the solar UV radiation (Gasparro et al., 1998). Benzophenones (BPs), camphors, and cinnamates are among well-known organic UV filters. These chemical filters are generally used in combination because no single active agent, used at levels currently permitted by legislation, would provide sufficient protection against UV (Díaz-Cruz et al., 2008; U.S. FDA Department of Health and Human Services, 2013a). UV filters are also used in other cosmetic products such as lipsticks, skin lotions, facial creams, and fragrances (Environmental Working Group, 2013; Liao and Kannan, 2014), and in various personal care products, including shampoos, body washes, toilet soaps, hair sprays, and insect repellents (Liao and Kannan, 2014; National Library of Medicine, 2011), because they can prevent polymer degradation or pigmentation.

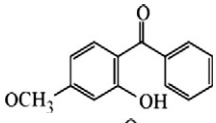
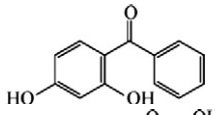
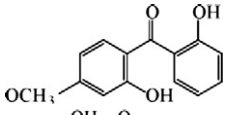
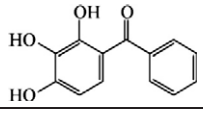
Benzophenone-3 (BP-3, 2-hydroxy-4-methoxybenzophenone, oxybenzone) is one of the most widely used BP type UV filters and has been available as a sunscreen agent for over 40 years. BP-3 can be used at levels of up to 5–6% as an active ingredient in sunscreen in Japan and the U.S.A. (The Society of Japanese Pharmacopoeia, 1985; U.S. FDA Department of Health and Human Services, 2013b), while up to 10% can be used in Europe (EEC Directive, 1983). In Korea, a maximum of 5% BP-3 can be used as a cosmetic ingredient (Korea Food and Drug Administration, 2012). BP-3 is approved by the U.S. Food and Drug Administration also as an indirect food additive (U.S. FDA Department of Health and Human Services, 2013a).

Other BP type UV filters are also commercially used. For example, benzophenone-1 (BP-1, or 2,4-dihydroxybenzophenone) which is a major metabolite of BP-3 in both experimental animals and humans (Kadry et al., 1995; Kunisue et al., 2012; Okereke et al., 1993; Wang and Kannan, 2013), is employed as a UV stabilizer in plastic surface coatings on food packages (Suzuki et al., 2005).

Widespread use of BP-3 has led to the release of this compound and its derivatives into aquatic environment such as lakes and rivers around the world (Balmer et al., 2005; Cuderman and Heath, 2007; Kameda et al., 2011; Loraine and Pettigrove, 2006). Up to 125 ng/L of BP-3 has been detected in surface water (Poiger et al., 2004). Wastewater treatment plant (WWTP) effluents are reported to contain higher levels of BP-3 (Loraine and Pettigrove, 2006). Therefore, the potential impact on aquatic ecosystems is of concern.

BP-3 has been frequently reported for endocrine disruption (Fent et al., 2008; Heneweer et al., 2005; Kunz et al., 2006; Schreurs et al., 2002; Sieratowicz et al., 2011). Experimental animal and in vitro studies have shown that BP-3 influences reproduction and sex hormone signaling (Blair et al., 2000; Kunz et al., 2006; Schlumpf et al., 2001; Schreurs et al., 2005; Schultz et al., 2000; Suzuki et al., 2005). BP-1 is reported to

Table 1
Structure and some physico-chemical properties of benzophenone-3 and its relevant derivatives.

Compound	CAS. number	Abbreviation	Chemical structure	Formula	Molecular weight	Boiling point (°C)	Log Kow[a]	pKa[a]	Vapor pressure[a] (mm Hg) at 25 °C	Bioconcentration factor (BCF) at pH 7, 25 °C
Oxybenzone, 2-hydroxy-4-methoxy benzophenone, benzophenone-3	131–57–7	BP-3 HMB		$C_{14}H_{12}O_3$	228.24	370.3	4.00	7.56	5.26×10^{-6}	502
2,4-Dihydroxy benzophenone, benzophenone-1	131–56–6	BP-1 DHB		$C_{13}H_{10}O_3$	214.22	409.0	3.15	7.53	2.84×10^{-7}	113
Dioxybenzone, 2,2′-dihydroxy-4-methoxybenzophenone, benzophenone-8	131–53–3	BP-8 DHMB		$C_{14}H_{12}O_4$	244.24	375.0	4.31	7.11	3.73×10^{-6}	524
2,3,4-Trihydroxy benzophenone	1143–72–2	THB		$C_{13}H_{10}O_4$	230.22	439.7	1.70	7.51	2.42×10^{-8}	7.75

Kow, octanol–water partition coefficient.
[a] Values obtained from SciFinder Scholar Database, http://www.cas.org.products/sfacad/.

possess even greater estrogen receptor binding affinity compared to BP-3 (Kunz et al., 2006; Molina-Molina et al., 2008). Furthermore, BP-3 and BP-1 are suspected to influence on hormone-dependent diseases, and are associated with the birth outcome of humans (Kunisue et al., 2012; Wolff et al., 2008).

This paper reviews the available literature on various aspects of BP-3 and its major metabolites, including their chemical properties, toxicokinetics, environmental occurrence, endocrine disrupting potential, and ecological risks. Knowledge gaps, environmental health implications, and the future direction of research are identified.

2. Characterization of BP-3 and its major derivatives

2.1. Physico-chemical properties and fates

BP has two benzene rings joined by a carbonyl group. Twelve substituted derivatives of BP, i.e., BP-1 to BP-12, have been used in various commercial products because of their UV absorption properties (Park et al., 2013). Among them, BP-3 is the most well-known compound. The physico-chemical properties of BP-3 and its relevant BP derivatives are shown in Table 1.

As with other organic UV filters, BP-3 is a photostable, lipophilic and potentially bioaccumulative compound. The relatively high log-Kow value of BP-3, i.e., 4.0, suggests its slow biodegradation, tendency to adsorb to suspended solids and sediments, and low volatilization potential from water surfaces. BP-3 has been shown to degrade by about 4% after 28 d in water (Chemicals Inspection and Testing Institute, 1992) indicating its persistence in aquatic environment. During summer, the half-life of BP-3 in surface water was estimated at a few weeks, and the persistence appeared to be 7 to 9 times greater in winter under mid-latitude conditions (Vione et al., 2013). In oxic conditions, BP-3 is reported to produce BP-1 as a biodegradation product (Liu et al., 2012). The biodegradation of BP-3 is favored under anaerobic (4.2 d half-life) compared to aerobic conditions (10.7 d half-life). BP-3 is relatively stable under UV light and artificial sunlight (Gago-Ferrero et al., 2012; Rodil et al., 2009a).

BP-1 has lower log-Kow value (3.15) and bioconcentration factor (BCF) compared to its parent compound. Unlike BP-3, BP-1 is readily photodegraded, and disappears after 24 h under UV radiation (Gago-Ferrero et al., 2012). Another BP derivative is 2,3,4-trihydroxybenzophenone (THB), but this compound has not been commercially used. THB has never been detected in environmental media. BP-8 (2,2′-dihydroxy-4-methoxybenzophenone, dioxybenzone), another metabolic product of BP-3, has similar chemical properties to BP-3 and is often detected in soil or sediments due to its high lipophilicity. Analytical methods for BP-3 and its derivatives are beyond the scope of this review, and related information can be found in Díaz-Cruz et al. (2008) and Giokas et al. (2007).

2.2. Toxicokinetics

BP-3 can be rapidly absorbed after oral, intravenous, or topical skin administration in rats and piglets (El Dareer et al., 1986; Kadry et al., 1995; Kasichayanula et al., 2007; Okereke et al., 1993). In male rats, BP-3, BP-1, and BP-8 were detected in plasma 5 min after gavage feeding (Okereke et al., 1993) and the absorption half-life was 0.71 h (Kadry et al., 1995). Maximum plasma concentration of BP-3 was found at 2 h after the topical skin application of BP-3 contained sunscreen products in piglets (Kasichayanula et al., 2007). In humans, milligrams of BP-3 were absorbed to systemic circulation following a topical application of sunscreen products containing BP-3 (Hayden et al., 1997; Janjua et al., 2004). While a skin permeability coefficient of BP-3 is not available, it was shown that about 1 to 2% of the topically applied amount could be absorbed through skin over 10 h period (Hayden et al., 1997).

