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# Dermal absorption and hydrolysis of methylparaben in different vehicles through intact and damaged skin: Using a pig-ear model in vitro

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ABSTRACT

Currently, there is a trend to reduce of parabens use due to concern about the safety of their unmetabolised forms. This paper focused on dermal absorption rate and effectiveness of first-pass biotransformation of methylparaben (MP) under in-use conditions of skincare products. 24-h exposure of previously frozen intact and tapestripped (20 strips) pig-ear skin to nine vehicles containing 0.1% MP (AD, applied dose of 10 μg/cm$^2$), resulted in 2.0–5.8%AD and 2.9–7.6%AD of unmetabolised MP, and 37.0–73.0%AD and 56.0–95.0%AD of *p*-hydroxybenzoic acid, respectively, in the receptor fluid. The absorption rate of MP was higher from emulsions than from hydrogels, from enhancer-containing vehicles than from enhancer-free vehicles, and when skin was damaged. Experiments confirmed that the freezing of pig-ear skin slightly reduces hydrolysis of MP.After 4-h exposure of intact freshly excised and intact frozen stored skin, amount of <LOQ-2.3%AD and 2.3–3.3%AD unmetabolised MP, respectively, were found in the receptor fluid. Taking into account the number of useful properties of MP, but also the potential of systemic availability of unmetabolised MP, we consider that MP is more suitable for preserving rinse-off topical products than for leave-on products. Risk of systemic absorption of parabens should also be explored via the skin with damaged barrier.



## 1. Introduction

Roughly 70 years, the parabens, alkyl esters of *p*-hydroxybenzoic acid (PHBA), were considered to be substances having low toxicity and some other properties of ideal preservatives. Parabens possess a broad spectrum of antimicrobial activity and water/oil solubility, excellent stability over a wide pH range, and low price (Soni et al., 2002, 2005; CIR, 2008). Furthermore, products containing parabens can be autoclaved. Therefore, either alone or in combination with other preservatives, parabens are used in a wide range of cosmetic, pharmaceutical, and partially also food products.

In our market survey conducted in 2011–2012 in the Slovak Republic (SR), according to the ingredients listed on the label, among 430 evaluated leave-on cosmetics at least one paraben was found in 39% products. Parabens were most frequently present in emulsion such as body-care and sunscreen creams and milks (including products intended for baby), hydrogels, lotions, and makeups. Among 159 topical medications registered in the SR, at least one paraben was listed in 10% of prescription and 5% of over-the-counter drugs. Methylparaben (MP) was present in 98% and 88%, propylparaben (PP) in 67% and 50%, respectively of paraben-positive cosmetics and medicines, while ethylparaben (EP) and butylparaben (BP) were listed sporadically only (Hojerová et al., 2013).

At recent years, the safety of parabens has become questionable. However, studies investigating the health effects of parabens are conflicting. Using a wide variety of assay systems in vitro and in vivo, a large number studies have demonstrated, that parabens may affect human health due to their endocrine disrupting activity (Routledge et al., 1998; Byford et al., 2002; Lemini et al., 2003; Pugazhendhi et al., 2005; Akomeah et al., 2007; Prusakiewicz et al., 2007; Darbre and Harvey, 2008; Boberg et al., 2010; Vo et al., 2010, 2011; Hu et al., 2013). Other researchers (van Meeuwen et al., 2008; Shaw and deCatanzaro, 2009; Witorsch and Thomas, 2010; Sciali, 2011; Aubert et al., 2012; Kirchhof and

*Abbreviations:* AD, applied dose; ANOVA, one-way analysis of variance; BP, butylparaben; BLOQ, below the limit of quantification; CIR, US Cosmetic Ingredient Review; E, enhancer; EP, ethylparaben; FDA, Food and Drug Administration; FTS, full-thickness skin membrane; $J_{ss}$, steady state flux; LOD, limit of detection; LOQ, limit of quantification; OECD, Organization for Economic Co-operation and Development; MP, methylparaben; PG, propylene glycol; PHBA, *p*-hydroxybenzoic acid; RF, receptor fluid; SC, stratum corneum; P, permeability coefficient; PP, propylparaben; SCCS, EU Scientific Committee on Consumer Safety; SD, standard deviation; SED, systemic exposure dosage; TC, Transcutol® CG; TEC, transcutaneous electrical conductivity; unmMP, unmetabolised MP; UR, urea.

