Food and Chemical Toxicology 83 (2015) 237–250

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Skin absorption and human exposure estimation of three widely discussed UV filters in sunscreens – In vitro study mimicking real-life consumer habits

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ARTICLE INFO

Article history:
Received 20 February 2015
Received in revised form
17 June 2015
Accepted 22 June 2015
Available online 4 July 2015

Keywords:
Benzophenone
Ethylhexyl Methoxycinnamate
Butyl Methoxydibenzoylmethane
Dermal permeation
Systemic Exposure Dosage
Margin of Safety

ABSTRACT

Due to health concerns about safety, three UV-filters (Benzophenone-3, BP3, 10%; Ethylhexyl Methoxycinnamate, EHMC, 10%; Butyl Methoxydibenzoylmethane, BMDBM; 5%) were examined *in vitro* for absorption on full-thickness pig-ear skin, mimicking human in-use conditions. Kinetic profiles confirmed the rapid permeation of BP3; after the first hour of skin (frozen-stored) exposure to 2 mg/cm^2 (W/O sunscreen; recommended but unrealistic amount), about 0.5% of the applied dose passed into the receptor fluid. The absorption rate of filters was higher from W/O than from O/W emulsions. The fresh/frozen-stored skin permeability coefficient (0.83–0.54) for each UV filter was taken into account. Systemic Exposure Dosage of BP3, EHMC, BMDBM for humans as a consequence of (i) whole-body and (ii) face treatment with 0.5 mg/cm^2 of W/O sunscreen for 6-h skin exposure followed by washing and subsequent 18-h permeation (a realistic scenario) were estimated to be (i) 4744, 1032 and 1036 μg/kg-bw/day, and (ii) 153, 33 and 34 μg/kg-bw/day, respectively. From Margin of Safety for BP3, EHMC and BMDBM (i) 42, 485 and 192 as well as (ii) 1307; 15,151 and 5882, respectively, only the value of 42 (<100) for BP3 indicated a possible health risk. Escalation of a phobia towards all organic UV filters is undesirable.

1. Introduction

Topical application of personal care products (PCPs) containing ultraviolet (UV) filters is a preferred protection against various detrimental effects associated with excessive sun exposure, including sunburn, immunosuppression, photoageing, and skin cancer. Historically, UV filters (initially UV-B filters) were designed to be used by adults and children in sun protection products. Modern sunscreens contain one or several UV-B filters enriched with UV-A filters. At present, UV filters are not only used in sunscreens but are also important ingredients of various leave-on PCPs for daily use such as skin-, lip-, and hair-care, as well as makeup preparations. This often results in daily application of products containing UV filters without the user making a conscious decision to use any sunscreen agent.

For many years, attention has focused on the effectiveness of UV filters to mitigate the negative impact of solar radiation. Currently, the safety and usefulness of sunscreens is being questioned. Opinions that sunscreens may be dangerous are supported by certain media, and according to Nohynek and Schaefer (2001), also by controversial interpretations of some scientific studies, resulting in a "sunscreen phobia". It should be emphasized that at present, before a new UV filter is allowed on the market in the European Union (EU), a stringent toxicological safety evaluation is carried out. Safety and efficacy requirements for UV filters are comparable with those of human dermatological drugs (Nohynek et al., 2010). Only those

List of symbols: AD, applied dose (sunscreen); BMDBM, Butyl Methoxydibenzoylmethane; BP3, Benzophenone-3; CID, compound identifier; EC, European Commission; EHMC, Ethylhexyl Methoxycinnamate; EP, exposure period (skin); FTS, full-thickness skin; HPLC, high performance liquid chromatography; INCI, International Nomenclature of Cosmetic Ingredients; LoQ, Limit of quantification; MoS, Margin of Safety; MW, molecular weight; NOAEL, No Observed Adverse Effect Level; OECD, Organisation for Economic Co-operation and Development; O/W, oil-in-water (emulsion); PCPs, personal care products; Po/w, partition coefficient n-octanol/water (log); RF, receptor fluid; SC, stratum corneum; SCCP, Scientific Committee on Consumer Products (EC); SCCS, Scientific Committee on Consumer Safety (EC); SED, Systemic Exposure Dosage; SPF, Sun Protection Factor; TEC, Transcutaneous Electrical Conductivity; USFDA, United States Food and Drug Administration; UV, ultraviolet; WHO, World Health Organisation; W/O, water-in-oil (emulsion).