Absorbed BP-3 can be hydroxylated to form metabolic byproducts such as BP-1, BP-8, or THB (Jeon et al., 2008; Kasichayanula et al., 2005; Nakagawa and Suzuki, 2002; Okereke et al., 1993). Of these metabolites, BP-1 has been most frequently detected in rats (El Dareer et al., 1986; Okereke et al., 1993). BP-1 is formed via *O*-demethylation of the methoxy side chain on ring A of BP-3, whereas BP-8 is formed via the aromatic hydroxylation of ring B at the ortho position. A small portion of BP-1 can be further converted into THB via the aromatic hydroxylation of ring A at the *meta* position (Fig. 1).

Even though the study on the toxicokinetics of BP-3 tends to be limited to the experiments using rats after oral administration, BP-1 is also believed to be one of the major metabolites of BP-3 in fish. During a 14 d exposure of adult male zebrafish to BP-3, only BP-1 and BP-3 were detected in water and fish samples while BP-2, 4,4′-dihyroxybenzophenone, and 4-hydroxybenzophenone (4-OH-BP) were not found (Bluthgen et al., 2012). In addition, as the nominal concentration of BP-3 in fish decreased, the ratio between the concentrations of BP-1 to BP-3 in fish notably increased. Increased proportion of BP-1 may explain greater endocrine disrupting potential at lower BP-3 concentrations in fish. In contrast to the adult male fish, biotransformation of BP-3 to BP-1 had not occurred in hatched fry fish at 5 days post-hatch (dph). Less developed metabolic functions at the eleuthero-embryo stage may account for this observation.

BP-1 is also the major metabolite in humans. Among women ($n = 625$ from Utah and California, U.S.A.), a positive correlation ($\beta = 0.59$,

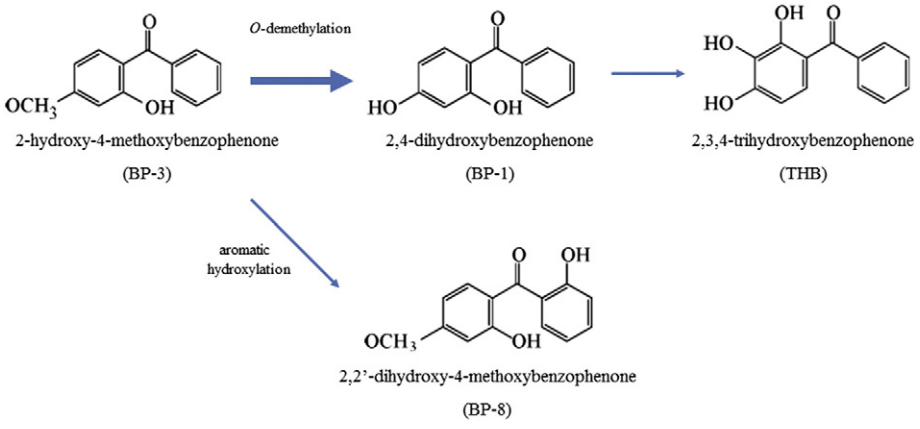


Fig. 1. Metabolic pathways of benzophenone-3 in rats.

r = 0.92) was found between BP-3 and BP-1 concentrations in urine samples with BP-1 levels notably high (Kunisue et al., 2012). The profiles of BP derivatives in human urine suggest that demethylation to BP-1 is the major metabolic pathway (Wang and Kannan, 2013). However, other types of BPs, e.g., BP-2 and 4-OH-BP which were not detected in animal studies, have been found in human urine. Some of the BPs might have originated from the direct use of those compounds but also might be explained by human-specific metabolism of BP-3. In urines collected from the children and adults of the U.S.A., the total concentrations of four BP derivatives including BP-1, BP-2, BP-8, and 4-OH-BP were positively correlated with the concentration of BP-3 (Wang and Kannan, 2013). Interestingly, a recent study (Zhang et al., 2013) reported no significant correlations between urinary BP-3 and 4-OH-BP concentrations, suggesting sources other than BP-3. Sex dependent differences in metabolism have been reported. After repeated topical application of BP-3, blood and urine concentrations of BP-3 in men were higher than in women (Janjua et al., 2008).

Like other ketonic compounds (Westphal, 1986), BP derivatives bind with plasma proteins and are transported through blood vessels. After oral administration of BP-3 in male Sprague–Dawley rats, tissue analysis at 6 h revealed that BP-1 was present in most tissues including the liver, kidney, testes, intestine, spleen and skin (Okereke et al., 1993). Using the same study design, Kadry et al. (1995) demonstrated that liver contained the highest concentrations of total and free BP-3, in the liver, 6.47% and 0.18% of the initial total and free BP-3 doses were detected, followed by the kidney with 0.97% and 0.02%, respectively. A similar distribution pattern was found also for BP-1 (Okereke et al., 1993). High levels of both BP-3 and BP-1 in liver samples imply that the liver is a major organ of BP-3 biotransformation. In hepatocyte suspensions, BP-3 can be converted enzymatically to BP-1 and probably to BP-8 (Nakagawa and Suzuki, 2002). While it is uncertain which enzymes exactly are responsible for the metabolism of BP-3, cytochrome P450 (CYP) enzymes are thought to play a role (Porter and Coon, 1991). In rat liver microsomes, the oxidation to BP-1 was mainly catalyzed by CYP2C6 and to a lesser extent by CYP1A1 (Kamikyouden et al., 2013). However, metabolism of BP-3 in fish may be different, because fish lack CYP2C homologs (Smith et al., 2010). In the brain of male zebrafish, transcripts of *cyp1a1* were significantly up-regulated by exposure to BP-3 (Bluthgen et al., 2012) suggesting the potential role of this CYP isozyme in metabolism of BP-3.

Conjugation with glucuronic acid and expulsion in the urine are one of the major routes of BP-3 excretion (El Dareer et al., 1986; Kadry et al., 1995; Okereke et al., 1993). In rats, the removal of BP-3 from blood was reported to be faster than that of BP-1, and the biological half-life of BP-3 was determined at 4.58 h (Jeon et al., 2008). Following topical application on skin in piglets, the elimination half-lives of BP-3 ranged between 7.14 and 8.04 h (Fediuk et al., 2012; Kasichayanula et al., 2005, 2007). Prolonged absorption phase through skin may explain longer elimination half-life following topical application.

3. Occurrences in environment and biota

3.1. Occurrence in aquatic environment

The concentrations of BP-3 and other UV filters fluctuate significantly by location, levels of public access, season, and sampling conditions such as water depth or flow (Díaz-Cruz et al., 2008; Fent et al., 2010b; Poiger et al., 2004). These compounds can reach the ambient water through both direct and indirect inputs. The direct inputs involve removal from the skin during recreational activities such as swimming or bathing in water bodies. Indirect inputs include releases via WWTPs, which may originate for example from processes such as laundry or showering (Balmer et al., 2005; Hernández Leal et al., 2010; Langford and Thomas, 2008).

In lake Hüttnersee, a recreational lake of Switzerland, notably higher levels of BP-3 were detected compared to other UV filters, such as (3-(4-methylbenzylidene) camphor (4-MBC), ethylhexyl methoxycinnamate (EHMC), or octocrylene (OC) (Balmer et al., 2005). Similarly, in a lake and a swimming pool in Slovenia, BP-3 was present at the highest level and frequency among the six UV filters measured which include 4-MBC, OC, octyl methoxycinnamate (OMC), octyl dimethyl PABA (OD-PABA), and homosalate (Cuderman and Heath, 2007). In ambient freshwater, BP-3 has been detected up to 125 ng/L. This maximum level was reported in lake Hüttnersee, Switzerland during the summer of 1998 where no indirect input other than public recreational access was expected, and the level was consistent with the predicted value based on the survey of sunscreen usage (Poiger et al., 2004). BP-3 was detected in other popular outdoor bathing areas. In outdoor swimming pools (n = 5) and recreational ponds (n = 6) of South Bohemia during the peak summer season of 2011, BP-3 has been found at concentrations as high as 620 and 550 ng/L, respectively (Grabicova et al., 2013). In beach seawaters, high levels of BP-3 have been reported. Up to 577.5 ng/L BP-3 was found in coastal waters of Majorca Island, Spain, during summer 2011 (Tovar-Sanchez et al., 2013). Higher levels of BP-3 were detected in surface nearshore beach waters of semi-enclosed or densely populated resort areas. These levels are several times greater than those reported for rivers or lakes. These observations underline the importance of human recreational activities as a source of BP-3 in water.

