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Review

The controversies of parabens – an overview nowadays

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Effects of paraben toxicity, *i.e.*, endocrine-disruption properties, are in the focus of researchers for decades, but still – they are a hot subject of debate. Parabens are aliphatic esters of *p*-hydroxybenzoic acid, which are widely used as antimicrobial agents for the preservation of cosmetics, pharmaceuticals and foods. Mostly used parabens are methyl-, ethyl-, propyl- and butylparaben. Although the toxicity of parabens is reported in animals and in *in vitro* studies, it cannot be taken for granted when discussing hazards for human health due to an unrealistic exposure--safety profile. Many studies have demonstrated that parabens are non-teratogenic, non-mutagenic, non-carcinogenic and the real evidence for their toxicity in humans has not been established. For now, methyl-, ethyl- and propylparaben are considered safe for use in cosmetics and pharmaceuticals within the recommended range of doses. Regarding alternatives for parabens, a variety of approaches have been proposed, but every substitute would need to be tested rigorously for toxicity and safety.

Keywords: parabens, toxicity, safety, endocrine disruption, health risk

INTRODUCTION

Human health depends on homeostasis where hormones as chemical messengers, regulate many physiological functions. Such regulation is defined and organised on the molecular and biochemical levels. In recent times, numerous publications reported side-effects of various xenobiotics, *i.e.*, chemical compounds from the environment which can directly modulate hormonal homeostasis and signalling. This certainly raises the question of the safety and extent of human exposure to the chemicals, which are classified as endocrine-disrupting chemicals (EDCs) (1). According to the World Health Organization (WHO), EDCs are substances that are mostly man-made, found in various materials such as pesticides, metals, additives or contaminants in food, and personal care products. EDCs have been suspected to be associated with altered reproductive function in males and females, increased incidence of breast cancer, abnormal growth patterns and neurodevelopmental delays in children, as well as changes in immune function (2). There are many

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examples of low dose effect endocrine-disrupting chemicals and one of them is the group of parabens (3). Parabens, a group of esters of *p*-hydroxybenzoic acid that are used as antifungal and antibacterial agents, are widely used in personal care products (PCPs), foodstuffs, pharmaceuticals and various types of cosmetics (4, 5).

As some scientific studies linked endocrine-disruption with parabens, widely used as a preservative for more than 70 years, parabenophobia started to spread around the globe. Sometimes media and press made things worse because of their mostly high influence on public opinion. Such a bad reputation of parabens resulted that nowadays some people are buying only paraben-free products, without actually thinking what is used as an alternative.

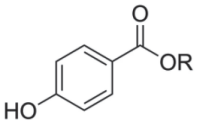
Bearing in mind medical and health professionals who are daily confronted with suspicions about parabens safety and evidence of their proven toxicity (6, 7), it is important to approach to this controversial topic *cum grano salis* and highlight from toxicological and pharmacological aspect what we know so far, and if we should be worried. These topics of current knowledge of parabens are focused on and discussed in this review.

WHAT ARE PARABENS AND WHAT IS THEIR USE?

Parabens are aliphatic esters of *p*-hydroxybenzoic acid (pHBA) (Fig. 1) widely used as preservatives in cosmetics, pharmaceuticals and the food industry. In cosmetics, they are mostly found in topical preparations, while in pharmaceuticals they are part of various formulations (Table I). Mostly used parabens are methyl-, ethyl-, propyl- and butylparaben. Earlier, due to their antimicrobial efficacy, parabens were found in injections and ophthalmic preparations while today such use is reduced to avoid potential irritations (8).

Parabens are chemically stable and effective over a wide pH range, with wide antimicrobial activity, particularly against Gram-positive bacteria and fungi (including molds). Parabens are odourless and tasteless, which also makes them highly preferred (8). In the past, quality, efficacy and safety were investigated only for an active pharmaceutical ingredient (API), whereas today the efficacy and safety of any excipient, including parabens, must be investigated and monitored by International Pharmaceutical Excipients Council (IPEC) (9).