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http://dx.doi.org/10.1016/j.fct.2015.06.025
0278-6915/

compounds that are safe and effective may obtain approval for human use by the competent authorities (Nohynek et al. 2010; SCCS, 2012). Similar safety assessment procedures before registration of UV filters are necessary in the United States (US-FDA, 2014), Australia (ARGS, 2012), and Japan (MHW, 2000). So, the new *UV filters* introduced in the last decades, have improved safety and efficacy.

However, some synthetic UV-absorbing filters have been in use for several decades, but their safety, efficacy and toxicological profile are still not clear. *Especially* there is an increasing concern regarding possible harmful consequences of exposure to Benzophenone-3, Ethylhexyl Methoxycinnamate, 3-Benzylidene Camphor, 4-Methylbenzylidene Camphor, Homosalate, Ethylhexyl Dimethyl-PABA, and Butyl Methoxydibenzoylmethane (Krause et al., 2012; Axelstad and Hass, 2013; Oz̀aez et al., 2013; Urek et al., 2013; Kim et al., 2014). Three possible side effects are the most problematic: (i) permeation into the viable layers of the skin; (ii) interference with the endocrine system in humans; (iii) photounstability (Klimová et al., 2013). This article is focused on the first of these hot issues.

In principle, sunscreens are intended for external application to the skin. To ensure effectiveness, UV filters should adhere to the skin surface like a protective film and have a high affinity for the *stratum corneum* (SC). But, to avoid toxicity, UV filters should permeate the skin as little as possible. Ideally, no amount of UV filters should be accumulated in the viable skin and be systemically available through the vascular system (lymph and/or blood vessels) (Klinubol et al., 2008; Scalia et al., 2011; Klimová et al., 2013).

Using a wide variety of *in vitro* and *in vivo* assay systems, many studies have demonstrated that certain organic UV filters can be absorbed into and across the skin, further metabolized in the body and excreted (Giokas et al., 2007; Kim et al., 2014). These processes may result in some local adverse effects, e.g. allergic contact dermatitis (Heurung et al., 2014), and some systemic effects, e.g. mutagenic and estrogenic activity (Chisvert et al., 2012; Oz̀aez et al., 2013; Roussel et al., 2015). That is the reason why studies that monitor the transport of these chemicals from the outer surface of the skin both into the skin and into the systemic circulation are important.

The filters studied in this work were three UV-absorbing synthetic chemicals: Benzophenone-3 (BP3); Ethylhexyl Methoxycinnamate (EHMC); Butyl Methoxydibenzoylmethane (BMDBM). The compounds were chosen because they have been (i) worldwide used in sunscreens and other PCPs for decades; (ii) authorized by legislation in many countries, although to a different maximum allowable concentration (see Table 1); (iii) often used together in the same PCP; (iv) the subject of increasing debate about their possible adverse effects on humans.

