EUROPEAN COMMISSION

Directorate-General for
Health & Consumers

Scientific Committee on Consumer Safety

SCCS

Clarification on Opinion SCCS/1348/10

in the light of the Danish clause of safeguard banning the use of parabens in cosmetic products intended for children under three years of age

Scientific Committees

- on consumer safety
- on emerging and newly identified health risks
- on health and environmental risks

The SCCS adopted this opinion by written procedure on

10 October 2011

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In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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1. BACKGROUND

On 21 March 2011, the Commission received the notification of a decision taken by the Minister of the Environment of Denmark to ban propyl and butyl paraben, their isoforms and their salts in cosmetic products for children up to three years of age, in light of article 12 of the Cosmetics Directive[1]. The ban entered into force on 15 March 2011.

According to article 12 (2), the Commission "*shall as soon as possible consult the Member States concerned, following which it shall deliver its opinion without delay and take the appropriate steps*".

The Commission's services have already written to the Member States in order to inform them and ask for any further information they may have and will discuss the issue at the next Working Party on Cosmetic Products in June 2011. However, in order to deliver an opinion and take appropriate steps, which may include amending the annexes to the Cosmetics Directive, the Commission's services would like to request the assistance of the SCCS.

Denmark's decision makes reference to the scientific data presented in the SCCS opinion published for public consultation in December 2010 and finally adopted on 22 March 2011[2]. While fully agreeing with the contents, the approach, argumentation and the conclusions of the SCCS, the Danish authorities take a precautionary approach for children under three years of age relying heavily on the inherent endocrine properties of these parabens which have shown experimentally *in vitro* and the lack of high-quality *in vivo* data. In its opinion, the SCCS concluded there were no reasons for concern as it took a risk assessment approach based on the inherent properties of parabens and the anticipated consumer exposure levels from the use of parabens in cosmetics. Furthermore, the opinion, however, did not highlight specific concerns for the health of children or other population subgroups. More details about the justification of this decision are to be found in the documents attached.

On the basis of the SCCS opinion the Commission's services were already preparing a proposal reflecting the conclusion of the SCCS opinion on parabens before being informed of Denmark's clause of safeguard. The measure under consideration included a reduction to 0.19% of the allowed maximum concentration of propyl- and butylparabens, used individually or combined, and a ban of five parabens, for which there was insufficient information to conduct a safety assessment (isopropyl -, isobutyl -, pentyl -, phenyl - and benzyl esters of 4-hydroxybenzoic acid and their salts).

Denmark's decision to ban parabens in products intended for children under three years of age opened the question of whether the same measure should be taken at EU level or not. In order to propose an appropriate risk management measure, the Commission's service would like to request the position of the SCCS on the scientific justification for the Danish measure.

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[1]   Council Directive of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (76/768/EEC), OJ L 262, 27.9.1976, p. 169.
[2]   SCCS/1348/10 Revision 22 March 2011.

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2. TERMS of REFERENCE

1. Can the SCCS confirm that its opinion of 22 March 2011 (SCCS/1348/11) on parabens addresses all safety concerns (including potential for endocrine disruption) which may be associated with the use of propyl and butyl parabens in cosmetics at levels of 0.19% for all consumers including children under the age of three and other potentially sensitive consumer sub populations (e.g. pregnant women)?

2. If this should not be the case, does the SCCS consider that parabens (and in particular butyl- and propylparaben) present a risk when used in products intended for children under three (or other specifically vulnerable groups)?

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3. Opinion

3.1. INTRODUCTION

This document responds to the scientific rationale given by the Danish authorities for the ban of propyl- and butyl parabens in products intended for use in children under three years of age. The concern of the Danish authorities relates to potentially increased susceptibility and exposure of children to certain endocrine disrupters such as propyl- and butylparaben compared to adults. In the scientific argumentation on possible effects of endocrine disrupters in children, general reasons with regards to young children have been stated, which can be summarized as follows:

• Different absorption and distribution factors due to the immaturity of physiological functions of young children may cause ineffective inactivation and elimination kinetics and thus higher internal exposure to the same external dose of certain chemicals in young children compared to adults (Makri et al. 2004; Renwick et al. 2000; Schwenk et al. 2003).

• In addition, infants have a higher body surface area to body mass ratio compared to older children and adults (US EPA 2009), which may be a cause of higher exposure per kg body weight to dermal applied compounds (Makri et al. 2004).

• Potentially enhanced target organ sensitivity in the young organism and effects induced in childhood may result in increased severity compared to adult effects, as impaired development of an organ may be irreversible and therefore more severe (Renwick et al. 2000).

Apart from the above general reasons, the specific reasons raising concern with regards to parabens by the Danish authorities can be summarized as follows:

• Parabens affect reproductive or endocrine endpoints in both male and female immature rats and mice, and both boys and girls at exposure may be at risk of endocrine disruption. Furthermore, the estrogenicity of parabens and their metabolites in vivo has not been fully determined and will also need to be compared to the possible risk of exposure to other sources of estrogens (Boberg et al. 2009 and 2010).

• For parabens (including butyl- and propylparaben), no adequate reproductive and developmental studies are available, and the possible effects of parabens may be of an irreversible nature. Therefore, according to Renwick et al. 2000, an additional uncertainty factor for children might be necessary.

• In addition to a high body surface area to body mass ratio of young children, during the summertime, children in the age up to 3 years spend many hours outside and therefore are exposed to a high amount of sunscreen products likely to contain propyl- and butylparaben (Danish EPA 2009).

3.1.1.   SCCS opinion SCCS/1348/10 on parabens

In its Opinion SCCS/1348/10, the SCCS reiterated its previous conclusion that the continued use of methylparaben and ethylparaben as preservatives in cosmetics at the maximum authorized concentrations (0.4% for one ester or 0.8% when used in combination) is considered safe for human health.

Concerns were expressed with respect to the potential endocrine modifying effects of propylparaben, butylparaben and their related iso compounds and benzylparaben, as these properties appeared to increase with increasing chain length. For the frequently used compounds, propylparaben and butylparaben, considered as having a weak endocrine modifying potential, the deduction of an adequate NO(A)EL value was hampered by

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considerable shortcomings of the reproductive toxicity studies carried out in rodents. In rats it was found that longer chain parabens are metabolized in a fast and complete way into p-hydroxybenzoic acid (PHBA) which is considered to be an inactive metabolite (rationale is given in the document). In humans, on the other hand it is possible that parent (unmetabolized) parabens become systemically available, even if in limited amounts. As properly conducted dermal absorption and/or toxicokinetic studies in humans were lacking, a quantitative risk assessment was carried out incorporating several layers of conservatism. The risk assessment was done for the most lipophilic compound butylparaben using the very low NOEL value of 2 mg/kg bw/day derived from a study where juvenile rats were exposed after subcutaneous administration of 2 mg butylparaben/kg/day for 17 days (postnatal days 2-18), a high dermal absorption value of 3.7% and a cumulative human exposure value of 17.4 g/day to cosmetic products containing lipophilic parabens. As a consequence, the use of propylparaben and butylparaben as preservatives in cosmetic products was considered as safe to the consumer as long as the sum of their individual concentrations does not exceed 0.19%. This conclusion was drawn in a conservative way due to the lack of scientifically sound data on the pivotal link between dermal absorption in rats and humans, in particular in relation to the metabolism of the parent compound in the skin. The latter can only be addressed through additional human data. As no or only limited information was available for their safety evaluation, human risk could not be evaluated for isopropyl-, isobutyl-, phenyl-, benzyl-and pentylparaben.

3.1.2.   Relevant age groups to be considered

"Children" are developing organisms at various stages of immaturity and maturation up to nearly two decades with age-dependent different susceptibilities and sensitivities[3] compared to adults. In the Danish document no clear definition of the term "*children"* has been given and there is no universally agreed age range for what constitutes childhood. Article 1 of the United Nations Convention on the Rights of the Child defines "children" as persons up to the age of 18. However, in many reports of the United Nations (UN) and the World Health Organization (WHO), the term "children" refers to persons up to the age of 14 years (e.g. UN 2010, WHO 2010). The term "infant" often refers to children between the ages of 1 month and 12 months (Berk 2009, WHO 2010b); however, other definitions vary between birth and 3 years of age. The term "toddler" refers to children who are learning to walk, so it is typically used for children aged 1 to 2 years (Berk 2009), but sometimes also up to 3 years.

Essential functional changes occur in the period between the first week and the first few months after birth (Makri et al. 2004; Lemper et al. 2009; Scheuplein et al. 2002). It seems therefore necessary to use a discriminating terminology for this period of childhood. From a survey of the literature, it appears that a variety of age-related terms are commonly used. The SCCS will use the following terminology in the further discussion[4]:

- Full-term neonate (<1 week)

- Newborn 1 wk–2 months

- Early infant 2–6 months

- Crawlers/toddler (6 months–2 years)

- Preadolescent (2–12 years)

- Adolescent (12–18 years)

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[3]   According to Makri et al. (2004), "*susceptibility* is defined as a capacity characterized by biological (intrinsic) factors that can modify the effect of a specific exposure, leading to higher health risk at a given exposure level. The term *sensitivity* is used to describe the capacity for higher risk due to the combined effect of susceptibility (biological factors) and differences in exposure."