Antimicrobial activity of parabens increases with the length of their aliphatic chain, from methylparaben to butylparaben. Chain length has an impact on their solubility, which decreases as the length of the aliphatic chain increases, but that is overcome by using different salts of parabens. The stability of parabens is markedly decreased in the presence of non-ionic surfactants, like polysorbate 80. Moreover, there have been observed incompatibilities with some other compounds such as hydroxypropyl cellulose, methylcellulose, bentonite, magnesium trisilicate, talc, tragacanth, sodium alginate, sorbitol and atropine. Hence, to achieve



Paraben	R
Methylparaben	$-CH_3$
Ethylparaben	$-CH_2CH_3$
Propylparaben	$-CH_2CH_2CH_3$
Butylparaben	$-CH_2CH_2CH_2CH_3$

Fig. 1. Structures of common *p*-hydroxybenzoic acid (pHBA) esters, *i.e.*, parabens (8).

Table I. Use of methylparaben and propylparaben in various pharmaceutical preparations (8)

Application	Concentration (%)	
	Methylparaben	Propylparaben
Injections (*i.m.*, *i.v.*, *s.c.*)	0.065–0.25	0.005–0.2
Inhalation solutions	0.025–0.07	0.015
Intradermal injections	0.10	0.02–0.026
Nasal solutions	0.033	0.017
Ophthalmic preparations	0.015–0.02	0.005–0.01
Oral solutions and suspensions	0.015–0.02	0.01–0.02
Rectal preparations	0.1–0.18	0.02–0.1
Topical preparations	0.02–0.3	0.01–0.6
Vaginal preparations	0.1–0.18	0.02–0.1

i.m. – intramuscular, *i.v.* – intravenous, *s.c.* – subcutaneous

better antimicrobial efficacy, parabens are often used in combination with other antimicrobial agents. Also, as parabens are aliphatic esters of pHBA, their hydrolysis can occur in formulation itself, which consequently affects the antimicrobial activity (8).

It is important to mention that parabens were never declared to be allergenic or carcinogenic, although sporadic cases of hypersensitivity reactions, like contact dermatitis were reported, while immediate reactions with urticaria and bronchospasm have occurred rarely. In the latest edition of the Handbook of Pharmaceutical Excipients (8), parabens are classified as non-teratogenic, non-mutagenic and non-carcinogenic. It is worth mentioning that parabens are naturally present in some foods (fruits and vegetables) and white wine in very low concentrations, thus making paraben intake from plant sources negligible (10, 11).

"STIRRING THE POT"

After isolation of parabens in cancerous breast tissue by Darbre *et al*. (12) parabens became the focus of research for many scientists, consequently tagging them to be bad. Further discoveries of parabens in various biological samples, such as urine, blood, semen, adipose tissue, placenta, amniotic fluid and breast milk, only made matters worse (13–15), despite the fact that based on the available repeated-dose toxicity studies, repeated oral exposure to methyl-, ethyl- or propylparaben is not considered to cause serious effects to health (16–18).

What should be addressed from the start is that scientific studies which point parabens as EDCs, mostly have an oral route of exposure, while the topical route is less prevalent (19). The topical route should not be neglected, because numerous cosmetic and pharmaceutical preparations are used only in that way, which makes the interpretation of the paraben safety-exposure profile over-exaggerated and unreal. Additionally, successful translation of paraben toxicity data from animals to humans, or extrapolation from *in vitro* to *in vivo*, is quite questionable as well in terms of providing truthfulness (20). Having that in mind, one

question arises: is reported toxicity of parabens and endocrine disruption, clearly and undeniably linked with health hazards in humans? Well, according to the provided information based on scientific papers and presented in this review, the answer is — no.