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de Gannes, 2013) have published a radical opposition toconcerns about health risks from doses of parabens commonly used. In 2004 Darbre's team (Darbre et al., 2004; Harvey and Darbre, 2004) firstly measured trace residues (nanograms/g of tissue) of intact parabens, particularly MP, in human breast cancer tissues, and suggested that their presence in the human body might originate from topical application of body-care cosmetics such as underarm deodorants and antiperspirants. Although some public health authorities (SCCP, 2005a,b, 2011a,b; FDA, 2007) and some cancer experts (Gikas et al., 2004; Rageth, 2005; CIR, 2008; Namer et al., 2008) rejected these considerations, the study has sparked controversy and also stimulated new international research on parabens effects on human health.

The Food and Drug Administration (FDA) in the USA, as well as the European Scientific Committee on Consumer Safety (SCCS) reopened the safety assessment for parabens, to request exposure estimates and risk assessment for cosmetic use. According to the opinion of the SCCS (2011a), parabens may really exert a weak estrogen-like activity but its potency is from 1000 (for MP) to 1,000,000 (for BP) times below the potency of the positive control 17β-estradiol. So the SCCS (2011a, 2013) considers that for general cosmetic products containing parabens, excluding specific products for the nappy area, there is no safety concern in children (any age group) and adult consumers. Regarding personal care products, FDA (2007) states parabens safe at concentrations up to 0.8% (mixtures of parabens) or up to 0.4%(single paraben). The SCCS recognises a mixture of parabens safe also at concentrations up to 0.8%, MP and EP at concentrations up to 0.4% (single paraben), but PP and BP up only to 0.19% individually or in combination (SCCS, 2011a,b, 2013).

However, general view on the safety of parabens is based on the assumption that the ester bonds in the parent compounds are quickly and nearly completely hydrolysed by carboxylesterases (EC 3.1.1.1) in the common metabolite, a non-specific PHBA (Soni et al., 2005; Boberg et al., 2010). Since PHBA is considered to be a compound without an endocrine effects (SCCS, 2011), carboxylesterases activity appears to be crucial for detoxification parabens. Several studies (Harville et al., 2007; Janjua et al., 2008; Boberg et al., 2010; Shirai et al., 2013) confirmed that orally administered parabens are indeed readily metabolised by carboxylesterases in the intestines and liver and then excreted without significant accumulation in the body. However, metabolism of parabensadministered dermally may be incomplete for some reasons. The main reason is a lower capacity of skin carboxylesterases compared to mammalian liver carboxylesterases (Harville et al., 2007; Prusakiewicz et al., 2007). Another reason may be the negative effect of skin esterase inhibitor (Bando et al., 1997; Seko et al., 1999; Prusakiewicz et al., 2007; Harville et al., 2007; Jewell et al., 2007a,b), long-term use of a wide range of paraben-positive topical preparations, as well as inter-individual variations of human skin (Darbre et al., 2004; Harvey and Darbre, 2004; Ishiwatari et al., 2007; Darbre and Harvey, 2008). So it is generally accepted that unmetabolised (intact) forms of parabens in the body tissues are more likely the result of dermal applications than oraladministration (Oh et al., 2002; Prusakiewicz et al., 2007; El Hussein et al., 2007; Harville et al., 2007; Janjua et al., 2007; Darbre and Harvey, 2008; Williams, 2008; Barr et al., 2012; Shirai et al., 2013).