Wastewater effluents are another major source of BP-3 into ambient water, although BP-3 is removed efficiently (68 to 96%) in conventional WWTPs (Balmer et al., 2005; Li et al., 2007). BP-3 concentrations in WWTP influents and effluents ranged from <5 to 10,400 ng/L, with the highest concentrations reported in a wastewater influent at San Diego County in the U.S.A. (Loraine and Pettigrove, 2006). Along the River Glatt, Switzerland, BP-3 was first detected downstream of the Dubendorf WWTP, which indicates the contribution of WWTP as an important source of BP-3 release (Fent et al., 2010b; Zenker et al., 2008). In contrast, BP-1 was detected in only one sample (47 ng/L) among 25 river and lake water samples collected during spring of 2003 in South Korea (Jeon et al., 2006). BP-1 concentrations of <0.3–17 ng/L were found in the Rivers Taff and Ely in South Wales regions, UK (Kasprzyk-Hordern et al., 2008). The reason for less frequent detection and lower concentrations of BP-1 in water can be found from its lesser persistence in water compared to BP-3.

Although contamination in sediment or soil samples has received lesser attention compared to that in natural waters, BP-3 and its byproducts are likely to exist at higher levels in sediments or soil due to their lipophilic properties. Schlenk et al. (2005) performed an in vivo vitellogenin assay for sediment samples collected from the Southern California Bight, U.S.A. using male or juvenile fish, and identified the samples with estrogenic potential. From those samples with estrogenic activity, only BP-3 was unequivocally detected among 62 PPCP analytes. In soil or sediments, the levels of BP-3 ranged between <0.5 and 27 ng/g dw. For BP-1, the levels ranged between 0.26 and 0.61 ng/g dw, and for BP-8, the range was 0.13 and 4.17 ng/g dw. Other BP derivatives, such as BP-2 and 4-OH-BP, have also been detected in the soil and sediment of the U.S.A. (Zhang et al., 2011).

3.2. Occurrence in biota

Only few studies are available on BP derivatives in aquatic biota. BP-3 and BP-4 have been reported in fish lipid (Balmer et al., 2005; Fent et al., 2010b; Zenker et al., 2008). In fish from lakes in Switzerland, BP-3 and 4-MBC were most frequently detected among four UV filters (Balmer et al., 2005).

Reports on occurrence of BP-3 and related compounds among biological samples are mostly limited to humans. As shown in Table 3 and Fig. 2, BP-3 was detected in the majority of urine samples. In the 2003–2004 National Health and Nutrition Examination Survey (NHANES), BP-3 was found at >0.4 μg/L in the urine of 96.8% of 2517 participants aged 6 years or older (Calafat et al., 2008). BP-3 was also found in breast milk samples from Germany and Switzerland (Hany and Nagel, 1995; Schlumpf et al., 2010). BP-1 and 4-OH-BP have also

Table 2
Environmental levels of benzophenone-3 and its relevant derivatives.

Compounds	Matrix		Country	Detect. freq.[a]	Median	Lowest level	Sampling date/site	Highest level	Sampling date/site	References
BP-3										
	Water (ng/L)									
	Surface water	River	Slovenia	1/2	–	<54	Nadiza-Soca	114	Kolpa	Cuderman and Heath (2007)
		River	Spain	–	–	<7	Jan. and Jul., 2007/ River Mero	27 ± 3	Mar., 2007/River Mero	Rodil et al. (2008)
		River	Spain	1/2	–	<LOD	May, 2007/River Elsterbecken	30 ± 3	May, 2007/River Parthe	Rodil and Moeder (2008)
		River	Switzerland	–	–	<LOD	Jul., 2006/River Glatt, Upstream WWTP Dübendorf	96 ± 93 (ng/ POCIS)	Jul., 2006/River Glatt, Downstream WWTP Dübendorf	Zenker et al. (2008)
		River	Switzerland	–	–	56	Sep., 2007/River Glatt	68	Oct., 2007/River Glatt	Fent et al. (2010b)
		River	UK	–	–	<0.3	River Taff	17	River Taff	Kasprzyk-Hordern et al. (2008)
		River	Brazil	0/3	–	<2	Oct., 2012 and Mar., 2013	<2	Oct., 2012 and Mar., 2013	Silva et al. (2013)
		Rivers heavily polluted by industrial and domestic wastewaters	Japan	1/6	4[b]	–	Summer, 2008	–	Summer, 2008	Kameda et al. (2011)
		Moderately polluted rivers	Japan	8/12	6	4	Summer, 2008	12	Summer, 2008	Kameda et al. (2011)
		River Tama	Japan, Tokyo	–	14	–	–	–	–	Kawaguchi et al. (2008)
		Rivers where the municipal wastewater is directly discharged	Taiwan	2/2	–	12.3	–	15.4	–	Wu et al. (2013)
		River	South Korea	2/8	2[b]	1.2	–	2.7	–	Kim et al. (2007)
		River	Unknown	1/1	52 ± 5	–	Sep., 2008	–	Sep., 2008	Negreira et al. (2009)
		Streams with direct inputs of domestic wastewater	Japan	2/2	25[b]	16	Summer, 2008	41	Summer, 2008	Kameda et al. (2011)
		Lake Cospuden (Recreational water)	Leipzig, Germany	–	40 ± 3	–	Summer	–	Summer	Rodil et al. (2009b)
		Lake	Slovenia	4/5	45	<28	Bakovci	85	Rakitna	Cuderman and Heath (2007)
		Lake	Spain	1/2	–	<LOD	May, 2007/Lake Cospudener	27 ± 4	Jun., 2007/Lake Bagger	Rodil and Moeder (2008)
		Lake Zurich	Switzerland	–	–	<2	Jul., 1998	4	Jul., 1998	Poiger et al. (2004)
		Lake Hüttnersee	Switzerland	–	–	5	Jul., 1998	125	Jul., 1998	Poiger et al. (2004)
		Lake	Switzerland	–	–	14	Sep., 2002/ Zurichsee (direct and indirect input)	35	Jul., 2002/Hu¨ttnersee (direct input only)	Balmer et al. (2005)
		Swimming pool	Slovenia	2/2	251.5	103	Kdeljevo	400	Portoroz	Cuderman and Heath (2007)
	Influent	–	Leipzig, Germany	–	234 ± 41	–	Summer	–	Summer	Rodil et al. (2009b)
		WWTP influent	Italy	–	–	6	Aug. and Sep., 2011	163	Jul., 2011	Magi et al. (2013)
		Raw waste water	Spain	–	–	<7	Jan., 2007	168 ± 7	Jul., 2007	Rodil et al. (2008)
		WWTP influent	Switzerland	–	–	700	Apr., 2002/Meilen	7800	Jun., 2002/Thalwil	Balmer et al. (2005)
		Wastewater influent	San Diego county, California, U.S.A.	–	6870	5300	Aug. to Nov., 2001	8300	Aug. to Nov., 2001	Loraine and Pettigrove (2006)
		Wastewater influent	San Diego county, California, U.S.A.	–	6240	110	Jan. to Jun., 2002	10,400	Jan. to Jun., 2002	Loraine and Pettigrove (2006)
		Stanley WWTP influent	Hong Kong	–	258	–	–	–	–	Yu et al. (2012)

(continued on next page)

Table 2 (*continued*)