Namely, Darbre *et al.* (12) investigated the assumption that creams and lotions, containing parabens as preservatives and used under the armpits and breasts, could increase the incidence of breast cancer in women. After breast tumour tissue analysis, results revealed that methylparaben was most prevalent in the analyzed tissue samples (among ethyl-, propyl-, butyl- and isobutylparaben).

Given its most common use as a preservative, this is not surprising at all, but before making any assumptions, a few facts should be considered for a valid conclusion. What should have been done is finding the route by which parabens entered the body at the first place, and how they accumulated in breast tissue because they could also enter by consuming food. Moreover, the medical history of patients was not known, neither was the breast tumour type. Was that estrogen receptor-positive breast cancer? It is not known. Most importantly, there were no control tissues, and parabens were found in samples of tissue used as a blank. Also, the sample size is a very important parameter during every experiment in order to achieve statistical strength.

BIOTRANSFORMATION, PHARMACODYNAMICS AND BIOMONITORING OF PARABENS

Paraben biotransformation studies reported that more than 90 % of parabens are excreted in the urine as conjugates (glucuronide and sulphate conjugates), whereas only a small fraction is left unconjugated (21). It is also considered that conjugated parabens have no biological activity (21, 22). The penetration of parabens through the skin varies, and also shows the difference between species (16–18). Additionally, only up to 0.9 % of the topically applied propylparaben (to the whole human body) could be recovered in the urine in a free form (19). The explanation for this lies in variable lipophilicity of parabens which increases as aliphatic chain becomes longer, variable thickness of the skin layers and the solvents used in formulation, limited duration of exposure, relatively small amount of the product itself, and the presence of the esterase in the skin which hydrolyzes parabens. Parabens absorbed from the oral route of exposure, or by the skin, are hydrolyzed to pHBA and *p*-hydroxyhipuric acid (pHHA) (19). pHHA is a biotransformation product of parabens found in animals and humans. Humans even produce pHHA endogenously, as a product of tyrosine metabolism (23).

Shin *et al.* (21) developed a propylparaben (PP) pharmacokinetic model, which can also be used as a tool for pharmacokinetic and toxicokinetic assessments of other parabens. In brief, orally given PP was rapidly absorbed (< 2 h), and quickly and completely eliminated; terminal half-life, *i.e.*, time taken to eliminate half of the remaining fraction of the substance in the body, was 2.9 h. pHBA, pHHA and free PP were eliminated even slightly faster than conjugated PP, while the oral PP bioavailability of 39 % (expressed as a fraction of the dose absorbed) is a rough estimate only.

Considering all of the above, it could be concluded that the degree of paraben absorption through the skin is not high and consequently bioavailability is expected to be low, whereas biotransformation data count against suggestions of paraben accumulation in the body. On the other hand, some data are indicating that *in vivo* dermal absorption of para-

bens is more effective than it is claimed (24), while regarding *in vitro* dermal absorption studies, Scientific Committee on Consumer Safety (SCCS), consider that they have low scientific quality (16–18). Hence, there is absolutely a need for more pharmacokinetic and toxicokinetic studies, to give a definite answer on that matter.

Additionally, new data for total daily exposure to parabens (*via* foodstuffs, cosmetics and pharmaceuticals) based on the measurement of real samples in Chinese females (25), report exposure to be 0.326 mg kg^{-1} daily, which is much less than the previous results provided by Soni *et al*. (10), of 1.26 mg kg^{-1} daily.

The classic premise in toxicology *the dose makes the poison*, cannot be applied to EDCs, because their pharmacological effect is not governed by a typical mechanism of receptor-mediated action. In other words, even the smallest dose of EDC can show effect like cell proliferation, whereas a higher dose may have the opposite effect – inhibition of cell growth. The latter has been shown for phytoestrogens, but never for parabens. Moreover, *in vitro* assay with radiolabeled estradiol (^{3}H-estradiol), designed to measure a competitive estrogen receptor (ER) inhibition, showed that concentration of parabens should be several orders of magnitude larger than estradiol to have any effect on the ER. The explanation lies in the fact that parabens have a lower binding affinity for the ER (1).