[4]   Note that premature babies are not considered in this opinion

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- Adult

This terminology reflects the normal changes in development of the child, in particular the skin maturation and dietary changes. Up to the age of 2 years, the occluded nappy area is frequently exposed to topically apply cosmetic products. Episodic acutely inflamed skin, nappy dermatitis, may occur particularly when the diet switches from solely milk, breast or formula, to the introduction of solid food; reports of potential effects of teething are not consistent. This could increase skin absorption from this area. Nappy dermatitis is treated with topical pharmaceutical creams or ointments in addition to cosmetics. After the first months, nappy dermatitis is less common. The SCCS considers the suggested terminology reflects more accurately the rapid physiological changes between neonates and newborns, early infants, crawlers/toddlers and other "children" up to 3 years.

It is difficult to follow the rationale of the Danish authorities to include in the ban of parabens all age groups of children up to three years without further differentiation, as no reasons for choosing this age range are given. It might be in analogy to a previous ban on phthalate esters in children's toys and childcare articles, where the threshold of 3 years was chosen because of the specific behavioural habits (hand-mouth contacts, sucking and chewing on toys etc.) in this particular age group, which would result in high exposure to the phthalate esters. These behavioural habits, however, are not applicable in the case of parabens in cosmetics, and hence choosing the age group of 3 years for parabens appears quite arbitrary from a scientific point of view.

The SCCS recognises the Danish argument that high exposure to sunscreens for the general age group of children up to 3 years can occur as a result of repeated use. However, children of this age group should not be exposed to direct sunlight, and if exposed, should be covered by appropriate clothing[5]. Sunscreens then need only to be applied on those areas that are exposed to sun and that cannot be protected by clothing. The SCCS considers the scenario of over-exposure to sunscreens as the result of product misuse and hence not applicable to risk assessment which considers normal uses of a product.

3.2.   GENERAL CONSIDERATIONS

3.2.1.   General susceptibilities/sensitivities of children – need for an extra safety factor?

Several reviews have dealt with the potential physiological differences between children of different age groups, and between children and adults. Moreover the various methodological difficulties in determining toxic effects and assessing risks in a continuum of developmental stages, functions and susceptibilities as well as sensitivities (e.g., enhanced exposures due to age-specific behavioural habits) have been considered. In these articles, in the light of the available data, the necessity of an additional safety factor for children or subgroups of children (e.g., SF 10) beyond the usual factor of 100, covering intra-and interspecies differences, was extensively discussed (Makri et al. 2004; Renwick et al. 1998 and 2000). Likewise, authorities have developed guidance how to deal with deficiencies or uncertainties in the database of chemical substances or with genotoxic and carcinogenic exposures in childhood (US-EPA 2005). For instance the REACH guidance document on information requirements (ECHA 2010) recommends that:

"A higher intraspecies assessment factor for children (US-EPA 1996, recommends from 10 up to 100 when assessing pesticides in relation to food safety) should be considered when the following two criteria are both fulfilled:

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[5] http://ec.europa.eu/health-eu/news/sun_uv_en.htm

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- There are indications, obtained from, for example, experiments in adult animals, epidemiological studies, *in vitro* experiments and/or SARs (structure activity relationships), of effects on organ systems and functions that are especially vulnerable under development and maturation in early life (in particular the nervous, reproductive, endocrine and immune systems and also the metabolic pathways), and

- There are deficiencies in the database on such effects in young animals."

Dermal exposure of the newborn and early infant: general differences and potential risk factors compared to adults

In general, a full-term baby possesses all skin structures of adult skin, and anatomically these structures do not undergo dramatic changes after birth. As outlined in **Annex 1**, the dermal absorption in newborn skin is similar to that observed in adult skin. For babies during their first weeks and months, however, a number of underlined typical differences and potential risk factors exist which are not present in the adult. These are:

(i) The surface area/body weight ratio is 2.3-fold higher in newborns than in adults, changing to 1.8- and 1.6-fold at 6 and 12 months, respectively. This ratio is covered by the intraspecies factor of 10 used in exposure-based risk assessment (in MoS)

(ii) Toxicokinetic parameters differ between various age groups of children and adults and can result in reduced clearance and/or longer half-life of bioavailable substances, thus increasing the potential risk for adverse reactions in babies. Depending on the specific substance under consideration, half-lives in premature and full-term newborns might be three-to nine times longer compared to adults (Renwick et al. 2000)

(iii) *In –use* conditions of topical products also play a role since baby skin care products are often applied to most of the body surface compared with selective sites in adults. This should be considered in exposure-based risk assessment of the finished product.

(iv) The nappy area: the nappy area and non-nappy regions are indistinguishable at birth but show differential behaviour over the first 14 days, with the nappy region having a higher pH and increased hydration. With respect to *skin hydration* in the nappy zone, newborns tend to have somewhat higher water content in the horny layer and a greater variation than newborns, infants and crawlers up to one year. The pH is stabilized at a slightly acidic range of 5-6, but is not much different from the adult. However, the buffering capacity is smaller in the newborn making baby skin more susceptible to pH changes, in particular in the case of rash and damaged skin. The latter may occur in particular during the first months by so-called nappy dermatitis, which consists of episodic acute skin inflammation (mean duration 2 to 3 days) caused by physical, chemical, enzymatic, and microbial factors in the nappy environment, for example it is seen with diet switches (breast feeding, bottle feeding, solid food).

According to the SCCS Notes of Guidance, with respect to points (i) - (iii) above, there is no need for a general additional uncertainty factor for children when intact skin is involved. There might be the need for an additional safety factor if substance-specific data clearly demonstrate that inter-individual variability would result in a value higher than 10.

Cosmetic products used in the nappy area

The special circumstances associated with the nappy area (resulting from the close confining clothes and nappies, uncontrolled urination and defecation and resulting problems with potential damage of the skin in the nappy zone) are outlined in **Annex 1**. Modern nappy technology has shown to provide increasingly good skin compatibility, leading to a decline in the frequency and severity of nappy dermatitis. However, irritant nappy

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dermatitis cannot be completely avoided and might enhance dermal absorption of substances.

Baby cosmetics can be subdivided in 2 groups: cleansing and protecting cosmetics:

Baby cleansing products consist of bath products, shampoos, soap bars and syndets (synthetic detergents), cleansing milk, baby wipes. Baby protecting cosmetics consist of face/body creams and body lotions, powder and sunscreens.

Protective creams for the nappy zone are preventive or protect against damage from urine, feces and their interactions. O/W creams are the first choice when no damage is present, but in cases of skin damage, mostly W/O creams or even water-free ointments on the basis of ZnO are used. As cosmetic products are meant to be used on intact skin medical consultation is necessary in the case of real skin damage and pharmaceutical products (and not cosmetics) should be used.

For the development of baby cosmetics, and the risk assessment of products intended to be used in the nappy area, the potential for irritation in this area which may lead to higher absorption needs to be considered by the manufacturers who are responsible for the final quantitative risk assessment of their products.

> 3.2.2. Sub-conclusions regarding general aspects of susceptibility/sensitivity of neonates/newborns and infants up to 6 months - need for an extra safety factor?

From the above, the following two main conclusions can be drawn:

• The skin structure of full-term neonates/newborns and early infants is similar to that of adult skin and the dermal absorption is comparable. However, distinction should be made between the skin of the nappy zone and the rest of the baby skin, since for this particular area risk factors exist which are not present for the rest of the body. Therefore, the nappy zone should be further considered, independent of the substance(s) under question.

• The SCCS is of the opinion that in general no additional safety factor needs to be included for ingredients used in children's cosmetics used on intact skin as an intra-species assessment factor of 10, covering the toxicokinetic (3.2) and toxicodynamic (3.2) differences between children and adults, is already included in the MoS calculated for individual ingredients (Notes of Guidance, 2010).

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3.3. SPECIFIC ISSUES REGARDING PARABENS

3.3.1. Endocrine modifying effects by parabens (and their metabolites)

The position of the Danish authorities has been based on a recent review of Boberg et al. (2010). The SCCS has considered the main arguments of the authors and has come to the following conclusions:

Possible effects on the developing organism

The toxicity of parabens, in particular butylparaben, has been investigated in previous and more recent studies, with exposure in utero, during lactation and in juvenile animals[6]. The lowest available critical effect level (NOAEL) chosen in the safety assessment (opinion SCCS/1348/10) was based on such studies.

The study chosen by SCCP/S is that of Fisher et al. (1999) with a NOEL for butylparaben of 2 mg/kg bw/day (no other doses studied) in <u>male</u> juvenile rats.

In this study, male rats received subcutaneous injections on postnatal day 2, 4, 6, 10 and 12 of either diethylstilbestrol (DES), ethinylestradiol (EE), bisphenol A, genistein, octylphenol or butylparaben. Numerous parameters were assessed during and after treatment (up to 75 days). The more potent estrogens (DES, EE) caused (dose-related) changes in testis weight, distension of the rete testis and efferent ducts, epithelial cell height in the efferent ducts and expression of aquaporin-1; minor effects were seen with the less potent estrogenic compounds. However, administration of butylparaben (2 mg/kg bw/day) had no detectable effects on any parameter on day 18.

In other studies in <u>female and male</u> rodents often (much) higher dose levels (several hundred mg/kg bw) were administered (e.g. Kang et al. 2002; Taxvig et al. 2008). This (and not the lack of any studies) makes it difficult to derive a NO(A)EL. Although a multigeneration OECD guideline study is missing, the main endpoints of reproductive toxicity are covered by the available studies.

The SCCS considers that the question of possibly increased susceptibility of children is sufficiently covered by the available data on reproductive toxicity. Potential remaining uncertainties have been addressed by introducing several layers of conservative assumptions in the assessment (summarized in the final conclusions).