Compounds	Matrix		Country	Detect. freq.[a]	Median	Lowest level	Sampling date/site	Highest level	Sampling date/site	References
		STP influent	Unknown	4/4	–	216 ± 27	Feb., 2008	462 ± 74	Sep., 2008	Negreira et al. (2009)
	Effluent	WWTP effluent	Italy	–	–	5	Apr. and Jun., 2011	28	May., 2011	Magi et al. (2013)
		Treated wastewater	Spain	2/2	–	42 ± 3	May, 2007	54 ± 6	Jul., 2007	Rodil and Moeder (2008)
		WWTP effluent	Switzerland	–	–	<10	Sep., 2003/Kloten-Opfikon	700	Jun., 2002/ Wadenswil	Balmer et al. (2005)
		Sewage treatment plant (STP) effluent	South Australia	–	32.7 ± 1.7	–	–	–	–	Liu et al. (2011)
		STP effluents	Japan	4/4	54	29	–	164	–	Kameda et al. (2011)
		WWTP effluent	Hon Kong	–	323	–	–	–	–	Yu et al. (2012)
		Industrial drainage	South Korea	1/7	–	<5	Apr., 2003	27	Apr., 2003	Jeon et al. (2006)
		WWTP effluent	South Korea	5/7	11	1.0	–	30	–	Kim et al. (2007)
		WWTP effluent	Taiwan	2/2	–	12.5	–	21.4	–	Wu et al. (2013)
		STP effluent	Unknown	2/4	–	13 ± 4	Feb., 2008	44 ± 8	Sep., 2008	Negreira et al. (2009)
	Sediment, soil, and sludge (ng/g dw)									
	Sediment	–	Northeastern Spain	13/20		<LOQ	Dec., 2009	27	Dec., 2009	Gago-Ferrero et al. (2011)
		From Saginaw River (2002) and Detroit River (1998)	Michigan, U.S.A.	6/6	2.34	0.728	1998 and 2002	4.66	1998 and 2002	Zhang et al. (2011)
		From Songhua River	China	6/6	0.380	0.272	2009	0.545	2009	Zhang et al. (2011)
		–	Japan	0/29	–	–	Summer, 2008	–	Summer, 2008	Kameda et al. (2011)
		–	South Korea	0/15	–	<0.5	Apr. to May, 2003	<0.5	Apr. to May, 2003	Jeon et al. (2006)
	Ground soil	–	South Korea	5/33	2.650	0.73	Apr. to May., 2003	3.880	Apr. to May., 2003	Jeon et al. (2006)
	Sludge	From WWTPs serving five large cities	Northeastern China	5/5	12.8	2.05	Jul., 2009	13.3	Jul., 2009	Zhang et al. (2011)
	Fish (ng/g lipid)									
	Andalusian Barbel	Along the Guadalquivir river basin	South Spain	2/2	20.4 ng/g dw	16.5 ng/g dw	2010	24.3 ng/g dw	2010	Gago-Ferrero et al. (2013)
	Roach	–	Switzerland	5/5	–	66	Sep., 2002/ Huttnersee	118	Aug., 2002/ Greifensee	Balmer et al. (2005)
	White fish	–	Switzerland	2/4	–	<54	Sep., 2002/ Pfaafikersee	<240	Jan., 2002/Thunersee	Balmer et al. (2005)
	Brown trout	From River Ergolz	Switzerland	–	–	<LOD,	Sep., 2006	151	Sep., 2006	Fent et al. (2010b)
BP-1										
	Water (ng/L)									
	Surface water	River	UK	–	–	<0.3	River Taff	17	River Taff	Kasprzyk-Hordern et al. (2008)
		River	UK	–	–	<0.3	River Ely	13	River Ely	Kasprzyk-Hordern et al. (2008)
		River	South Korea	1/31	47	–	–	–	–	Jeon et al. (2006)
		Rivers where the municipal wastewater is directly discharged	Taiwan	1/2	6.1	–	–	–	–	Wu et al. (2013)
		River	Unknown	1/1	37 ± 6	–	–	–	–	Negreira et al. (2009)
	Influent	STP influent	Unknown	4/5	–	161 ± 11	–	245 ± 20	–	Negreira et al. (2009)
	Effluent	STP effluent	Unknown	1/4	41 ± 2	–	–	–	–	Negreira et al. (2009)
		WWTP effluent	Taiwan	2/2	–	7.7	–	16.8	–	Wu et al. (2013)

Table 2 (*continued*)

Compounds	Matrix		Country	Detect. freq.[a]	Median	Lowest level	Sampling date/site	Highest level	Sampling date/site	References
	Sediment, soil, and sludge (ng/g dw)									
	Sediment	From Saginaw River (2002) and Detroit River (1998)	U.S.A., Michigan	4/6	0.454	0.259	1998 and 2002	0.607	1998 and 2002	Zhang et al. (2011)
	Sludge	–	Northeastern China	5/5	32.7	4.41	Jul., 2009	91.6	Jul., 2009	Zhang et al. (2011)
BP-8										
	Water (ng/L)									
	Surface water	Rivers where the municipal wastewater is directly discharged	Taiwan	0/2	–	–	–	–	–	Wu et al. (2013)
	Effluent	WWTP effluent	Taiwan	2/2	–	9.8	–	10.1	–	Wu et al. (2013)
	Sediment, soil, and sludge (ng/g dw)									
	Sediment	From Saginaw River (2002) and Detroit River (1998)	U.S.A., Michigan	4/6	0.424	0.133	1998 and 2002	0.796	1998 and 2002	Zhang et al. (2011)
		–	South Korea	12/15	0.95	0.5	Apr. to May, 2003	2.14	Apr. to May, 2003	Jeon et al. (2006)
	Ground soil	–	South Korea	5/33	1.670	0.5	Apr. to May, 2003	4.170	Apr. to May, 2003	Jeon et al. (2006)

[a] Detection frequency.
[b] Mean value detected.

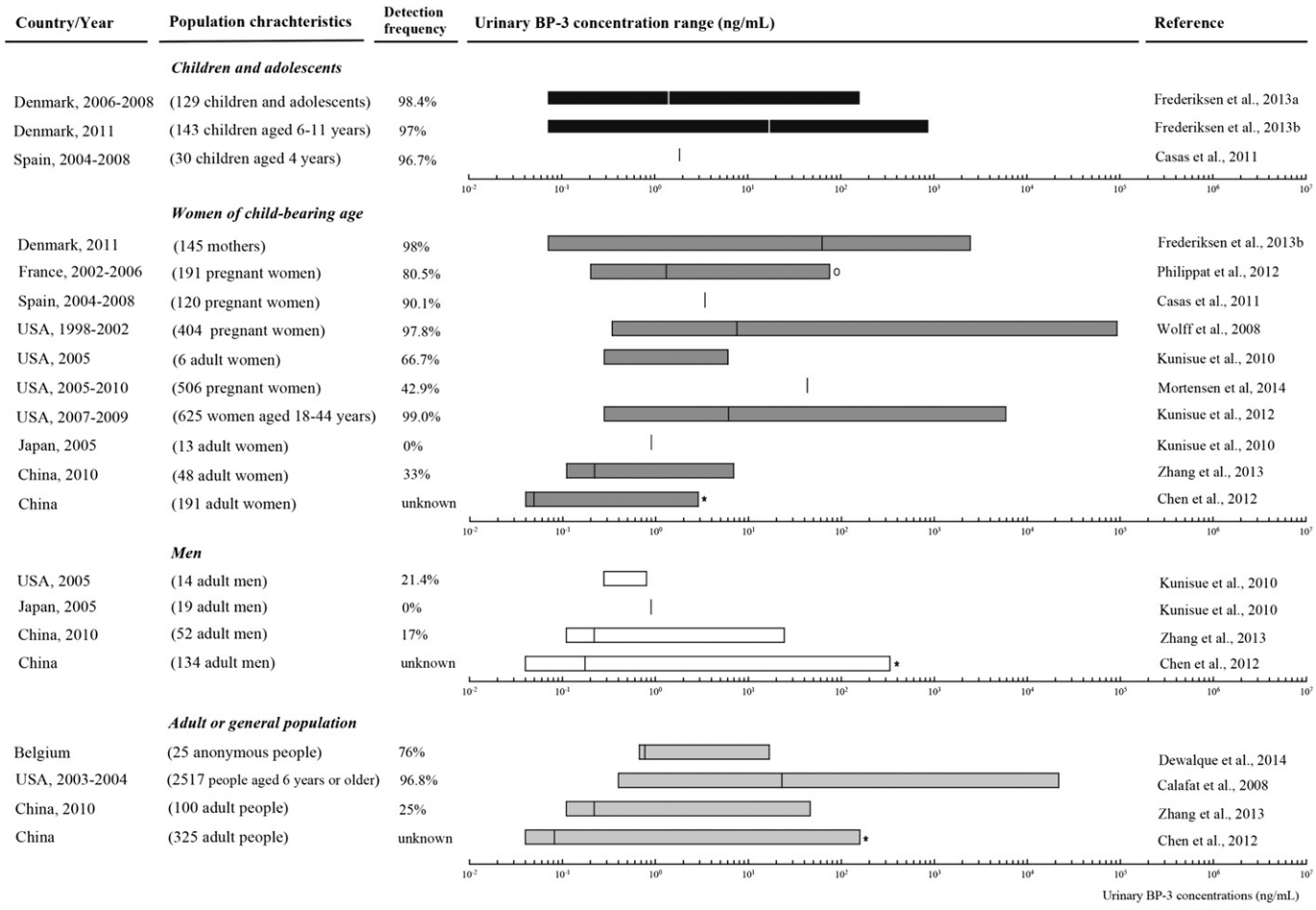


Fig. 2. Urinary benzophenone-3 (BP-3) concentrations (ng/mL) reported worldwide. The left and right edges of the horizontal bar graph indicate the lowest and the highest levels of detection, respectively. The vertical line shown within the box represents the median concentrations. If the highest level of detection is not available in the literature, the 95th (open circle) or 99th (star) percentile was shown.

Table 3
Levels of human exposure to benzophenone-3 and its relevant derivatives.