It is generally accepted that *in vitro* chemical safety testing methods and animal laboratory data offer the potential for efficient and economical tools to provide relevant assessments of human health risk. However, simple extrapolation is not possible. It was stated that there is a need to refine currently available *in vitro* to *in vivo* extrapolation (IVIVE) approaches before they can be utilized for regulatory decision-making (26). It was proposed to combine *in vivo* and *ex vivo* studies of paraben toxicity to build more comprehensive, scientifically sound strategies for paraben safety testing (27).

Analysis of human urine reflects directly the exposure to parabens and serves as a good method of biomonitoring from time to time. One such study revealed that parabens are detected in 99 % of the American population. From the whole group of mostly used parabens, methyl- and propylparaben were the most frequently detected ones. After statistical stratification, interesting results showed up: women had a 3-fold higher value of methylparaben and a seven-fold higher value of propylparaben, compared to men. Furthermore, the black population had a greater exposure compared to white, and most importantly, no differences in health status among subjects were observed (28). Despite paraben detection in urine, the authors agree that the measurable level of parabens in urine does not immediately imply a risk for human health (28, 29). Additionally, parabens have been detected in human matrices such as breast tumour, seminal plasma, adipose tissue, human breast milk, placenta and cord blood (12, 30–34) showing the general population is inevitably exposed to parabens in daily life.

PARABENS AND REPORTED FINDINGS OF THEIR TOXICITY – TWO SIDES OF THE SAME COIN

For a long time, parabens were considered harmless and therefore they have been widely used as preservatives. However, over 20 years, a vivid discussion on paraben safety is ongoing because of the main concern from their endocrine-disrupting potential.

As stated previously, parabens do have estrogenic activity *in vitro*, but it is very low. Although estrogenic activity increases with the length of paraben's aliphatic chain, its

relevance relies on the fact that methyl-, ethyl-, propyl- and butylparaben have 2.5×10^6, 1.5×10^5, 3×10^4 and 1×10^4 times lower potency than estradiol, the physiological agonist for ER (35). Moreover, their low estrogenic activity and a high tendency for hydrolysis were already confirmed (36, 37).

The mechanism of xenoestrogenicity of parabens is not well understood. Paraben esters are rapidly metabolized *in vivo* to the relatively inactive metabolite pHBA, and to what extent micromolar concentrations of parabens disturb normal estrogen activity or function as mimicker *in vivo* is unknown (38). One hypothesis relates the potential biologic activity to the inhibition of the enzymatic activity of 17β-hydroxysteroid dehydrogenase type 2 (estrogen and androgen-converting enzyme), at micromolar concentrations that would increase conversion of estradiol to the weaker estrone (20). Another study found that parabens inhibit aromatase, an enzyme involved in a rate-limiting step in steroidogenesis, by one order of magnitude less than that which induces MCF-7 human breast cancer cell line proliferation, potentially indicating a more potent antiestrogenic effect (39). It was shown that isopropyl-, butyl- and benzylparaben are aromatase inhibitors, but such inhibition required micromolar paraben concentration which is several orders of magnitude higher than the concentration (in nmol L^{-1}) previously found in human breast tissue (40). Hence, toxicological relevance cannot be determined.

The study of paraben effects on mRNA levels and protein expression of ER-α (ESR1) and ER-β (ESR2) and the progesterone receptor (PGR) conclude that, *via* these effects on hormone receptor expression and stimulation, the estrogenic effect of parabens and putative initiation and progression of breast cancer may be explained (41).