Safety assessment based on endocrine activity

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[6]

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The approach taken by Boberg et al. (2010) to use values from uterotrophic assays for NOAEL/LOAELs derivation (for MOS calculations, section 8.3) is problematic for two reasons:

i. Only one study in immature mice is referred to (Lemini et al. 2003), but other data from similar uterotrophic assays by other groups (e.g. Shaw & deCatanzaro 2009) which indicate higher values are neglected.

ii. It is inappropriate to refer to these results as NOAEL/LOAEL, since the endpoint provides data on estrogenic activity/potency; but this cannot simply be "translated" to an adverse effect. Note that widely accepted definitions of an "endocrine disruptor" or a "potential endocrine disruptor" make a clear distinction.

In the review on possible endocrine disrupting activity of parabens the „estrogenic burden" of parabens was estimated based on estrogenic potency (*in vitro* and in uterotrophic assays) and human blood concentration of estradiol, parabens and PHBA (Boberg et al. 2009, Table 6; Boberg et al. 2010 Table 5). The comparison is worth discussing, however, it is weakened by the following facts: a) the data base on child estradiol levels is poor (see Bay et al. 2004), b) the human plasma levels of butylparaben were determined following application of a cream containing 2% butylparaben whereas only 0.4% is currently permitted and c) the assumption that 5 mg/kg/d of PHBA is effective is questionable (see below and **Annex 2**).

Exposure to endocrine active substances such as parabens should be assessed by comparison with exposure to other endocrine active compounds in the diet (Bolt et al. 2001). By use of estimated daily intakes and the relative potencies, a hygiene-based margin of safety may be derived for endocrine active compounds. Taking the phytoestrogen daidzein as reference and assuming a daily systemic intake of about 0.1 mg/k bw butylparaben the estrogenic burden of daidzein is about 20 times higher (CIR 2008).

Estrogenicity of the common metabolite PHBA

Concerning the assumed estrogenic activity of PHBA, the experimental results of PHBA showed no endocrine activity *in vitro* while the results *in vivo* are contradictory. PHBA was tested negative in most uterotrophic bioassays with mice and rats (Hossaini et al. 2000; Twomey et al. 2000; Lemini et al. 2003) with subcutaneous administration of doses up to 100 mg/kg bw/d and more; only one study (Lemini et al. 1997) reports a positive response at 5 mg/kg bw/d. This unusual finding for PHBA may be due to differences in rodent chow and experimental procedures (Shaw & de Catanzaro 2009; references and details in Annex 2). PHBA is the common metabolite of all parabens. The different parabens exhibit large differences in estrogenic activity *in vitro* (see Table in Annex 2) and *in vivo* and also in toxicity. When assuming endocrine activity also for the main metabolite PHBA, such differences are not plausible.

The weight of evidence supports the generally accepted view that the metabolite PHBA lacks estrogenic activity and does not contribute to endocrine activity of parabens.

Estrogenicity of paraben conjugates

According to Boberg et al. (2010), conjugated parabens are assumed to be rapidly excreted, but it has not been clarified whether these conjugated parabens may have any potential endocrine disrupting effects.

Regarding the potential effects of conjugated parabens, no experimental data is available. The observed structure-activity relationships of estrogen receptor activation for a series of parabens (e.g. Byford et al. 2002; Okubo et al. 2001; Gomez et al. 2005; van Meeuwen et al. 2008) and molecular modeling of their receptor binding (Byford et al. 2002) may serve as an argument against the view that paraben conjugates may be biologically active in terms of estrogenicity. Similarly, the conjugated metabolites of bisphenol A are devoid of

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binding to the nuclear estrogen receptor *in vitro* whereas this estrogenic activity has been demonstrated for the parent compound (Matthews et al. 2001; Shimizu et al. 2002). On the basis of the available scientific evidence, the SCCS concludes that an estrogenic activity by paraben conjugates is highly unlikely, particularly since the steroid conjugates themselves are inactive at the receptor.

Inhibition of sulfotransferases

Boberg et al. (2010) argue that inhibition of sulfotransferases in human skin and liver by parabens may contribute to the estrogenic effects of parabens.

The influence of parabens including butylparaben and PHBA on estrogen levels by inhibiting estrogen sulfotransferases (SULT) in skin was studied using skin and liver cytosol and human epidermal keratinocytes (Prusakiewicz et al. 2007). SULT activity (estradiol, estrone) was inhibited in skin cytosol by methyl-, ethyl-, propyl-, and butylparaben, but **not** by PHBA. Potency increased with chain length (IC_{50} butylparaben in skin: 37 µM = 7.2 µg/ml; IC_{50} propylparaben: about 0.2 mM = 36 µg/ml). With methyl- and ethylparaben, the inhibition in skin was weak, so that no IC_{50} value could be derived. No inhibition of androgen sulfation was detected. In human epidermal keratinocytes, butylparaben displayed an IC_{50} of 12 µM (2.3 µg/ml). No positive control was included.

Although exact concentrations of parabens in human skin cells after topical application are not known, it would seem scientifically plausible that the concentrations of free propyl- and butylparaben could cause a marked inhibition of estrogen SULT (if any) only in cells of the skin area of the topical application. Available data indicate that concentrations of free propyl– and butylparaben in human body fluids (serum, seminal fluid and urine) are on average 1-3 orders of magnitude lower, 95-percentiles at least 1 order of magnitude lower than IC_{50} values of the parabens (see **Annex 4**). As IC_{50} values in human liver were similar to those in human skin, a marked inhibition of systemic estrogen sulfotransferases by long-chain parabens is regarded as not likely.

3.3.2. Metabolism and toxicokinetics of parabens

The SCCS has re-assessed the role of metabolism of parabens, as there is increasing evidence that rat and humans markedly differ in this respect and that the rat appears to be a model of limited value when predicting the toxicokinetics of parabens in humans (reviewed by Boberg et al. 2009, 2010 and in the SCCS Opinion 2011).

While parabens are efficiently hydrolysed to PHBA in the skin (and possibly in the systemic circulation) of rats, free and predominantly conjugated parabens (glucuronides and sulfate esters) can be detected in human serum or urine after dermal application. The extent of hydrolysis to PHBA has not been quantified in these studies (Janjua et al. 2007 and 2008). From *in vitro* studies with human skin from adults, an uptake of about 3.7% free butylparaben has been derived (although the studies have some shortcomings; see the discussion in SCCS/1348/10). It is assumed that the parabens dermally taken up into the systemic circulation are further metabolized to PHBA and parabens conjugates in the liver and other organs of the human body before the remaining free parabens and their metabolites are excreted into the urine.

As the efficiency of the metabolic pathways determines the level of free parabens in the body, in the first postnatal months (neonates/newborns and infants) the immaturity of drug metabolising enzymes involved in the metabolism of parabens in humans (carboxylesterases, UDP-glucuronosyltransferases and sulfotransferases) may influence the level of unconjugated parabens circulating in the human body (for details see **Annex 3**).

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3.3.2.1. Role of esterases and hydrolysis of parabens

Human skin expresses carboxylesterases hCE1 and hCE2 at a much lower level when compared to liver. Other forms of carboxylesterases may also be expressed in humans, but are less well characterised. Both hCE1 and hCE2 are developmentally expressed in the human liver. If this developmental expression is also evident in skin, it can be assumed that expression of both hCE1 and hCE2 is lower in the skin of children when compared to adults. The difference is more pronounced for hCE1 which preferentially metabolises methylparaben and ethylparaben. For hCE2, which preferentially metabolises propyl-, butyl- and benzylparaben, the age difference is less pronounced.

For hepatic hCE1and hCE2, age differences were most pronounced between adults and children under the age of 1 year. No further differentiation between the first 12 months of life has been made in this study. Thus, if age dependency of carboxylesterases as observed in the liver holds also true for skin, ester cleavage of parabens can be assumed to be lower in the skin of children age <1 year when compared to adults.

3.3.2.2. Role of glucuronidation and sulfation of parabens

In neonates/newborns and early infants up to 6 months, glucuronidation activity is known to be reduced, whereas older children mostly have similar activities compared to adults (see Annex 3 for details).

It has been shown *in vitro* that several UDP-glucuronosyltransferase (UGT) isoenzymes are capable of glucuronidation of parabens in the liver of adult humans. Although glucuronidation of parabens in human skin appears possible, the contribution of glucuronidation to the inactivation of parabens in adults, neonates and infants remains to be elucidated. In addition, there is only little information available on the ontogeny and development of the UGT isoenzymes conjugating parabens in neonates, newborns and early infants below six months (**Annex 3**).

Of the sulfotransferase (SULT) isoenzymes accepting exogenous phenols as substrates, SULT1A1 is the only SULT enzyme form with proven (although low or moderate) catalytic activity towards one of the parabens, namely butylparaben, and is considered so far as the only established defence among the SULT isoenzymes against this member of the parabens in adults, neonates and infants. The role of sulfation of parabens in human skin and systemic circulation remains to be elucidated.

Overall, the existing data suggest that the glucuronidation of parabens may be reduced in neonates and infants at least up to six months of age. Of the sulfotransferases, only SULT1A1 has been shown to convert parabens to sulfate esters *in vitro* so far. Because of the patchy data in neonates and infants, it is questionable whether sulfate ester formation of parabens by SULT isoenzymes can counterbalance the reduced glucuronidation. Hence, neonates, newborns and early infants exposed to parabens might have higher internal exposures than adults and thus be potentially at higher risk (at comparable dermal/external exposure) due to reduced glucuronidation and prolonged half-lives of parabens circulating in the body.