Sample type	Country/region	Year	Test subjects	M/F[d]	Compounds	Detect. freq. [a] (%)	Median conc. (ng/mL)	Range (ng/mL)	Note	Reference
Urine										
	Belgium	–	25 anonymous Belgian donors	–	BP-3	76	0.77	<0.67–16.8	–	Dewalque et al. (2014)
	Denmark	2006–2008	104 children aged 6–16 years and 25 adolescents aged 17–21 years	65/64	BP-3	98.4	1.41	<0.07–162	Children were recruited from Copenhagen puberty study and adolescents were recruited among pupils in a high school from the Northern Copenhagen area.	Frederiksen et al. (2013a)
	Denmark	2011	145 mothers 143 children aged 6–11 years	0/145 –	BP-3	98 97	62[c] 17[c]	<0.07–2442 <0.07–885	Children and their mothers were recruited from schools in Gentofte (urban area, n = 70) and Viby Sj. (rural area, n = 75)	Frederiksen et al. (2013b)
	France	2002–2006	191 pregnant women	0/191	BP-3	80.5	1.3	5th: 0.2 95th: 74.5	EDEN Mother–Child cohort; recruited before gestational week 28	Philippat et al. (2012)
	Greece, Athens	2012, Mar. to Apr.	100 people aged 2.5–87 years	50/50	BP-1 BP-8 BP-2 4-OH-BP	78 24 40 23	1.8 1.0 1.1 1.6	<1–1117 <2–24.7 <1–54.3 <0.7–46.8	–	Asimakopoulos et al. (2014)
	Spain	2004–2008	120 pregnant women 30 children aged 4 years	0/120 30/0	BP-3	90.1 96.7	3.4 1.9	– –	INMA (INfancia y Medio Ambiente [Environment and Childhood]) Spanish project	Casas et al. (2011)
	U.S.A., Albany, New York	1998–2002	404 pregnant women	0/404	BP-3	97.8	7.5	<0.34–92,700	The Children's Environmental Health Study: a prospective ethnically diverse birth cohort of 404 mother–infant pairs	Wolff et al. (2008)
	U.S.A.	2003–2004	2517 people aged 6 or older	1229/1288	BP-3	96.8	22.9[c]	0.4–21,700	National Health and Nutrition Examination Survey (NHANES) Study	Calafat et al. (2008)
	U.S.A., Albany, New York	2005, Feb. to Mar.	6 women 14 men	0/6 14/0	BP-3 BP-1 4-OH-BP BP-3 BP-1 4-OH-BP	66.7 100 33.3 21.4 42.6 7.1	– – – – – –	<0.28–6.0 0.9–9.0 <0.28–0.8 <0.9–5.0 <0.28–6.0 0.61–0.8	–	Kunisue et al. (2010)
	U.S.A.	2005–2010	506 pregnant women	0/506	BP-3	100	42.9 59.5[c]	–	National Children's Study (NCS) Vanguard Study	Mortensen et al. (2014)
	U.S.A., Utah and California	2007–2009	625 women aged 18–44 years	0/625	BP-3 BP-1 BP-8	99.0 93.3 2.6	6.1 6.1 –	<0.28–5900 <0.082–3200 –	ENDO (Endometriosis, Natural history, Diagnosis, and Outcomes) Study	Kunisue et al. (2012)
	Japan, Matsuyama	2005, Feb. to Mar.	13 women 19 men	0/13 19/0	BP-3 BP-1 4-OH-BP BP-3 BP-1 4-OH-BP	0 15.4 0 0 0 0	– – – – – –	<0.9 <0.28–1.0 ND[b] <0.9 <0.28 ND[b]	–	Kunisue et al. (2010)
	China	2010, Feb. to May	100 adults	52/48	BP-3 BP-1 4-OH-BP	25 57 61	<0.22 0.14 0.14	<0.11–46.1 <0.07–14.6 <0.06–5.14	General adult population in Tianjin (n = 50), Shanghai (n = 26), and Qiqihar (n = 25)	Zhang et al. (2013)

			48 adult women	0/48	BP-3	33	<0.22	<0.11–6.91		
					BP-1	65	<0.33	<0.07–14.3		
					4-OH-BP	69	<0.33	<0.03–8.11		
			52 adult men	52/0	BP-3	17	<0.22	<0.11–24.3		
					BP-1	50	0.11	<0.07–20.3		
					4-OH-BP	54	0.25	<0.06–2.82		
	China	–	325 adults	191/134	BP-3	–	0.082	95th: 2.179 99th: 157.820	Non-occupational Chinese adults were recruited from affiliated hospitals of Nanjing Medical University	Chen et al. (2012)
			191 adult women	0/191		–	0.049	95th: 1.013 99th: 2.881		
			134 adult men	134/0		–	0.175	95th: 5.070 99th: 332.923		
Serum										
	U.S.A.	2001–2002	936 children aged 3–11 years	–	BP-3	63	–	<0.5–8.7 (free conc.)	NHANES Study	Ye et al. (2012)
Whole blood										
	China	2010, Feb. to May	10 children aged 1–5 years	5/5	BP-3	30	<0.52	<0.52–2.20	Whole blood samples were collected from children in Nanchang; matched maternal blood and fetal cord blood samples were collected from women who were delivering in Tianjin; paired blood and urines samples were collected from adults in Tianjin.	Zhang et al. (2013)
					BP-1	10	<0.06	<0.06–0.09		
					4-OH-BP	100	0.32	0.23–0.40		
			20 pregnant women	0./20	BP-3	35	<0.41	<0.41–2.30		
					BP-1	0	<0.12	<0.12		
					4-OH-BP	100	0.58	0.32–1.78		
			23 adults	12/11	BP-3	83	2.09	<0.52–3.38		
					BP-1	4	<0.06	<0.06–0.15		
					4-OH-BP	100	0.35	0.26–1.29		
Cord blood										
	China	2010, Feb. to May	22 fetuses at the time of delivery	11/11	BP-3	55	0.59	<0.41–2.55		
					BP-1	0	<0.12	<0.12		
					4-OH-BP	100	0.41	0.26–0.51		
Human breast milk										
	Switzerland	2004–2006	54 mothers	0/54	BP-3	12.96	26.70 ng/g lipid	7.30–121.40 ng/g lipid	Cohorts; mothers who gave birth to a single child at the University, Women's Hospital Basel	Schlumpf et al. (2010)
Placental tissue										
	Spain	–	16 pregnant women	0/16	BP-3	0	ND[b]	ND[b]	Placenta samples were collected during different deliveries in the Maternity Unit of San Cecilio University Hospital of Granda	Vela-Soria et al. (2011)
					BP-1:	87.5	2.6 ng/g	0.5–9.8 ng/g		

[a] Detection frequency; percentage of samples containing BP derivatives above analytical detection limit.
[b] Not detected.
[c] Geometric mean.
[d] Sex ratio; male/female.

Table 4
Endocrine disrupting activities of benzophenone-3 and its relevant derivatives in vitro.