Estrogenic activity of parabens *in vivo* was assessed by monitoring the enlargement of female rat uterus. After orally (or *s.c.*) administered methyl- and butylparaben for three days, it was observed that neither methylparaben (up to 800 mg kg^{-1} daily) nor butylparaben (up to 1200 mg kg^{-1} daily) caused enlargement of the uterus when given orally. However, after *s.c.* administration of butylparaben at doses of 600–800 and 1200 mg kg^{-1} daily, uterine enlargement was 30–40 % and 70 %, resp. (35, 40). If these data were translated to the human, an equivalent dose of parabens is not realistic.

In a study where methyl-, propyl- and butylparaben were orally administered to female rats for 5 weeks (100 mg kg^{-1} daily), results showed that parabens accelerated ovarian dysfunction (previously induced by another chemical) and disrupted folliculogenesis and steroidogenesis, *i.e.*, induced premature ovarian failure (42). For humans, such exposure is not of toxicological relevance according to established No Observed Adverse Effect Level (NOAEL) of 1000 mg kg^{-1} b.m. daily for methyl- and ethylparaben by the European Food Safety Authority (EFSA) and the Scientific Committee Consumer Safety (SCCS); this was done based on the absence of reproductive effects from four repeated-dose toxicity studies. Propylparaben is not allowed as a food additive in the EU and more data is needed for its NOAEL. But, in the meantime, the SCCS proposes that the NOEL for butylparaben of 2 mg kg^{-1} b.m. daily can be very conservatively used for propylparaben (16–18).

Another study associated the menstrual cycle duration with parabens exposure. Results showed that a shorter menstrual cycle is associated with higher parabens measured in the urine (43). However, the subjects were asked to keep a diary for recording their menstrual bleeding on their own, which could affect statistics. A mechanism for the observed relationship between menstrual cycle length and exposure to parabens could not be explained and subjects with smaller intra-individual variability of cycle duration seemed to be exposed to

higher levels of parabens, for which the underlying mechanism of the relationship was not clear either. The authors concluded that they might have failed to also include some other potential covariates (like psychological stress) in addition to sample size (43).

Oishi *et al.* studied the effects of butylparaben (44) and propylparaben (45) on the male reproductive system. In brief, rodent males (rats and mice) 4–6 weeks old were fed with parabens up to 15, 150 and 1500 mg kg^{-1} daily, for 4 weeks. The study consisted of measuring sperm production, sperm reserve, masses of epididymis, testicles and prostate, and the level of testosterone. The lowest intake of parabens did not show any effects, whereas higher intake decreased testosterone in both species. Furthermore, rats with the highest intake of butylparaben had a 15 % decrease of epididymis mass, while mice had a 15 % increase (44). Such deviation in the results is not explained by the authors, so the valid conclusions cannot be drawn. Additionally, high doses of parabens used in this study are unrealistic in terms of exposure for humans, so toxicological importance of these data, again, cannot be given. Most importantly and interesting, Hoberman *et al.* (46) performed the same experiment and concluded that there were no changes in testosterone levels, spermatogenesis, nor were any effects dependent on doses of parabens. Furthermore, testosterone levels and measurement of sperm concentration in Hoberman's study were consistent with the historical National Toxicology Program (NTP) data, while in Oishi's study (44) testosterone was 4–5 times higher, along with a deviation in sperm concentration as well.

Although theoretical concerns regarding paraben activity and xenoestrogenicity are supported by a body of *in vitro* and animal *in vivo* evidence, the actual impact, if any, to human health is far from clear, especially given the margin of exposure safety data (margin of exposure/margin of safety, MOE/MOS) (16–18). Thus, it is not surprising that there are no studies in humans confirming the harmful effects of paraben exposure from the estrogen mimicry standpoint (38).

One might surely ask themself, what about reported evidence of paraben's carcinogenicity and genotoxicity in some studies?