Considerable number of studies through intact human and various animal skin in vivo and in vitro have documented many parameters influencing the overall dermal absorption rate of parabens, i.e. without detection of individual quantities of unmetabolised parabens and their metabolite, PHBA (Pozzo and Pastori, 1996; Kitagawa et al., 1997; Oh et al., 2002; El Hussein et al., 2007; Janjua et al., 2007, 2008; Jewell et al., 2007a; Mbah, 2007; Pedersen et al., 2007; Wilkinson et al., 2007; Caon et al., 2010; Romonchuk and Bunge, 2010). Unfortunately, the available studies on the degree of hydrolysis of parabens during percutaneous permeation are limited. Bando et al. (1997) reported that after application of BP and PP to the rat skin in vitro, about 4% of intact BP and about 30% of intact PP fromthe total permeants in the receptor fluid was detected. Ishiwatari et al. (2007) evaluated the influence of daily exposure to MP containing formulations to human skin. At 1 h after a single application of 0.15% MP in emulsion to the forearm of human volunteers, unmetabolised MP (unmMP) concentrations about 18% of the application quantity of the parent MP were found in the stratum corneum (SC), but after 12 h the concentration of unmMP was decreased at 10 pmol/cm$^2$ (approximately 0.028%). However, the authors confirmed that repeated application of MP containing topical products significantly increases the amount of unmMP in the SC. The same researchers studied also the metabolism of MP through Yucatan micropig skin in vitro. 2-h exposure to an aqueous solution (10 μg/cm$^2$) containing 0.1% of MP resulted in 2.06 mgof unmMP and 0.36 mg of PHBA expressed to 1 g tissue of full-thickness skin (Ishiwatari et al., 2007). Aubert et al. (2012) measured the content of unmMP in excretes from rat following dermal application for 6 h at a dose of 100 mg/kg of MP. No parent ester, only metabolite PHBA in the urine and feces (14–27% and <2% of the applied dose, respectively) was determined.

According to Aubert et al. (2012) in line with our view, the pivotal question of the safety assessment of parabens-containing topical products is the fate after human skin exposure, (a) their dermal absorption rate and (b) whether they absorbed intact or after first-pass hydrolysis in the skin. So the first aim of this study was to assess systemic exposure of unmMP and its main metabolite PHBA as a result of single topical application of different MP-containing products to ex-vivo intact skin (frozen prior to the experiments). Because of anatomical, physiological and biochemical similarity to human skin (Sekkat et al., 2002; Singh et al., 2002; Godin and Touitou, 2007; Jacobi et al., 2007; Klang et al., 2012; Lau et al., 2012), excised pig-ear skin as a skin model was chosen.

When investigating dermal absorption values of substances, risk assessment is generally focused on intact skin. The OECD (2004), as well as the SCCS (2010) guidelines for studies on in vitro dermal absorption prescribe also optimal barrier integrity of the excised skin. Such membrane is suitable to predict dermal absorption values through intact skin but may not be relevant for situation, where the topical product is applied to barrier-impaired skin due to mechanical, physical, chemical or biological reasons. Several studies (Jacobi et al., 2007; Lademann et al., 2009; Weigmann et al., 2009; Klang et al., 2012, 2013) have shown that stripped ex-vivo pig-ear skin is very representative to the in vivo human skin with barrier impaired due to mechanical trauma. To our knowledge, skinbarrier damage due to stripping in relation to the permeation of parabens has not been studied yet. Therefore, the second aim of this study was to assess the same objectives as in the first aim, but through stripped skin (frozen prior to the experiments).

Conflicting reports concerning the effect of storage conditions of the skin on percutaneous absorption of chemicals and degree of hydrolysis of ester bonds are published. While several reports suggest that the freezing of the skin alters the permeability of certain compounds (Hadzija et al., 1992; Shaikh et al., 1996; Wester et al., 1998; Ahlstrom et al., 2007; Payne et al., 2013), Harrison et al. (1984) has shown that the permeability of human skin ex-vivo is no significantly affected after prolonged freezing at −20 °C up to 466 days. Two studies have demonstrated reduced activity of non-specific esterases in the suspension of frozen stored (at −20 °C) pig ear skin towards retinyl ascorbate (Abdulmajed et al., 2006) and acetylsalicylate (Lau et al., 2012) compared to freshly excised pig-ear skin. Conversely, a number of studies have shown for snake, human, rat, rabbit, guinea-pig, and mouse skin, that