Compounds	Hormonal activity	Cell line	Assay	Endpoints	Conc. (μM)	Reference
BP-3	Estrogenic	MCF-7 cells	MCF-7 cell proliferation assay	6 d, cell proliferation EC50	3.73	Schlumpf et al. (2001)
			MCF-7 cell proliferation assay	6 d, cell proliferation LOEC	>100	Nakagawa and Suzuki (2002)
			pS2 protein assay	pS2 protein secretion, LOEC	10	Schlumpf et al. (2001)
			ERE-luciferase reporter assay	Estrogenic EC50	19.5	Suzuki et al. (2005)
		Recombinant yeast	Recombinant yeast assay	Agonism toward rtER, EC50	21.9	Kunz et al. (2006)
			Recombinant yeast assay	Agonism toward hERα, EC50	18.6	Kunz and Fent (2006b)
		MELN cells	Luciferase assay	Transactivation for hERα, EC50	20.315	Molina-Molina et al. (2008)
		HELN cells	Luciferase assay	Transactivation of hERα, NOEC	10	Gomez et al. (2005)
			Luciferase assay	Transactivation of hERβ, NOEC	10	Gomez et al. (2005)
			Luciferase assay	Transactivation for hERα, EC50	>30	Molina-Molina et al. (2008)
			Luciferase assay	Transactivation for hERβ, NOEC	0.01	Molina-Molina et al. (2008)
			Luciferase assay	Transactivation for rtERα, EC50	18.426	Molina-Molina et al. (2008)
		HEK293 cells	Gene expression assay	Transactivation for hERα, EC50	2.9	Schreurs et al. (2005)
			Gene expression assay	Transactivation for hERβ, EC50	25	Schreurs et al. (2005)
			Gene expression assay	Transactivation for hERα, LOEC	10	Schreurs et al. (2002)
			Gene expression assay	Transactivation for hERβ, LOEC	10	Schreurs et al. (2002)
	Antiestrogenic	Recombinant yeast	Recombinant yeast assay	Antagonism toward hERα, IC50	17.8	Kunz and Fent (2006b)
			Recombinant yeast assay	Antagonism toward hERα, IC50	3.68	Kunz and Fent (2006b)
		HELN cells	Luciferase assay	Antagonism toward hERα, NOEC	0.01	Molina-Molina et al. (2008)
			Luciferase assay	Antagonism toward hERβ, NOEC	0.01	Molina-Molina et al. (2008)
			Luciferase assay	Antagonism toward rtERα, NOEC	0.01	Molina-Molina et al. (2008)
	Antiprogestagenic	U2-OS cells	Gene expression assay	Transrepression for hPR, IC50	5.2	Schreurs et al. (2005)
	Antiandrogenic	U2-OS cells	Gene expression assay	Transrepression for hAR, IC50	2	Schreurs et al. (2005)
		NIH3T3 cells	ARE-luciferase reporter assay	Antiandrogenic IC50	>100	Suzuki et al. (2005)
		HEK293 cells	Gene expression assay	Transrepression for hAR, IC50	3.1	Nashev et al. (2010)
BP-1	Estrogenic	MCF-7 cells	MCF-7 cell proliferation assay	6 d, cell proliferation, LOEC	0.01	Nakagawa and Suzuki (2002)
			ERE-luciferase reporter assay	Estrogenic EC50	1.26	Suzuki et al. (2005)
		Recombinant yeast	Recombinant yeast assay	Agonism toward rtER, EC50	0.799	Kunz et al. (2006)
		MELN cells	Luciferase assay	Transactivation for hERα, EC50	9.192	Molina-Molina et al. (2008)
		HELN cells	Luciferase assay	Transactivation for hERα, EC50	8.513	Molina-Molina et al. (2008)
			Luciferase assay	Transactivation for hERβ, EC50	3.965	Molina-Molina et al. (2008)
			Luciferase assay	Transactivation for rtERα, EC50	3.48	Molina-Molina et al. (2008)
	Antiestrogenic	Recombinant yeast	Recombinant yeast assay	Antagonism toward hERα, EC50	1.15	Kunz and Fent (2006b)
		HELN cells	Luciferase assay	Antagonism toward hERα, IC50	3.19	Molina-Molina et al. (2008)
			Luciferase assay	Antagonism toward hERβ, IC50	1.59	Molina-Molina et al. (2008)
			Luciferase assay	Antagonism toward rtERα, IC50	2.45	Molina-Molina et al. (2008)
	Antiprogestagenic	Recombinant yeast	Recombinant yeast assay	Antagonism toward hAR, IC50	0.692	Kunz and Fent (2006b)
	Antiandrogenic	NIH3T3 cells	ARE-luciferase reporter assay	Antiandrogenic IC50	10	Suzuki et al. (2005)
		HEK293 cells	Gene expression assay	Transrepression for hAR, IC50	5.1	Nashev et al. (2010)
BP-8	Estrogenic	MCF-7 cells	MCF-7 cell proliferation assay	6 d, cell proliferation LOEC	0.1	Nakagawa and Suzuki (2002)
			ERE-luciferase reporter assay	Estrogenic EC50	>100	Suzuki et al. (2005)
	Antiandrogenic	NIH3T3 cells	ARE-luciferase reporter assay	Antiandrogenic IC50	>100	Suzuki et al. (2005)
THB	Estrogenic	MCF-7 cells	MCF-7 cell proliferation assay	6 d, cell proliferation, LOEC	1	Nakagawa and Suzuki (2002)
			ERE-luciferase reporter assay	Estrogenic EC50	11.8	Suzuki et al. (2005)
		MELN cells	Luciferase assay	Transactivation for hERα, EC50	4.012	Molina-Molina et al. (2008)
		HELN cells	Luciferase assay	Transactivation for hERα, EC50	2.272	Molina-Molina et al. (2008)
			Luciferase assay	Transactivation for hERβ, EC50	0.827	Molina-Molina et al. (2008)
			Luciferase assay	Transactivation for rtERα, EC50	0.578	Molina-Molina et al. (2008)
	Antiandrogenic	NIH3T3 cells	ARE-luciferase reporter assay	Antiandrogenic IC50	>100	Suzuki et al. (2005)

been detected in human samples. Kunisue et al. (2012) reported BP-3 in 99.0%, BP-1 in 93.3%, and 4-OH-BP in 83.8% of urine samples (n = 625 U.S.A. women). In placental tissue samples of pregnant women, BP-1 was present in 87.5% (n = 16) (Vela-Soria et al., 2011). Frequent detection of BP-3 and BP-1 in breast milk or placental tissues implies potential transfer to fetus and breastfed infant. Trans-placental transfer ratios of BP-3, i.e., 0.48 (geometric mean), were also suggested by Zhang et al. (2013) employing whole blood samples.

Urinary levels of BP-3 appeared to be affected by several factors. The urinary concentrations of BP-3 are reported to be higher in females than in males of the U.S.A. (Calafat et al., 2008; Ye et al., 2012) while no gender differences were found in urine of Danish children and adolescents (Frederiksen et al., 2013a). Furthermore, urinary or serum concentrations of BP-3 tend to be higher in urban mothers and in individuals of higher socioeconomic status (SES) (Frederiksen et al., 2013b; Tyrrell et al., 2013), probably reflecting their greater frequency of usage. Differences by sampling countries also have been reported. Detection frequencies of BP-3 appeared to be much higher in the U.S.A. and Europe (80.5–99.0%, Table 3) than in Asian countries such as Japan (0%, n = 32) and China (25%, n = 100) (Kunisue et al., 2010; Zhang et al., 2013). These results might be attributable to different life styles or to the different pattern of UV filter use among countries. For example, both concentrations and detection rates of BP-3 in personal care products of the U.S.A. (1200 ng/g product weight with 99.1% of detection frequency) were reported higher than those of China (20.1 ng/g product weight, 64.1%) (Liao and Kannan, 2014).

Recent studies indicate that BP-3 exposure may occur from sources other than sunscreens or PCPs (Krause et al., 2012). Frederiksen et al. (2013b) reported BP-3 in almost all urine samples (97–98%) that were collected during seasons when sunscreens are not generally used. In addition, in the river or lakes near WWTP, levels of BP-3 in water were generally higher during spring or fall than in summer (Fent et al., 2010b; Rodil et al., 2008). BP-3 was detected at a similar frequency as adults in the urines of young children aged 6–16 years (Frederiksen et al., 2013a, 2013b). These observations indicate the presence of BP-3 sources other than sunscreen or cosmetics.

Dust ingestion is thought to be one of major sources of BP-3 exposure. Five types of BPs were detected in indoor dust samples from the

U.S.A., China, Japan, and Korea, and the detection rates of BP-3 and BP-1 were 100% (Wang et al., 2013). Concentrations of BP-3 in dust were 64.5–1190 ng/g in the U.S.A. and 9.72–1690 ng/g in Korea.

4. Adverse effects of BP-3 in vitro and in vivo

4.1. Endocrine disruption potentials in vitro

A number of in vitro studies indicate that UV filters such as BP-3, 4-MBC, OMC, and OD-PABA have endocrine-disrupting capacities (Gomez et al., 2005; Kunz and Fent, 2006b; Morohoshi et al., 2005; Schlumpf et al., 2001; Schreurs et al., 2005). Table 4 summarizes endocrine disrupting activities of BP-3 and its metabolites reported from in vitro studies.

BP-3 was determined to be slightly estrogenic in an MCF-7 human breast cancer cell proliferation assay and pS2 protein assay; the maximal MCF-7 cell count increase was 95.09% of 17β-estradiol (E2) and the maximal weight increase was 7.60% of 17α-ethinylestradiol (EE2) (Schlumpf et al., 2001). Weak estrogenicity of BP-3 was also found in a yeast bioassay transfected with estrogen receptor (ER), estrogen responsive elements (ERE), and a *lacZ* reporter gene (Miller et al., 2001). BP-1, a major metabolite of BP-3, was found to possess stronger estrogenicity than its parent compound. Molina-Molina et al. (2008) examined four types of BP derivatives for the transcription activation toward ERs. BP-1 and other BP derivatives such as BP2 and THB had considerably lower EC50 values than those of BP-3 in MELN (MCF-7-ERE-Luciferase-Neo) and HELN (HeLa-ERE-Luciferase-Neo) cell lines. In a similar human breast cell line, MCF-7 cells, BP-1 induced cell proliferation at concentrations of 0.01–1 μM, whereas BP-3 did not induce the proliferation even at 100 μM (Nakagawa and Suzuki, 2002). In a yeast two-hybrid assay, Kawamura et al. (2003) reported that estrogenic activities of BP-3 and its metabolites were in the order of BP-1 > THB ≫ BP-3 > BP-8. Relative to E2, estrogenic activity of BP-1 was calculated at 1/5000 fold, while that of BP-3 was 1/45,000 fold in an in vitro yeast sex hormone receptor transactivation study (Kunz and Fent, 2006b). For BP-8 and THB, significant MCF-7 cell proliferation was found (Nakagawa and Suzuki, 2002) although estrogenic activity of BP-8 was not evident in a yeast bioassay (Kawamura et al., 2003; Miller et al., 2001). The ER binding affinity of BPs could be explained by the hydroxyl group at the *para*-position of BP (Kawamura et al., 2003; Miller et al., 2001; Schultz et al., 2000).