Since the first detection of parabens in human breast tumour tissue in 2004 (12) with many recognized shortcomings, recent *in vitro* short-term and long-term studies performed on human breast lines exposed to parabens reported that parabens can influence not only proliferation but also migratory and invasive properties of human breast cancer lines (47). Recently, the review paper summarised current evidence data of environmental chemicals, termed obesogens, that may be able to interfere in the endocrine regulation of energy metabolism and adipose tissue structure. This paper confirmed the obesogenic potential of parabens and offered a possible explanation of why obesity is an underlying risk factor for so many diseases including cancer (48). However, current scientific knowledge is insufficient to demonstrate a clear cancer risk due to the topical application of cosmetics that contain parabens on normal intact skin (38).

From the available *in vivo* carcinogenicity studies on methyl-, ethyl- and propylparaben it can be concluded that they are not considered to be carcinogenic (8, 10, 16, 17, 22).

The results of the *in vivo* studies are equivocal in terms of the potential of parabens to exhibit harmful effect in the animals, but on the other hand, *in vitro* data on cultured human peripheral lymphocytes from Bayülken *et al.* (49, 50) showed that parabens are cytotoxic and genotoxic, which may indicate also a genotoxic potential for humans. Taken altogether, *in vitro* studies often have a big impact on providing a piece of new knowledge, but solely they cannot

be used as a reference point, hence scientific community hopes that more studies in future will provide new and stronger insights about paraben genotoxicity and carcinogenicity.

Concerning reproductive male and female health, recently contradictory results were presented. Smarr *et al.* (51) suggested that specific urinary parabens found in consumers' goods (methyl-, ethyl- and butylparaben) may adversely impact sperm quality parameters among reproductive-age male partners of couples trying for pregnancy. Also, a positive association of couples' urinary concentration of parabens (methyl- and ethyl-) in the context of reduced fecundity was obtained (52) while the other study showed no relationship between urinary paraben concentrations (methyl-, ethyl- and butyl-) and *in vitro* fertilisation outcomes among women undergoing infertility treatments (53).

It was stated that medications are among the three major sources of paraben. In oral pharmaceutical formulations, methylparaben and propylparaben prevail (Table I) whereas other parabens are also used to a lesser extent, such as ethylparaben and butylparaben. The latter is predominantly used in pharmaceutical formulations for the cutaneous route (8, 54). The contribution of medicinal products to aggregate exposure could be estimated only very roughly and worst-case for methyl- and propylparaben. For ethylparaben, there was insufficient data available to estimate the contribution by medicinal products to total exposure (16, 17). Dodge *et al.* (55) showed that paraben-containing medicines contributed to higher urinary paraben concentrations within hours of use which is in agreement with data from the National Health and Nutrition Examination Survey (NHANES) 2005–2006 showing that nearly all subjects had detectable levels of methyl- and propylparaben in their urine, while only 40 % of subjects had detectable levels of butylparaben (56). Despite this, data from NHANES 2005–2012 showed no clear overall evidence of the association between the use of paraben-containing medications and increases in urinary paraben concentrations among participants in NHANES 2005–2012. Therefore, the authors pointed out that these results highlight the difficulties inherent in the proper assessment of exposures with short half-lives based on a single cross-sectional biologic sample (57).

Considering that humans are often exposed to a mixture of chemicals rather than a single compound, nowadays, particular interest is given to realistic scenarios of exposure to chemical cocktails in which parabens, as a type of EDC, interact with other types of chemicals with a possible synergistic or additive effect (38, 39, 58).

ARE PARABENS NECESSITY FOR CHILDREN? WHAT ABOUT ALTERNATIVES AND REGULATORY OPINION?

Parabens are used in different applications as preservatives. Therefore, there is a large overlap of their use in cosmetics, food, cleaning products and pharmaceuticals as well as in regards to available alternatives. In EU parabens are regulated by the cosmetics regulation (59), the food additives regulation (60) and the detergents directive (61). They are allowed in the EU for use in pharmaceuticals and monographs can be found within the authoritative source of information, The Handbook of Pharmaceutical Excipients (8) which is an internationally accepted comprehensive guide to uses, properties and safety of excipients.