Consistent with a reduced metabolic capacity in very young children, in spot urine samples of hospitalized preterm neonates/newborns, 3- to 5-fold higher proportions of free methylparaben or propylparaben (about 10-15% of the total parabens fraction, free and conjugated) were found compared to 2-5 % in adults. The preterm neonates/newborns in the study had an assumed gestational and postnatal age of less than 44 weeks and had an active (although probably immature) UGT1A1 because individuals with hyperbilirubinaemia had been excluded from the study (Calafat et al. 2009). Although the paraben conjugates were considered stable under controlled conditions of storage for several years, according to

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the authors the estimated urinary concentrations of the free parabens must be interpreted with caution.

Conclusions

The level of free parabens in the body is determined by the efficiency of the drug metabolising enzymes involved in the metabolism of parabens in humans (carboxylesterases, UDP-glucuronosyltransferases and sulfotransferases). The UDP-glucuronosyltransferase enzyme family is not fully developed until an age of 6 months and data suggest reduced carboxylesterase expression in children below 1 year. Therefore it cannot be excluded that the internal dose and the half-life of the unmetabolised parabens may be higher in children up to 6 months of age when compared to adults after topical application of cosmetics containing parabens.

Whether such enhanced internal exposures to parabens also imply enhanced risks to neonates/newborns and early infants remains uncertain and has yet to be determined. In any case, the missing data regarding parabens metabolism in adult humans, neonates/newborns and early infants require particular consideration in the risk assessments.

Compared to neonates/newborns or early infants, the unborn foetus will be better protected by the relatively efficient systemic parabens inactivation by the mother than the neonate/newborn or early infant exposed dermally to parabens.

The SCCS emphasizes that relevant human data regarding metabolism, required for reducing uncertainties and for a sound risk assessment of parabens, is missing so far. This data could be gained for instance by a human toxicokinetic study *in vivo* (e.g., by use of deuterated parabens) or by an approach combining *in vitro* data on the metabolism of parabens and toxicokinetic modelling, similar to the case of bisphenol A (Mielke and Gundert-Remy 2009; Mielke et al. 2011). For toxicokinetic modelling of parabens metabolism in humans of different age groups, relevant *in vitro* data regarding hydrolysis and phase II metabolism of parabens in human skin and liver would be needed.

3.3.3. Dermal absorption and exposure of parabens

Based on the exposure calculation made for adults in opinion SCCS/1348/10, an extrapolation can be made for children on the basis of the body surface area, assuming a concentration of 0.19% for butylparaben in the finished cosmetic product.

The cumulative exposure to preservatives used in all cosmetic product categories is considered to be 17.4 g/day on a surface of 1.75 m^2 for an adult. For a child of 3 months of age (5.3 kg and a surface area 0.31m^2)[7] the cumulative exposure would then result in 17.4 *0.31/1.75= 3.08 g/day.

Accordingly, the MOS would then be:

Dermal absorption: 3.7%

Intended concentration in finished product: 0.19%

Typical body weight: 5.3 kg

Cumulative exposure to preservatives: 3.08 g/day

NOEL (subcutaneous, rat, 17 days): 2.0 mg/kg bw/day

SED = 3080 mg/day * 0.19/100 * (3.7/100* 5.3) kg = 0.0408mg/kg bw/day

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[7] http://www.rivm.nl/bibliotheek/rapporten/320005005.pdf

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MoS = NOEL / SED = 49

However, it is not realistic to assume that children are exposed to all the cosmetic products that adults use. Therefore, this exposure calculation needs to be refined, using appropriate exposure information (data on amounts applied and use frequency) for children. Unfortunately, reliable information is not available.

COLIPA[8] was requested to provide exposure data for children existing in the cosmetics industry, but reported that data for children on use frequencies and amounts are currently not available. However, COLIPA suggested correcting the use data for adults for body weight of children.

One set of data was provided by the French Authorities which had been received from representatives of the cosmetic industry. The SCCS has no further information on how this data was generated.

According to these data, the following quantities of products are used daily for children:

- for leave-on products:

0.063 g/d for body care leave-on products,

1.34 g/d for leave-on products for nappy area,

0.55 g/d for wipes for nappy area

- for rinse-off products:

1 g/d for rinse-off products for body care

2.4 g/d for rinse-off products for nappy area,

This results in the following exposure, considering a child 3 month of age (5.3 kg bw):

Leave-on products			
	Body care products	**Products for buttock area**	
		Cream and other products	**Wipes**
Dermal absorption	3.7%	>3.7%	>3.7%
concentration	0.19%	0.19%	0.19%
Daily amount	0.063 g	1.34 g	0.55 g
Body weight	5.3 kg	5.3 kg	5.3 kg
SED (mg/kg/day)	0.000836	0.0177	0.0076
NOEL=2 (mg/kg/day)			
MOS	2393	<112	<275

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[8] The European Cosmetics Association

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Leave on body care products:

The MOS calculated for the body care products is considered acceptable. However, there is uncertainty with regard to the exposure data. The daily amount for body care products used by children was reported to be 0.063 g (according to the representatives from the French cosmetic industry) but no justification for this value was given.

An alternative approach would be to correct the amount of body lotion used by adults for a body weight of a child as suggested by COLIPA. For body lotion the value of 123.20 mg/kg/day is given[9]; resulting in a daily applied amount of 123.2X5.3= 0.6 g, i.e. 10 fold higher than the value used in the present calculation using the French data. The amount of body lotion used on children can also be calculated by correction for body surface area. This would result in an amount of 8 g * 0.31 /1.75= 1.4 g per day and a MOS of 107. As stated before, it is not clear whether it is appropriate to extrapolate from adult use to children. The range of results obtained by the different approaches demonstrates the uncertainty in the exposure data and urges the need for children specific exposure information. A realistic exposure is expected to be inside this range and the MOS is considered sufficient despite the uncertainties with regard to the metabolic capacity of the skin of newborn and early infants, as the value for the dermal absorption and the NOEL are conservative.

Leave-on products used in the nappy area:

A specific calculation has been made for products used for the nappy area. For this area it is expected that, especially in the case of irritated skin (see specific section on cosmetics products used in the nappy area above (section 3.2.1) the dermal absorption might be higher than the 3.7% used in the calculation above. In combination with the uncertainty associated with the exposure data, the likely simultaneous use of wipes and cream on the nappy area, and the fact that for children under 6 months of age the metabolic system in the skin may be immature, the calculated MOS is not considered acceptable for this age group.

Rinse- off products		
	Body care products	Products for buttock area
Dermal absorption	3.7%	> 3.7%
concentration	0.19%	0.19%
Retention factor	0.01	0.01
Daily amount	1 g	2.4 g
Body weight	5.3 kg	5.3 kg
SED (mg/kg/day)	0.0001326	0.000318
NOEL=2 (mg/kg/day)		
MOS	15078	<6282

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[9]   SCCS Notes of Guidance, § 4-2, Tab 3,
       http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_004.pdf

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Rinse-off-products:

For rinse-off products, the MOS is considered sufficient both for body care products and for products for the nappy area.

Paraben levels in urine and plasma

Information on exposure to parabens can be derived from human biomonitoring studies.

Concentrations in human biological fluids (human biomonitoring) account for both dietary intake (e.g. from foods with paraben preservatives) and dermal application of products with parabens; according to Soni et al. (2005) the latter is considered to be the major contributor. Thus, such measurements are of interest as they provide information on the frequency and the magnitude of an overall exposure.

Urinary paraben concentrations were assessed in the U.S. general population (adults and children above age 6 years) by Ye et al. (2006) and by Calafat et al. (2009, 2010), in U.S men attending an infertility clinic (Meeker et al, 2011) and in young Danish men (Frederiksen et al. 2011) in addition to premature infants (Calafat et al., 2009)..

There are also data on serum levels in consumers, one from a small sample size in the U.S. (Ye et al. 2008), and two from larger sample sizes in Danish males (Frederiksen et al. 2011) and in Norwegian females (Sandanger et al. 2011).

The results of these studies (see Annex 4 for details and references) indicate that the (average) systemic exposure dose is considerably lower than estimated in the previous paraben opinion for adults who use all types of cosmetic products with parabens at the authorized concentrations.

Exposure estimates based on biological monitoring data are considered by SCCS as useful additional information in their overall evaluation on the safety of parabens.

4. CONCLUSIONS

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 was based on very conservative assumptions, both with regards to toxicity and exposure. The risk assessment in opinion SCCS/1348/10 was carried out for the most lipophilic compound, butylparaben, using the very low NOEL value of 2 mg/kg bw/day in juvenile rats, a high dermal absorption value of 3.7% and a cumulative human exposure value of 17.4 g/day to cosmetic products containing lipophilic parabens. This approach is confirmed to be very conservative by recent human biomonitoring data from Europe and the United States (for adults and children above 6 years) suggesting that systemic exposure doses are considerably lower than estimated in the paraben opinion.

In the case of children below the age of 6 months, and with respect to parabens present in leave-on cosmetic products designed for application on the nappy area, a risk cannot be excluded in the light of both the immature metabolism and the possibly damaged skin in this area. Based on a worst case assumption of exposure, safety concerns might be raised. Given the presently available data, it is not possible to perform a realistic quantitative risk assessment for children in the pertinent age group as information on internal exposure in children is lacking.

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Scientifically sound data on the pivotal link between dermal absorption in rats and humans, in particular with regard to the metabolism of the parent parabens in the skin and specific exposure information for cosmetic products used for children would allow a refinement of the above assessment.

With regard to pregnant women, the unborn foetus will be better protected than the neonate/newborn or early infant exposed dermally to parabens by the more efficient systemic parabens inactivation by the mother.

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ANNEX 1 - Dermal exposure of the newborn and early infant: differences and risk factors compared to adults immature skin: leading to enhanced absorption of chemicals?