BP-3 can also elicit both antiestrogenic (Molina-Molina et al., 2008; Schreurs et al., 2002) and antiandrogenic activities (Ma et al., 2003; Nashev et al., 2010; Schreurs et al., 2005; Suzuki et al., 2005) in vitro. Antiestrogenic activity of BP-3 was calculated at 1/45 fold relative to that of 4-hydroxytamoxifen, and antiandrogenic activity relative to flutamide was calculated at 1/1.3 fold in an in vitro yeast sex hormone receptor transactivation assay (Kunz and Fent, 2006b). However, conflicting results have often been published regarding the antiestrogenic potential of BP-3: BP-3 showed no antagonistic effects at both human ERα (hERα) and hERβ transfected in human embryonal kidney (HEK293) cell line (Schreurs et al., 2002). Different binding affinities to ERs by species may explain such inconsistency (Krause et al., 2012). For BP-1, strong estrogenic and antiandrogenic activities were detected.

The binding affinity of BP-3 appears to also vary depending on the ER subtype, e.g., ERα and ERβ (Molina-Molina et al., 2008; Schreurs et al., 2002, 2005). In HEK293 cells, the EC50 value of transactivation for hERα was one order of magnitude lower than for hERβ (Schreurs et al., 2005). Estrogenic activity of BP-1 is likely to be ERα mediated rather than ERβ (Park et al., 2013). ERα activation is related to the cell proliferation while ERβ is reported to play an important role in cell differentiation (Förster et al., 2002; Helguero et al., 2005).

A few in vitro studies have been performed to understand the estrogenicity of BP-3 in fish. Based on a recombinant yeast assay expressing rainbow trout ERα (rtERα) as well as hERα (Kunz et al., 2006b; Molina-Molina et al., 2008), BP-1 displayed the highest binding affinity to both fish and human receptors among 23 UV filters. The binding activity of BP-3 was relatively higher in rtERα than in hERα (Kunz et al., 2006).

Toxicity interactions of UV filter mixtures are of concern because UV filters are generally used in combination and are present as such in the aquatic environment (Heneweer et al., 2005; Kunz and Fent, 2006a). Estrogenic compounds are expected to interact additively if they possess similar modes of action (Kortenkamp, 2007). Binary mixtures of BP-3 and BP-1, and a multi-component mixture of BP-1, BP-3, OMC, and 4-MBC showed additivity in ER binding in MCF-7 cells (Heneweer et al., 2005). While these observations suggest similar modes of endocrine disruption among BP based UV filters, other types of mixture interactions have also been reported. A tertiary mixture of BP-1, BP-2 and 3-benzylidene camphor (3BC) displayed additivity at high concentrations but antagonism at lower concentrations, in terms of fish vitellogenesis (Kunz and Fent, 2009). Contrary to these reports, synergistic estrogenic activities were detected for mixtures of four or eight UV filters in a recombinant yeast assay with hERα, indicating significant interactions among UV filters (Kunz and Fent, 2006a). Further investigation on the modes of action of BP-3 in mixture is warranted.

Table 5
Endocrine disrupting activities of benzophenone-3 and its relevant derivatives in vivo.

Compounds	Hormonal activity	Assay	Experimental animals	Exposure route/duration	Endpoints	Conc.	Reference
BP-3	Estrogenic	Uterotrophic assay	Immature Long-Evans rats	Oral, 4 d	Increase of uterine weights, ED50	1000–1500 mg/kg/day	Schlumpf et al. (2001)
		Uterotrophic assay	F344 female rats	Ip, 3 d	Increase of uterine weights, NOEC	500 mg/kg/day	Suzuki et al. (2005)
		Uterotrophic assay	Female Sprague–Dawley rats	Oral, 5 d	Increase of uterine weights, NOEC	250 mg/kg/day	Schlecht et al. (2004)
	Antiestrogenic	Competitive binding assay	Sprague–Dawley rats	–	ER competitive binding with [3H]-estradiol, IC50	$>1.00 \times 10^{-4}$ M	Blair et al. (2000)
BP-1	Estrogenic	Uterotrophic assay	F344 female rats	Ip, 3 d	Increase of uterine weights, LOEC	500 mg/kg/day	Suzuki et al. (2005)
		Uterotrophic assay	Female Crj:CD rats	Sc, 3 d	Increase of uterine weights, LOEL	625 mg/kg/day	Koda et al. (2005)
	Antiestrogenic	Competitive binding assay	Sprague–Dawley rats	–	ER competitive binding with [3H]-estradiol, IC50	3.65×10^{-5} M	Blair et al. (2000)
BP-8	Estrogenic	Uterotrophic assay	F344 female rats	Ip, 3 d	Increase of uterine weights, LOEC	300 mg/kg/day	Suzuki et al. (2005)
	Antiestrogenic	Competitive binding assay	Sprague–Dawley rats	–	ER competitive binding with [3H]-estradiol, IC50	$>1.00 \times 10^{-4}$ M	Blair et al. (2000)
	Androgenic	Hershberger assay	F344 male rats	Sc, 10 d	Increase of prostate gland and seminal vesicle	300 mg/kg/day	Suzuki et al. (2005)

Table 6
Acute and chronic effects of benzophenone-3 in aquatic organisms.

Compounds	Taxonomic group	Species	Test duration/endpoint	Conc. (mg/L)	Reference
BP-3	Algae	*Scenedesmus vacuolatus*	24 h, reproduction IC50	0.36	Rodil et al. (2009a)
		Desmodesmus subspicatus	72 h, growth IC10	0.61	Sieratowicz et al. (2011)
		Desmodesmus subspicatus	72 h, growth IC50	0.96	Sieratowicz et al. (2011)
	Invertebrate	*Daphnia magna*	48 h, immobilization EC50	1.67	Sieratowicz et al. (2011)
		Daphnia magna	48 h, immobilization EC50	1.9	Fent et al. (2010a)
		Dugesia japonica	48 h, immobilization EC50	0.9	Li (2012)
		Daphnia magna	21 d, reproduction NOEC	0.5	Sieratowicz et al. (2011)
	Fish	*Danio rerio*	14 d, vitellogenin induction NOEC	0.312	Bluthgen et al. (2012)
		Oncorhynchus mykiss	14 d, vitellogenin induction LOEC	0.749	Coronado et al. (2008)
		Oncorhynchus mykiss	14 d, vitellogenin induction NOEC	0.132	Coronado et al. (2008)
		Oryzias latipes	7 d, reproduction LOEC	0.62	Coronado et al. (2008)
		Oryzias latipes	7 d, reproduction NOEC	0.132	Coronado et al. (2008)
		Oryzias latipes	F1 hatchability LOEC following 21 d F0 exposure	0.62	Coronado et al. (2008)
		Oryzias latipes	F1 hatchability NOEC following 21 d F0 exposure	0.132	Coronado et al. (2008)
		Oryzias latipes	21 d, vitellogenin induction LOEC	0.62	Coronado et al. (2008)
		Oryzias latipes	21 d, vitellogenin induction NOEC	0.132	Coronado et al. (2008)
		Pimephales promelas	14 d, vitellogenin induction NOEC	3.9	Kunz et al. (2006)
BP-1	Invertebrate	*Dugesia japonica*	48 h, immobilization EC50	2.8	Li (2012)
	Fish	*Pimephales promelas*	14 d, vitellogenin induction LOEC	4.919	Kunz et al. (2006)
BP-8	Invertebrate	*Dugesia japonica*	48 h, immobilization EC50	4.4	Li (2012)
THB	Invertebrate	*Dugesia japonica*	48 h, immobilization EC50	34.6	Li (2012)

4.2. Endocrine disruption and other adverse effects in vivo

Relatively limited information is available on endocrine disruption of BPs in experimental animals (Table 5). Some hydroxylated BPs have produced both estrogen agonistic and antagonistic activities in an immature rat uterotrophic assay, but no antiandrogenic activity was observed in a Hershberger assay (Yamasaki et al., 2003). Compared to 4-MBC and OMC, uterotrophic effects of BP-3 in an ovariectomized rat are negligible (Schlecht et al., 2004; Schlumpf et al., 2001; Suzuki et al., 2005). BP-3 was also reported for significant down-regulation of *erα* mRNA in pituitary, and reduced *erβ* expression in uterus of female Sprague–Dawley rats (Schlecht et al., 2004). However, BP-1 appeared to have significant uterotrophic effects (Koda et al., 2005). BP-1 exposure stimulated the growth of BG-1 human ovarian cancer cells in the absence of endogenous ovarian estrogen in xenograft mouse models (Park et al., 2013). Based on an ER competitive-binding assay in Sprague–Dawley rats, estrogenic potential of BP-1 is also evident while those for BP-3 and BP-8 were not observed (Blair et al., 2000). These reports are generally consistent with the results of the in vitro studies.