Nowadays, there is a growing concern regarding potentially harmful consequences of exposure to xenobiotic compounds that are capable of modulating or disrupting the endocrine system. As the BP3 and EHMC structure are similar to estrogens, a number of experimental studies in a variety of screening systems (Schneider et al., 2005; Calafat et al., 2008; Schlumpf et al., 2008a,b; Zhang et al., 2011, 2013; Bluthgen et al., 2012; Oz̀aez et al., 2013; Kerdivel et al., 2013; Liao and Kannan, 2014; Watanabe et al., 2015) as well as review articles (SCCP, 2008; Axelstad et al., 2011, 2013; WHO, 2012; Krause et al., 2012; Urek et al., 2013; Kim and Choi, 2014; Manová et al., 2013) dealing with their influence on the endocrine system (oestrogen activity, progesterone activity, effect on reproduction, and other) were published in the past years. It should be emphasized, that conclusions on the potentially hormone-like activities of these filters were somewhat conflicting. One of the reasons may be variable designs and endpoints, the particular *in vitro* studies. Despite this, there is no doubt that for assessment of the systemic human exposure to both compounds via the PCPs, the rate of dermal absorption is important.

The formation of reactive intermediates with adverse side effects as a result of significant photounstability (Yang et al., 2008; Hojerová et al., 2011; Gaspar et al., 2013; Alfonso et al., 2014; Benevenuto et al., 2015) is the most undesirable side effect associated with the skin absorption of BMDBM.

Over the past years, several articles regarding dermal absorption of BP3, EHMC and BMDBM have been published. Studies have included experiments on human volunteers and animals *in vivo*, experiments on excised human, rodent, mouse, baby-mouse, pig, pig-ear, guinea-pig, etc. skin *in vitro*, and, more recently, *in vitro* experiments on a synthetic skin as well as a prediction using mathematical models. Several authors have demonstrated that BP3 can pass through the skin in significant amount (Gupta et al., 1999; Potard et al., 1999; Fernandez et al., 2000; Kurul and Hekimoġlu, 2001; Janjua et al., 2004; Gonzales et al., 2006; Calafat et al., 2008; Klinubol et al., 2008; SCCP, 2008; Gulbake et al., 2010; Kunisue et al., 2010, 2012; Liao and Kannan, 2014; Watanabe et al., 2015). Both significant (Gupta et al., 1999; Janjua et al., 2004; Jiménez et al., 2004; Iannuccelli et al., 2008; Klinubol et al., 2008; Montenegro et al., 2008; Durand et al., 2009; Vettor et al., 2010; Scalia et al., 2011) and insignificant (Potard et al., 1999; Simeoni et al., 2004) skin permeation of EHMC or BMDBM were reported. However, the experimental conditions used in some of the cited studies were far from the real-life habits of consumers (see Sections 3.3 and 3.4).

So, the aim of this work was to estimate the extent to which the three widely discussed UV filters in the sunscreens pose a health risk regarding dermal absorption under experimental conditions *in vitro* that mimic the real conditions *in vivo* as closely as possible.

Table 1
Physicochemical characteristics of the ultraviolet filters evaluated for dermal absorption.

INCI name[a]	INN name[b]	CAS no[c]	Molecular formula	Log Ko/w[d]	Molecular weight (g mol^{-1})	Absorption efficiency[e]	Maximum absorbance (nm)	Max. level in the EU[f] (%)
Benzophenone-3	Oxybenzone	131-57-7	$C_{14}H_{12}O_3$	3.79	228.25	UV-A II, UV-B	287.5	10
Ethylhexyl Methoxycinnamate	Octinoxate	5466-77-3	$C_{18}H_{26}O_3$	5.80	290.40	UV-B	308	10
Butyl Methoxydibenzoylmethane	Avobenzone	70356-09-1	$C_{20}H_{22}O_3$	4.51	310.39	UV-A I	358	5

[a] The name according to the International Nomenclature of Cosmetic Ingredients (CosIng, 2015).
[b] The International non-proprietary name recommended by the World Health Organisation (CosIng, 2015).
[c] The code number according to the Chemical Abstracts Service (CosIng, 2015).
[d] The partition coefficient n-octanol/water (PubChem, 2015).
[e] The main absorption efficiency in UV region (UV-B 290–320 nm; UV-A II 320–340 nm; UV-A I 340–420 nm).
[f] The maximum level allowed for cosmetic products in the European Union (EC, 2009; CosIng, 2015).