In general, a full-term baby possesses all skin structures of adult skin, and anatomically these structures do not undergo dramatic changes after birth. The skin of the newborn could be considered as an "unripe" skin which progressively adapts during the first weeks and months of life. These adaptations lay at the origin of the physiological differences observed between baby and adult skin (1).

On the basis of the functional measurements of TEWL (trans-epidermal water loss is an indirect measurement of the barrier function) and dermal absorption studies, term infants seem to possess a fully developed stratum corneum with adult barrier properties. Other parameters such as skin thickness, skin pH, stratum corneum hydration also show that neonatal skin is adjusting very well to the extra uterine environment (2). Thus the dermal absorption in newborn skin is similar to that observed in adult skin. For babies during their first weeks and months, however, a number of typical risk factors exist (3-5) which are not present in the adult. These are:

(i) **The surface area/body weight ratio** is 2.3-fold higher in newborns than in adults, decreasing to 1.8- and 1.6-fold at 6 and 12 months, respectively (6).This ratio is taken up in the intraspecies factor of 10 used in exposure-based risk assessment (in MoS).

(ii) **Pharmacokinetic parameters** differ widely between babies and adults and result in reduced clearance and/or longer half-life of bioavailable substances, thus increasing the potential risk for adverse reactions in babies. Premature and full-term neonates newborns tend to show a three-to nine times longer half-life than adults. However, these differences do not necessarily apply and are strongly dependent on the substance in question. Moreover, once the neonatal period is over, often a greater elimination and higher clearance are observed compared with adults bringing back the normal equilibrium (6, 7). This neonatal period coincides with the lactation period (6-10).

(iii) *In –use* **conditions of topical products** also play a role since baby skin care products are often applied on relatively larger surfaces than usually is done in adults. This factor is considered in exposure-based risk assessment.

(iv) **The nappy area**: the nappy area and non-nappy regions are indistinguishable at birth but show differential behaviour over the first 14 days, with the nappy region having a higher pH and increased hydration (11).

Cosmetic products used in the nappy area:

The nappy area shows a higher pH and increased hydration. Indeed, special circumstances arise because of the close confining clothes and nappies and the uncontrolled urination and defecation. The close-fitting nappy provides a warm nutritive environment for the proliferation of bacteria (12). Because of the interaction between the urine and the faeces, urease becomes activated and converts urea into ammonia, giving rise to alkaline skin pH. As a consequence, fecal enzymes such as lipases and proteases become activated and damage the skin in the nappy zone. Despite modern nappy technology, which has shown to provide increasingly good skin compatibility profile reducing the frequency and severity of nappy dermatitis (13, 14), irritant nappy dermatitis cannot be completely avoided, favouring dermal absorption of substances. A number of molecules are historically known to induce systemic toxicity in such a way, including hexachlorophene, dichlorophene, corticosteroids, boric acid, ethanol and others (4). These of course are forbidden or should be only used when medically indicated. In practice, when baby cosmetics are developed for use in the napkin area, the manufacturer often incorporates a 100% dermal absorption of

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the "actives" in the risk assessment carried out for the manufacturer, bringing in these particular cases a safe product on the market.

TEWL measurements show values for newborn skin of 6-8 g/m² (15), which are similar as for adults. This value, however, increases when skin damage occurs as can happen in the nappy zone when nappy dermatitis is present (16). It is also used to measure the capability of a nappy to keep the skin dry (17).

With respect to skin hydration in the nappy zone, newborns tend to present somewhat higher water contents in the horny layer and a greater variation than adults up to one year (18, 19).

The pH is stabilized at a slightly acidic range of 5-6, but again that is not much different from the adult. However, the buffering capacity is smaller in the newborn making baby skin more susceptible to pH changes, in particular in the case of rash and damaged skin.

Cosmetic products for babies

From the anatomical/physiological differences between baby skin and adult skin, it can be learned that frequent contact with xenobiotics should be avoided since they could damage the barrier function and change skin pH, which may be at the basis of dermal absorption, an increased TEWL and the onset of infections (15, 16). Therefore, exposure-based risk assessment for baby products in a so-called Technical Information File (TIF) is key to bringing safe baby cosmetics to the market. This is the responsibility of the manufacturer, first importer or marketer of the product under consideration (Dir 76/768/EEG).

Baby cosmetics can be subdivided in 2 groups: cleansing and protecting cosmetics. Baby cleansing products consist of bath products, shampoos, soap bars and syndets, cleansing milk, baby wipes. Baby protecting cosmetics consist of face/body creams and body lotions, powder and sunscreens. Protective creams for the nappy zone are preventive or protect against aggressions from urine, faeces and their interactions. O/W creams are the first use when no damage is present, but in case of starting skin damage mostly W/O creams or even water-free ointments are used on the basis of ZnO. As cosmetic products are meant to be used on intact skin, in the case of real skin damage, medical consultation is necessary and pharmaceutical products (and not cosmetics) should be used.

For the development of baby cosmetics, a number of criteria should be taken into consideration by the manufacturer such as using high quality raw materials, no use of irritant ingredients, no known sensitizers, limiting promotional additives, adjusting the pH to a skin friendly value, adding anti-oxidants whenever necessary and preservatives in well-determined correct amounts, etc. (1)…...The manufacturer is responsible for the final quantitative risk assessment that brings the cosmetic finished product safely on the EU market.

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ANNEX 2 - Estrogenicity of p-hydroxybenzoic acid (PHBA), the common metabolite of parabens

There is a proposal to ban the use of butylparaben and propylparaben in the EU for the use for children less than three years of age. In the argumentation for increased sensitivity of children to certain endocrine disrupters compared to adults it was argued that the estrogenicity of parabens and their metabolites *in vivo* is not fully determined, and that the common metabolite of all parabens, p-hydroxybenzoic acid (PHBA), contributes considerably to an endocrine activity ("estrogenic equivalency"). This statement was based on reviews of the Danish Environmental Protection Agency (Boberg et al. 2009 and 2010).

In all *in vitro* tests investigated (yeast screen, MCF-7 cells, estradiol binding in uterine cytosol, sulfotransferase inhibition in skin cytosol) PHBA reacted negative. Endocrine activity *in vivo* was negative in fish. Uterotrophic assays were performed in ovariectomized and immature mice and immature rats after oral and s.c. administration. Two publications (Hossaini et al. 2000 and Twomey 2000) reported no activity in both species with both routes. One group (Lemini et al. 1997, 2003) reported negative effects in rats but positive ones in mice. According to these authors the lowest effective dose was 5 mg/kg/d (1997) or 150 mg/kg/d (2003) using s.c. administration whereas 50 mg/kg/d were negative (2003) which is considered non-consistent. Shaw & deCatanzaro (2009) discuss as possible reasons for the discrepant findings differences in phytoestrogen content of rodent diets and in experimental procedures (vaginal smearing).

Conclusion

The experimental results of PHBA *in vitro* showed no endocrine activity while the results *in vivo* are contradictory. PHBA is the common metabolite of all parabens. The different parabens exhibit big differences in endocrine activity *in vitro* (see Table **A2-1**) and *in vivo* and also in toxicity. When assuming endocrine activity also for the main metabolite PHBA, such differences are not plausible. The weight of evidence supports the generally accepted view that the metabolite PHBA lacks estrogenic activity and does not contribute to endocrine activity of parabens.

Table A2-1: Summary of *in vitro* potency data of parabens in MCF-7 cells compared to estrogen (molar ratio); from *Golden et al. 2005*

Studies	Detection of competitive ligand binding to estrogen receptor	Regulation of CAT gene expression in transfected MCF-7 cells [1]	Proliferation
Byford et al. 2002	Estrogen (1) MePB (1,000,000)	Estrogen (1) MePB (10,000)	Estrogen (1) MePB (1,000,000)
Darbre et al. 2002, 2003	EtPB (1,000,000) PrPB (100,000)	EtPB (10,000) PrPB (10,000)	EtPB(1,000,000) PrPB (100,000)
Okubo et al. 2001	BuPB (100,000) i-BuPB (100,000) Benzyl (1000)	BuPB (1000) i-BuPB (1000) Benzyl (1000)	BuPB (100,000) i-BiPB (100,000) Benzyl (100,000)

[1] Chloramphenicol acetyl transferase gene expression after 7 d

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Studies on endocrine activity *in vitro* of PHBA

Routledge et al. 1998

The yeast estrogen screen assay with the parabens MePB, EtPB, PrPB and BuPB as well as PHBA was used. All parabens were tested positive, BuPB was 1/10.000 less effective than estradiol. In contrast, PHBA was negative.

Byford et al. 2002

MePB, EtPB, PrPB, BuPB and PHBA were investigated in MCF-7 cells (human-breast cancer derived cell line) and measured a) competitive inhibition of estradiol receptor binding, b) CAT gene expression and c) cell proliferation. The results were as follows:

a) molar ratio to estradiol PrPB and BuPB 1/100.000, MePB 1/1.000.000

b) MePB and EtPB 1/10.000; PrPB and BuPB 1/1.000

c) MePB 1/1.000.000; EtPB, PrPB and BuPB 1/100.000

PHBA was tested negative.

Lemini et al. 2003

A competitive estradiol receptor binding assay was used with cytosol from uteri of immature rats. All parabens investigated (MePB, EtPB, PrPB and BP) were able to displace estradiol, except MePB and PHBA, the relative binding activities were about 1/100,000 compared to estradiol.