For aquatic organisms, several studies have reported the adverse effects of BP-3 on reproduction and development (Table 6). Adverse acute and chronic effects on algae (*Scenedesmus vacuolatus* and *Desmodesmus subspicatus*), the freshwater flea (*Daphnia magna*) and planarian (*Dugesia japonica*) have been reported for BP-3 (Fent et al., 2010a; Li, 2012; Rodil et al., 2009a; Sieratowicz et al., 2011). BP-3 can alter the expression of an endocrine signaling gene, *ultraspiracle* (*USP*) in the aquatic invertebrate, *Chironomus riparius* (Ozaez et al., 2013).

A 21 d exposure to 620 μg/L BP-3 led to a decrease of reproduction and hatching success in Japanese medaka (*Oryzias latipes*). The exposure to a positive chemical, i.e., 0.05 μg/L E2, also led to the similar reproduction damages (Coronado et al., 2008). VTG induction by BP-3 varies by fish species. VTG concentrations in plasma samples from male Japanese medaka and rainbow trout (*Oncorhynchus mykiss*) increased by 21 d exposure to 620 μg/L and 14 d exposure to 749 μg/L, respectively (Coronado et al., 2008). In contrast, no significant alterations of plasma or whole body VTG were observed in male zebrafish (*Danio rerio*) and juvenile fathead minnow (*Pimephales promelas*) at concentrations of BP-3 as high as 312 and 3900 μg/L, respectively (Bluthgen et al., 2012; Kunz et al., 2006), while exposure to 0.1 μg/L E2 induced VTG in the same fish (Kunz et al., 2006). The interspecies differences in VTG synthesis by chemical exposure have already been reported by Van den Belt et al. (2003). The variation among species might be also explained in part by differences in the metabolic rate of BP-3 into BP-1. BP-1 induced VTG synthesis in juvenile fathead minnows at concentrations of 4919 μg/L (Kunz et al., 2006). The estrogenic activity of BP-3 such as VTG induction reported in certain aquatic species, could therefore be at least in part attributed to its metabolic product, i.e., BP-1, rather than by BP-3 alone which has shown only weak estrogenic activities in many in vitro studies. Among other BP derivatives, BP-2 has been reported to cause VTG induction and adverse reproduction effects in adult fathead minnows at high level of exposure ranging between 1200 and 9700 μg/L (Weisbrod et al., 2007).

Low level BP-3 can also inhibit steroidogenesis and affect hormonal pathways in male zebrafish (Bluthgen et al., 2012). Upon exposure to 84 μg/L BP-3, steroidogenic enzymes including *17β-hsd* were down-regulated, implying both antiestrogenic and antiandrogenic activities of BP-3 in the zebrafish. BP-1 is also believed to inhibit human *17β-HSD3*, and therefore could possibly reduce testosterone production (Nashev et al., 2010). A similarly designed study showed that BP-4

Table 7
Derivation of predicted no effect concentration (PNEC) for benzophenone-3 in fresh water environment.

Toxicity	Taxonomic group	EC50 or NOEC	Conc. (μg/L)	Reference	Assessment factor	PNEC (μg/L)
Acute	Algae	72 h, EC50	960	Sieratowicz et al. (2011)	100[a]	1.32
	Invertebrate	48 h, EC50	1670	Sieratowicz et al. (2011)		
Subchronic	Fish	21 d, NOEC	132[b]	Coronado et al. (2008)		
Chronic	Algae	72 h, EC10[c]	610	Sieratowicz et al. (2011)		
	Invertebrate	21 d, NOEC	500	Sieratowicz et al. (2011)		

[a] An assessment factor of 100 can be used in cases where the acutely most sensitive species has a lower toxicity than the two chronic toxicity data from two trophic levels (European Commission, 2003).
[b] Based on the lowest *Oryzias latipes* F1 hatchability NOEC after 21 days of parental exposure. The test duration of 21 days for fish was considered as acute with conservative perspectives.
[c] If no NOEC value is available for a long-term test, EC10 obtained by extrapolation using appropriate statistics can be considered as a NOEC (European Commission, 2003).

Table 8
Measured environmental concentrations (MECs) of benzophenone-3 in water environment and their hazard quotients (HQs).

Country	Source	Type	MEC (μg/L)	HQ	Reference
Unidentified	Surface water	Mean	0.052	0.04	Negreira et al. (2009)
Switzerland		Max	0.125	0.09	Poiger et al. (2004)
U.S.A.	Influent	Median	6.87	5.20	Loraine and Pettigrove (2006)
U.S.A.		Max	10.4	7.88	Loraine and Pettigrove (2006)

could also alter the expression of the genes involved in steroidogenesis in zebrafish (Zucchi et al., 2011).

5. Ecological risk assessment

To estimate the potential risk of BP-3 in aquatic environment, we performed an ecological risk assessment based on the available information, following European Commission (2003). In order to derive the predicted no-effect concentration (PNEC), only ecotoxicological data that are related to ecologically meaningful endpoints, e.g., survival, growth, or reproduction, were selected from the available toxicological information (Table 6), and were listed in Table 7. Considering the amount of available toxicological information, a factor application method which employs an assessment factor, was used to derive PNEC. As relevant toxicological information currently available includes only two chronic toxicity values from algae and water flea (Table 7), an assessment factor of 100 was chosen based on European Commission (2003). While results of 21 d fish exposure studies are often considered as chronic toxicity value (e.g., Fent et al., 2010a), we did not consider the results of the 21 d adult fish exposure test (Coronado et al., 2008) as "chronic". This was because a chronic toxicity test should include at least 10% of the organism's life span as an exposure duration (Suter and Barnthouse, 1993), or should include early life stage of the organism (Stephen et al., 1985).

Based on a hatchability no-observed-effect concentration (NOEC) of 132 μg/L in *O. latipes* following 21 d exposure of F0 (Coronado et al., 2008) and an assessment factor of 100, the PNEC was determined at 1.32 μg/L. This PNEC, however, should be used with caution, because this value reflects only the current knowledge of ecotoxicological information of BP-3, and is likely to be refined in the future with forthcoming toxicological data.

Hazard quotient (HQ) derived based on the measured environmental concentrations (MECs, Table 2) implies that direct impact of BP-3 would be negligible at the current levels in ambient waters (Table 8). However, in hotspots, e.g., wastewater influents of the U.S.A. and Switzerland, the HQ values for BP-3 often exceeded unity. For example, in wastewater influent in San Diego County, U.S.A., BP-3 was detected at as high as 10.4 μg/L (Loraine and Pettigrove, 2006), of which HQ is close to 8. Since the level of this compound varies by season and by human activities, and relatively limited information is available for environmental occurrences of this compound, systematic monitoring program and thorough toxicological studies are warranted to better understand risks of BP-3 in the aquatic environment.

6. Conclusion and future research

Widespread use of BP-3 in various personal care products including sunscreens and cosmetics, and its frequent detection in both water environment and biota have raised concerns on ecological risks of this compound. BP-3 and other BP derivatives have shown multiple endocrine disrupting activities. Animal studies have shown that BP-3 could be biotransformed into its hydroxylated forms, and some of which could exhibit greater estrogenic affinity both in vivo and in vitro. Based on the available in vivo toxicity studies, and the levels occurring in ambient water, BP-3 likely poses a negligible risk to freshwater ecosystems except for some hotspot areas. However, temporal and spatial pattern of environmental occurrences of BP-3 and its derivatives are not well understood. In addition, ecotoxicological information on long-term exposure to BP-3 and related derivatives is limited. Considering continuous release of this group of compounds into the water as well as their bioaccumulative potential, consequences following long-term exposure of aquatic organisms warrant further investigation.

Acknowledgment

This study was supported by the National Research Foundation of Korea (Project 2012R1A2A2A01015236).

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