The SCCS carried out a case-by-case safety assessment of the different parabens. While the safety for use in cosmetics of certain types of parabens (methyl-, ethyl-, propyl- and butylparaben) has been confirmed by the SCCS, the SCCS could not rule out the risk

for human health of other categories of parabens (isopropyl-, isobutyl-, phenyl-, benzyl- and pentylparaben) (62). For general cosmetic products containing parabens, excluding specific products for the nappy area, the SCCS considers that there is no safety concern in children (any age group) as the MOS value was based on very conservative assumptions, both concerning toxicity and concerning exposure (29, 62). The European Medicines Agency (EMA) claims that methylparaben up to 0.2 % in oral formulations is not dangerous, including children of any age (54). Moreover, methyl- and propylparaben in concentrations of up to 0.1 % have a GRAS (Generally Recognized as Safe) status in the USA (8). Risk assessments on parabens have been performed by several European expert panels including the European Food Safety Authority (EFSA) and SCCS. EFSA established a full-group acceptable daily intake (ADI) of 0–10 mg kg^{-1} b.m. for the sum of methylparaben, ethylparaben and propylparaben. The EFSA opinion dated July 2004 considered that propylparaben should not be included anymore in this group ADI due to effects on the male reproductive organs observed in juvenile rats and the lack of a clear NOAEL. As a consequence, from the year 2006, propylparaben was no longer allowed for use as a food additive within the European Union (54, 63). EU authorities, as a measure of precaution, limited some parabens in products intended for the diaper area for children under 3 years, whereas methyl- and ethylparaben are considered safe. Additionally, the EU allows the use of a single paraben in the concentration of 0.4 %, or 0.8 % in combination (54). As a precautionary measure, the rule of thumb should always be to use the lowest amount of any excipient, not just parabens.

The fetus, neonates and young children are considered to be most vulnerable to EDC exposures, drug treatments and the effects of active compounds and excipients present in pharmaceutical and other products due to their unique physiology (16–18, 64–66). That is, indeed, the rationale behind the EU decision to ban parabens in products intended to be applied to intertriginous areas in children younger than 3 years.

Recently, it was stated that the unborn fetus will be better protected from possible parabens effects than the neonate/newborn or early infant exposed dermally to parabens by the more efficient systemic parabens inactivation by the mother (29). However, the study that reported the levels of major parabens in the first urine of newborn infants (67) and cord blood of mother-child pairs (34) indicated evidence of fetus exposure and transplacental passage of parabens. The study of Park *et al*. (68) showed that the estimated daily intake of parabens in infants *via* breastfeeding appears to be negligible when compared to the acceptable daily intake values (ADI of total parabens, 0–10 mg kg^{-1} daily) set forth by the EFSA (63). However, considering the vulnerability of breastfed infants and ubiquitous sources of exposure from daily use of household and personal toiletries, efforts to identify sources and mitigate exposure are warranted.

The safety assessment of parabens present in pediatric oral pharmaceutical formulations needs to take into account their beneficial antimicrobial effects, so their withdrawal may increase the risk of infection in neonates (64). However, paraben's maximum daily intake, or exposure profile, is quite difficult to determine in this case, since the products do not indicate which amount of the specific excipient was used. Furthermore, while acceptable daily intake has been established for adults (54, 63), this has not been assigned to neonates. It is difficult to extrapolate data to neonates because of differences in their metabolism and changing physiology. Yakkundi *et al*. (69) showed the presence of parabens in the blood circulation of 196 neonates from the UK and Estonia and confirmed a systemic exposure to

these compounds following administration of routine medicines: this is of concern, being the exposure at high doses associated to hyperbilirubinemia and oestrogenic effects (70).

Hence, the best solution for avoiding systemic exposure to any preservative, not just parabens, is the use of single-dose containers (64). On the other hand, to avoid "harmful" parabens, the cosmetic industry uses alternatives. How can we be sure they are better and safer, compared to thoroughly studied parabens?