Pugazhendhi et al. 2005

Using the same techniques as Byford et al. (2002), the study compared the estrogenicity of MePB and PHBA in MCF-7 cells (human-breast cancer derived cell line) by measuring a) competitive inhibition of estradiol receptor binding, b) CAT gene expression and c) cell proliferation. Despite a similarity in oestrogen receptor binding between both compounds, the activity of PHBA in whole cells was clearly lower than that of MePB for all endpoints up to concentrations of 5×10^{-4} M. The authors interpret the findings as indicative of estrogenic activity of PHBA in these assays.

Gomez et al. 2005

This study investigated the activity of various parabens and PHBA in HeLa cell derived reporter cell lines expressing ERalpha or ERbeta and an ER negative cell line to account for non-specific binding: Estrogenic activity of parabens was ranked as BuPb > PrPb > EtPb, and similar for ERalpha and ERbeta. MePB and PHBA did not activate estrogenic responses up to 10^{-5} M. With the other parabens the magnitude of an estrogenic response increased with the alkyl group size, and at 10^{-6} M the ranking was EtPb < PrPb < BuPb.

Prusakiewicz et al. 2007

The influence of Parabens (MePB, EtPB, PrPB, BuPB) and PHBA on estrogen levels by inhibiting estrogen sulfotransferases (SULT) in skin was studied using skin and liver

cytosol and **human** epidermal keratinocytes. SULT activity (estradiol, estrone) was inhibited in skin cytosol by MePB, EtPB, PrPB, BuPB, **not** by PHBA. Potency increased with chain length (IC50 BuPB = 37 µM). No inhibition of androgen sulfation was detected. In human epidermal keratinocytes, BuPB displayed an IC50 of 12 µM. No positive control was included.

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Studies on endocrine activity *in vivo* of PHBA

Hossaini et al. 2000

Uterotrophic assays were performed in immature mice (B6D2F1 strain) and rats (Wistar strain). In **mice** MePB, EtPB, PrPB and BuPB as well as a mixture of MePB+EtPB+PrPB either at 100 mg/kg/d were administered s.c., PHBA doses were 5 and 100 mg/kg/d. In addition, oral doses of MePB 1 - 1000 mg/kg/d, PrPB 1 - 100 mg/kg/d and a mixture MePB+EtPB+PrPB 100 mg/kg/d were studied. No uterotrophic effect was reported for any of the parabens alone or in combination, either by oral or subcutaneous injection at levels up to 100 mg/kg/d. PHBA at 5 and 100 mg/kg/d sc reacted negative.

In **rats** BuPB was administered s.c. at 100, 400, and 600 mg/kg/d, PHBA at 5 mg/kg/d. An increase in wet and dry uterine weight at 600 mg/kg/d BuPB was observed. PHBA at 5 mg/kg sc reacted negative.

Twomey 2000 as cited in CIR 2008

Alpk:AP *f* CD-1 immature female mice (20-21 days of age) were used in an uterotrophic assay. PHBA single sc doses were injected at dose levels 0.5, 5.0, 50.0, and 100.0 mg/kg/d for three consecutive days, 10 animals/ group. As vehicle control arachis oil was given, as positive control group diethylstilbesterol at 0.01 mg/kg/d was used. Blotted uterus weights in animals administered diethylstilbesterol were significantly increased compared to controls. Uterus weights in animals administered PHBA were significantly decreased compared to controls, although no dose-response was reported.

Lemini et al. 1997

PHBA was investigated in an uterotrophic assay with both immature and ovariectomized CDI mice, positive control was estradiol. SC administration of 0.05, 0.5 and 5 mg/kg/d PHBA.

PHBA reacted positive at 5 mg/kg/d (both in ovariectomized and immature mice), the relative potency to estradiol was 0.0011 and 0.0018.

Lemini et al. 2003

PHBA was investigated in an uterotrophic assay with both immature and ovariectomized CDI mice and immature Wistar rats using SC dosages of 50 and 150 mg/kg/d. PHBA reacted positive in immature mice at 150 mg/kg/d and negative in immature rats.

Shaw and deCatanzaro 2009

The authors conducted an *in vivo* study with subcutaneous administration of butylparaben in early pregnancy and uterotrophic assays in two mouse strains (CF-1 and CD-1). The results indicate that the estrogen-sensitive period of implantation is not vulnerable to butylparaben exposure (up to 35 mg/kg/d), and that the *in vivo* estrogenicity may not be as potent as previously reported.

Studies on endocrine activity in fish of PHBA

Pedersen et al. 2000

Induction of yolk precursor protein vitellogenin in trouts was used to test EtPB, PrPB, BuPB and PHBA for oestrogenicity. All tested parabens were oestrogenic in doses 100 – 300 mg/kg while PHBA showed no activity.

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ANNEX 3 - Metabolism of parabens in humans after dermal exposure

Introduction

Parabens topically applied to the human skin are absorbed, partly/predominantly metabolized in the skin and during systemic circulation (mainly liver) and rapidly excreted into the urine predominantly as p-hydroxybenzoic acid (PHBA) and probably to a relevant part as glucuronides and sulfate esters. Some other minor conjugate metabolites as well as minor amounts of the parent parabens are also excreted into the urine. In addition, PHBA conjugates with glycine (p-hydroxyhippuric acid), glucuronide, and sulfate ester were formed after oral applications in humans, rats and rabbits (Andersen 2008) at mid and high doses. The PHBA conjugates were also formed in the rat after i.v. or duodenal application of 2 mg/kg b.w. ethylparaben (Kiwada et al. 1979 and 1980). Whether PHBA conjugates are also formed during low-dose dermal exposures in humans has yet to be determined. Overwhelming evidence indicates that the common metabolite of parabens, p-hydroxybenzoic acid, has no endocrine modulating activity. This is also assumed for the glucuronides and sulfate esters of parabens and the minor conjugates of PHBA. The interplay between the three main metabolic inactivation pathways (ester hydrolysis, glucuronidation and sulfation of the parent parabens), determines the level of free parabens in the body (see the metabolic scheme, **Fig. A3-1**). It is expected that the level of systemic exposure to free parabens determines the endocrine modulating activity of these compounds. Insofar, the main inactivating metabolic pathways may play a critical role in the availability of free parabens in the body of adults, neonates/newborns and infants.

Toxicokinetic animal studies, biomonitoring studies in humans, and investigations *in vitro* indicate that the metabolism of parabens differs between rats and humans (reviewed by Boberg et al. 2010 and in the SCCS Opinion 2011). In rats, after dermal exposure, parabens are efficiently hydrolysed to p-hydroxybenzoic acid in skin (and possibly in the systemic circulation) and no parent parabens (free or conjugated) were detected in serum or urine. In contrast, in humans, studies with dermal application of parabens revealed parabens in free and predominantly conjugated form (as glucuronides and sulfate esters) in serum or urine whereas the proportion of hydrolysis to p-hydroxybenzoic acid has not been determined in these studies and thus remains unclear. Concerning specifically butylparaben, absorption studies using rat skin *in vitro* showed a rapid hydrolysis of butylparaben by esterases, which was apparently more efficient than in human skin. Although the studies with human skin displayed a number of shortcomings they appeared to show a significant dermal absorption of parent butylparaben (Janjua et al. 2007 and 2008). These observations are supported by other studies in vitro showing that parabens are hydrolysed in human skin by up to three orders of magnitude slower than in rat skin (Harville et al. 2007).

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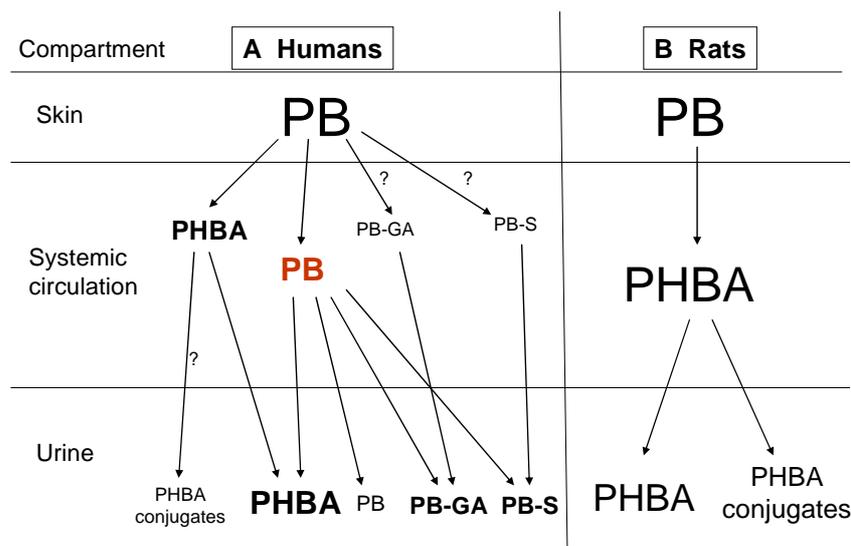**Main metabolic pathways of dermally applied parabens (PB) in humans and rats**

Fig. A3-1: Paraben metabolism in human and rat
PB, paraben; PHBA, p-hydroxybenzoic acid, PB-GA, paraben glucuronide; PB-S, paraben sulphate ester. PHBA conjugates in the rat: PHBA glycine, PHBA glucuronide, PHBA sulphate ester (amounts formed in decreasing order).

Humans in their early life may be considered as susceptible groups to endocrine modulating substances such as parabens (although their estrogenic activity is very low compared to endogenous estrogens). There is already some evidence that the metabolism of exogenous substances may be immature in neonates, newborns and early infants.[10] Therefore, the role of the main metabolizing enzymes involved in the inactivation of parabens in neonates, newborns and early infants is reviewed in order to determine whether and to which extent differences in paraben inactivation between adults and children of different age groups might be quantified or whether there are uncertainties and gaps of knowledge that hamper a sound risk assessment.

Carboxylesterases in human skin

There are five carboxylesterase genes listed in the human genome organization database, from which several variants may result, respectively. Their protein products have partially been characterized (Sanghani et al. 2009).

Lobemeier et al. (1996) identified three carboxylesterases of B-type in human skin, which were capable of hydrolysing parabens, and characterized their substrate specificities regarding parabens. Paraben esterase I is located in subcutaneous fat tissue and appears to correspond to the most prominent unspecific carboxylesterase in subcutaneous fat. It prefers methylparaben as substrate and its activity decreases with increasing chain length of the alcohol moiety. Paraben esterase II is also present in subcutaneous fat tissue and prefers butylparaben over methylparaben. Paraben esterase III was found in transformed keratinocytes (HaCaT cells) and also prefers butylparaben as substrate. Its activity decreases with decreasing chain length of the alcohol moiety. Another paraben esterase IV considered as an impurity in skin homogenates is probably an enzyme in human blood and was not further characterized.

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[10] For definitions see response to DK

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By using human skin from three individual female donors (age 28, 35 and 37 years), Jewell et al. (2007) demonstrated the presence of human carboxylesterase 1 (hCE1) and human carboxylesterase 2 (hCE2) in human skin by investigating hydrolysis of different parabens (methylparaben, ethylparaben, propylparaben, butylparaben and benzylparaben) in skin microsomes, skin cytosol and during skin penetration.

The authors confirmed earlier findings revealing that hCE1 preferentially hydrolyses substrates with small alcohol groups whereas hCE2 preferentially hydrolyses lipophilic substrates with large alcohol and small acyl groups. Thus, methylparaben was preferentially hydrolysed by hCE1 and butylparaben was preferentially hydrolysed by hCE2.

The involvement of hCE2 in the metabolism of butylparaben, benzylparaben and (partly) propylparaben was confirmed using the hCE2 specific inhibitor loperamide. A further finding of the study was that the expression of both hCE1 and hCE2 is by far higher in human liver when compared to human skin (activity approximately several 100-fold lower in skin).

It is nearby to assume that paraben esterase I in the study of Lobemeier et al. (1996) corresponds to hCE1 in the Jewell et al. (2007) study and that paraben esterase III in the Lobemeier et al. (1996) study corresponds to hCE2 in the Jewell et al. (2007) study.

Age dependency of Carboxylesterases

By using a small number of samples, Pope et al. (2005) observed that the expression and hydrolytic activity of carboxylesterases in the liver differs between children and adults. Yang et al. (2009) investigated the age dependency of carboxylesterases in human liver by using a larger number of individual liver samples from three different age groups (48 fetuses (gestation days 82 – 224), 34 children (age 0- 10 years) and 22 adults (> 18 years)).

The individual and/or pooled liver samples were investigated for the expression patterns of hCE1 and hCE2 by using RT-qPCR, Western Analysis (protein analysis) and enzymatic assays (cleavage of typical substrates for hCE1 and hCE2 such as aspirin, pyrethroids and oseltamivir).

The authors could demonstrate that at the mRNA, protein and enzyme activity level age differences in the expression of hCE1 and hCE2 exist. Age differences were more pronounced for hCE1 when compared to hCE2. For example, at the mRNA level, adults had an approximately 50% higher level of hCE1 when compared to children. The mRNA level of hCE2 in adults was about 40% higher in adults when compared to children.

An attempt has been made to compare mRNA levels with age in the group comprising all children. As correlation was not statistically significant, the group of children was further subdivided into smaller age groups. A statistically significant correlation between mRNA for both carboxylesterases and age was observed for the group between 0 – 1 years.

The observations of age differences between adults, children and fetuses were also confirmed by Western analysis and hydrolysis of substrates for hCE1 and hCE2.

As a further observation from the study, high interindividual variability in enzyme expression was observed in the different age groups (this might be due to the heterogeneity of the samples (with respect to age, sex and ethnicity) but in the case of hCE2 also to the polymorphic expression of the enzyme).

Conclusions on carboxylesterases and hydrolysis of parabens

Among carboxyl esterase enzymes in human skin, most information concerns the major forms hCE1 and hCE2. Scientific literature reveals that at lest one further form is expressed in human skin, but no statements can be made about its developmental regulation. Also in

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the liver a third carboxylesterase is expressed (Sanghani et al. 2004, cited in Yang et al. 2009), but whether parabens represent substrates for this third hepatic carboxylesterase and whether the expression of this carboxylesterase is developmentally regulated, remains to be established as well as its concomitant expression in human skin.

Human skin expresses hCE1 and hCE2 at much lower level when compared to liver. Maybe other forms of carboxylesterases might be expressed in humans.

Both hCE1 and hCE2 are developmentally expressed in the human liver. Assumed that this developmental expression is also present in skin, it can be assumed that expression of both hCE1 and hCE2 is lower in the skin of children when compared to adults. The difference is more pronounced for hCE1 which preferentially metabolises methylparaben and ethylparaben. For hCE2, which preferentially metabolises propyl-, butyl- and benzylparaben, the age difference is less pronounced. A good correlation between hCE1 and hCE2 mRNA levels and age was only found in the subgroup 0-1-year-old indicating that age difference is highest for children under the age of 1 year. Thus, as an approach to quantify the difference between adults and children age <1 in carboxylesterase expression, the mean value of mRNA levels in adults is compared to the lowest level observed in children (table 2 from Yang et al. 2009) which leads to a 87 fold higher hCE1 level in adults compared to children age <1 and to a 12.8 fold higher level of hCE2 in adults when compared to children age <1.

Thus, under the condition that age dependency as observed in the liver holds also true for skin and based on the assumption that parent parabens are responsible for the endocrine modulating activity, metabolism (cleavage) of parabens can be assumed to be lower in the skin of children age <1 when compared to adults. As metabolism by ester cleavage is regarded as inactivation of parent parabens, children at the age <1 year are at higher risk compared to adults from the ester cleavage point of view based on the information available on hCE1 and hCE2.

However, the interplay between all paraben-inactivation pathways has to be considered when addressing a potential higher risk of children towards the endocrine modulating effects of parabens. Thus the main alternative pathways, glucuronidation and sulfate ester formation (sulfation), and their role and age-dependency in the inactivation and elimination of free parabens in skin and systemic circulation of humans including neonates and infants have to be considered.

Glucuronidation and sulfate ester formation of parabens in humans

The data on glucuronidation and sulfate ester formation (sulfation) of parabens in humans is scarce. In most of the available biomonitoring studies, only the fractions of the parent parabens (free and/or conjugated) were determined in human serum or urine samples. Mostly, the frequently used parabens methyl-, ethyl, n-propyl- and butylparaben were determined (Boberg et al. 2010; Calafat et al. 2009; Calafat et al. 2010). In a population study, Ye et al. (2006) determined the paraben glucuronides and sulfate esters separately, besides free, unconjugated parabens, in spot urine samples from individuals with variable but unknown environmental exposures to parabens. They found that the relative proportions of the glucuronides and sulfate esters were similar and did not much differ when considering the whole range of exposures to parabens that were assessed by the concentrations of the parabens fraction (free and conjugated) found in urinary samples. The combined conjugates amounted to 95% or more and free, unconjugated parabens to 2 - 5% of the total parabens fractions. After topical application of butylparaben to adults, a similar proportion of free butylparaben in urine was determined (2.1%) (Janjua et al. 2008). In urinary spot samples of hospitalized preterm infants, 3- to 5-fold higher proportions of free methylparaben or propylparaben (about 10-15% of the total parabens fraction, free and conjugated) were found compared to 2-5 % in adults. The preterm infants in the study had an assumed gestational and postnatal age of less than 44 weeks and had an active

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(although probably immature) UGT1A1 because individuals with hyperbilirubinaemia had been excluded from the study (Calafat et al. 2009). Although the paraben conjugates were considered stable under controlled conditions of storage for several years, the estimated urinary concentrations of the free parabens must be interpreted with caution, according to the authors.

The data available may raise concerns about free parabens circulating in the human body and potentially exerting endocrine modifying effects in susceptible groups such as neonates or infants. In neonates and infants up to 6 months, glucuronidation activity is known to be reduced, whereas older children mostly have similar activities compared to adults (Allegaert et al. 2008; Edginton et al. 2006; Gow et al. 2001; Miyagi and Collier 2007; Renwick et al. 2000; Zaya et al. 2006). Hence, the question has to be solved whether neonates and infants exposed to parabens are at higher internal exposures than adults and therefore potentially at higher risk (at comparable dermal/external exposure) due to reduced glucuronidation and prolonged half-lives of parabens circulating in the body. In addition, the question has to be considered to what extent sulfation of parabens can counterbalance the reduced glucuronidation in neonates and infants.

It will be shown below that data on the conjugation of parabens in neonates and infants are patchy so far. Therefore, predictions on the fate of parabens in neonates and infants and on the degree of protection by conjugating enzymes are very difficult. As an approach to bridge the gaps, it is nearby to identify the isoenzymes of UDP-glucuronosyltransferase (UGTs) and sulfotransferase (SULTs) capable of conjugating parabens in adults, neonates and infants and to pursue their development and roles regarding parabens conjugation around parturition and early life after birth. The identification of these isoenzymes and the knowledge on their ontogenetic and kinetic properties may contribute to better assessments of the fate of parabens in neonates and infants or even enable more precise predictions when using suitable assessment tools such as PBPK modelling (de Zwart et al. 2004; Edginton et al. 2006).