Methylisothiazolinone (MI), as a paraben alternative, is used due to a strong biocidal effect. MI has been associated with high cytotoxic effects *in vitro* and is characterized as an allergen causing hypersensitivity (71). However, SCCS concluded that the concentration of MI of 15 ppm is safe and restricted its use in rinse-off products, whereas in leave-on products use of MI is prohibited (72). Another commonly used paraben alternative is triclosan (TCS). In some studies, topically applied TCS caused only mild irritation, whereas the others were reported for hepatotoxicity and liver damage, but such adverse effects have not been reported in humans (73). Just from the two previous examples, it is obvious that using parabens alternatives does not immediately imply safety and a better choice.

Additionally, the SUBSPORTplus Portal as a result of the SUBSPORT multicentric project financed partially by EU and with the participation of European Chemicals Agency (ECHA), national EU agencies concerning food, health and environment, partners from industry and independent non-profit organisations that advocate for substitution of toxic chemicals to safer alternatives, published a document, Specific Substances Alternatives Assessment – Parabens, that is mainly concentrated on the cosmetic use of parabens (74). This document gave a summarized profile of safety and toxicity data of possible paraben alternatives as well as further assessed data (pros and cons for different aspects: health, environmental, performance and cost) for chemicals (phenoxyethanol, sorbic acid, benzoic acid) that passed the set criteria.

A variety of approaches have been proposed to find alternatives for parabens, including, but not limited, to citrus extracts such as ascorbic acid, benzyl alcohol, synergistic blends of multifunctional natural ingredients including botanical extracts, honeysuckle extract, spice extracts, fragrances, the replacement of the aqueous component of creams with *Aloe vera* and single-use packaging system for products. Pure essential oils, such as rosemary, lavender, clove, and plant extracts such as *Calendula*, exhibit variable microbiostatic and microbicidal activities (38, 64, 74). Herbal preservatives and essential oils are generally considered as safe even for children, but regarding the toxicity of some oils, Eisenhut (75) pointed out that essential oils cannot be recommended for use in food preservation because like any artificial food additive, every component would need to be tested rigorously for toxicity before its contact with food for human consumption could be permitted. The same stands for cosmetics and recently the European Directorate for the Quality of Medicines and HealthCare of the Council of Europe published *Guidance on Essential Oils in cosmetic products* dealing with toxicity and safety of essential oils (76).

Overall conclusion regarding paraben alternatives is in line with a recent paper by Fransway *et al.* (38) which pointed out that having to remove parabens from consumer products could result in their substitution with alternatives that are less proven and possibly unsafe. The fact is that compiled data of paraben dermal toxicity have not shown their significant toxicity, thus eventual withdrawal from consumer products is not based on scientific knowledge.

CONCLUSIONS

Parabens are widely used and efficient preservatives. Their toxic effects, endocrine disruption, carcinogenicity or genotoxicity was never confirmed in humans. Furthermore, their affinity for estrogen receptors is from 2.5 million- to 10,000- times less compared to estradiol.

Although theoretical concerns regarding paraben activity, xenoestrogenicity and toxicity are supported by a body of *in vitro* and animal *in vivo* evidence, the actual impact, if any, to human health is far from clear. Also, findings of parabens in the human tissue and fluids showed no conclusive evidence of paraben-related toxicity, nor undoubtedly causal relationship was established. Careless removal of parabens from consumer products with alternative substitutes which are less investigated, possibly toxic or unsafe, can lead to serious adverse effects and human health risks.

As scientific evidence of paraben toxicity and endocrine disruption in humans is still missing, parabens remain to be considered safe. But, regarding safety as well as the toxicity of parabens, precautions should be considered because some doubts exist and still lack complete scientific knowledge. However, more research is needed to overcome scientific gaps and controversy about them in order to provide more accurate answers to unsolved questions, even when just potential concerns may arise.

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