In addition to the differences between rats and humans in the metabolism of parabens described above, the UGTs and SULTs responsible for glucuronidation and sulfate ester formation of exogenous substances including parabens often develop differently in humans and laboratory animals during intra-uterine life and after parturition (Coughtrie 2002; de Wildt et al. 1999; Gammage et al. 2006; Hines 2008; McCarver and Hines 2002). Because of the well-known ontogenetic differences between developing humans and laboratory animals regarding drug metabolizing enzymes, the emphasis of this review is on human data.

High inter-individual differences have been shown for UGT and SULT enzyme expression and enzyme activities in vitro and in vivo (see for instance Renwick et al. 2000). This is not unusual since such variability has been observed with many of the phase I and II enzymes of drug metabolism. Partly these differences can be explained by proven genetic polymorphisms. Prominent examples are some allelic variants in the UGT1A family and of the SULT1A1 isoenzyme. Apart from these genetic differences, other factors on the level of regulation not well understood so far may contribute to enzyme expression and activity. Therefore, inter-individual variations in glucuronidation and sulfate ester formation will not be considered here. In the future, inter-individual differences of drug metabolism will become an increasing challenge to risk assessors.

Human UDP-glucuronosyltransferases (UGTs)

UDP-glucuronosyltransferase enzymes (UGTs) (EC 2.1.4.17) are located in the membrane of the endoplasmic reticulum of cells and exhibit distinct but overlapping substrate specificities. Two UGT gene families were found in humans. Nine functional genes exist in the UGT1 family, UGT1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9 and 1A10, and seven within

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the UGT2 family, UGT2A1, UGT2B4, 2B7, 2B10, 2B11, 2B15 and 2B17. The majority of these enzyme forms are located in liver but most of them are also found in extrahepatic tissues, normally at lower expression levels compared to liver (Tukey and Strassburg 2000). Several of the isoenzymes also conjugate endogenous signalling substances such as steroid or thyroid hormones and thereby probably serve for the control and balance of endogenous hormone concentrations.

Identification of UGT isoenzymes conjugating parabens

Available data on parabens glucuronidation in humans is mainly derived from biomonitoring studies and is limited as delineated above. Apart from parabens (free and conjugated) in urinary samples from preterm infants, data on the glucuronidation of parabens in neonates and infants are missing so far. Abbas et al. (2010) have recently published an *in vitro* study on glucuronide formation and ester hydrolysis in liver samples from adult humans. The authors used commercially available human recombinant UGT isoenzymes and several parabens (methyl, ethyl, propyl, butyl, benzyl) and showed that these parabens are mainly conjugated by the UGT isoenzyme forms 1A1, 1A8, 1A9, 2B7, 2B15, and 2B17 (however, with different specific activities). Other isoenzymes investigated displayed lower or even very low specific glucuronidation rates, namely the UGTs 1A3, 1A4, 1A6, 1A7, 1A10, and 2B4. The authors concluded that the parabens are readily metabolized in human liver through glucuronidation by several UGT isoforms as well as by esterase hydrolysis and suggest according to their results that these parabens do not accumulate in human tissues. Apart from human liver samples, data on the glucuronidation of parabens from other relevant human extrahepatic organs such as gut, kidney, lung or skin are not available.

UGT isoenzymes capable of conjugating parabens in human skin

Glucuronidation of parabens in human skin has not been investigated in published studies. Existing information on UGT isoenzymes which are capable of forming glucuronides of parabens in human skin is scarce (Oesch et al. 2007). From the UGT1A family members, only UGT1A1 with bilirubin as a probe substrate and potentially another "phenol-UGT" have been detected in skin from adult humans (Peters et al. 1987; Pham et al. 1990) and human keratinocytes, respectively (Vecchini et al. 2005). In addition, the UGT2B family members UGT2B4, UGT2B11, UGT2B15 and UGT2B17 have been detected as gene transcripts in human skin (Lévesque et al. 1997; Lévesque et al. 1999; Luu-The et al. 2007; Tukey and Strassburg 2000).

Taken together, in adult human skin, only UGT1A1, UGT2B15 and UGT2B17 with proven catalytic activity towards parabens in liver have been detected, in part only as gene transcripts. It can be concluded that glucuronidation of parabens in adult human skin is possible but the contribution of glucuronidation to the inactivation of parabens in human skin remains to be determined.

Ontogeny of UGT isoenzymes in human development

Most of the human UGT isoenzymes conjugating parabens are not well expressed at birth up to several months or even some years of age. Strassburg et al. (2002) could not detect any gene transcripts of the UGT enzyme forms in human liver of two foetuses of week 20 of gestation. For most UGT isoenzymes capable of conjugating parabens, there is little knowledge on their development at birth and infancy (**Table A3-1**). Similar gaps of knowledge exist for the other UGT enzyme forms that are less relevant for the glucuronidation of parabens in humans (see reviews of de Wildt et al. 1999; Hines 2008; McCarver and Hines 2002).

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Table A3-1: Ontogeny of human UGT isoenzymes capable of forming paraben glucuronides

UGT isoenzyme (marker substrate)	Onset, gene transcript or protein levels, and enzyme activities in development compared to adult levels.	Remarks	References
1A1 (bilirubin)	Onset of activity at birth; the activity is fully developed to adult levels after 3-6 months of age.	Frequently jaundice (icterus) in newborns due to unconjugated hyperbilirubinaemia during first days after parturition	de Wildt et al. 1999; Hines 2008; Tukey & Strassburg 2000
1A8 (various phenols)	Ontogeny and activity at birth and during the first months of age are unknown. The gene transcript is fully developed to adult levels after 6 months of age.	Extrahepatic UGT isoenzyme. Predominantly located in gastrointestinal tract. Relevant only in case of oral parabens exposure	Strassburg et al. 2002
1A9 (estrogens, paracetamol)	Ontogeny and activity at birth and during the first months of age are unknown. The gene transcript of this isoenzyme reaches 30-40% of the adult activity after 6-12 months and 60-70% after 1-2 years of life.		Strassburg et al. 2002
2B7 (morphine)	With morphine as substrate, an onset of activity within the second trimester and adult levels of activity after 2-3 months of age were reported. With epirubicin as substrate: < 10% of adult levels at less than 1 year of age, 50-70% at an adolescent age and interjacent levels at 1 to 11 years.	Early development was not confirmed by a more recent study of Zaya et al. (2006). See text.	de Wildt et al, 1999; Hines 2008; Zaya et al. 2006
2B15 (propofol: testosteron**)**	Ontogeny and activity at birth and during the first months of age are unknown. The gene transcript is fully developed to adult levels after 6 months of age.		Strassburg et al. 2002
2B17 (androgenic steroids)	Enzyme activity of less than 10% in foetal liver samples and about 10% in neonates compared to adult levels was reported.		de Wildt et al. 1999; Hines 2008

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Regarding UGT2B7 with morphine as a typical substrate, the early foetal development previously described was not confirmed by a more recent study of Zaya et al. (2006) who investigated another substrate, the drug epirubicin, and both the expression of the UGT2B7 protein and its catalytic activity. They reported a much slower increase of this enzyme form and levels of enzyme activity in adolescent age coming closer to adult levels (Table 1).

Strassburg et al. (2002) observed that gene transcripts and proteins of all except two of the UGT isoenzymes investigated were shown to have reached adult levels after 6 months of age. However, UGT enzyme activities towards various substrates tested in vitro were low and did not correlate to the appearance or content of the UGT isoenzyme proteins. Understanding of the ontogeny and development of the UGT isoenzymes is complicated by the fact that after appearance of the gene transcripts and the proteins, UGT enzymes may need further post-translational maturation during the development of the neonate or infant (and in particular cases beyond) until adult levels of enzyme activity are reached.

Human sulfotransferases (SULTs)

Sulfate ester formation is also an important and potentially critical pathway of the inactivation and elimination of parabens from the human body as delineated above and is catalyzed by sulfotransferase (SULT) enzymes (EC 2.8.2.1); those involved in drug metabolism are located in the cytosol. In humans, four different SULT enzyme families are known, SULT1, SULT2, SULT4 and SULT6, comprising at least twelve distinct members of isoenzymes (Blanchard et al. 2004; Gamage et al. 2006, Lindsay et al. 2008). Similar to the UGTs, SULT isoenzymes exhibit distinct but overlapping substrate specificities towards exogenous substances. Several of the SULT isoenzymes also conjugate, e.g. endogenous steroid or thyroid hormones and thereby play a role in the control and balance of endogenous hormone concentrations.

Identification of human SULT isoenzymes conjugating parabens

Available evidence on sulfate ester formation of parabens in humans is primarily derived from biomonitoring studies and is limited as delineated above. Prusakiewicz et al. (2007) reported that butylparaben sulfate was formed *in vitro* in human liver and skin cytosols, and when using the recombinant human allozyme SULT1A1*2, respectively. They concluded that butylphenol is "not a very good SULT substrate". SULT1A1 is an isoenzyme mainly located in human liver and small intestine and also present in smaller amounts in other extrahepatic tissues (Riches et al. 2009). The allozyme SULT1A1*2 normally has lower specific activity than the wild type enzyme. In the past often termed as "phenolsulfotransferase", SULT1A1 has been characterized to possess broad substrate specificity towards many exogenous and endogenous phenolic substrates (Gamage et al. 2006; Lindsay et al. 2008). It is not known which of the other SULT isoenzymes are capable of forming sulfate esters of parabens. In addition to human SULT1A1, SULT1A3, SULT1B1 and SULT1C2 have also been shown to sulfate exogenous phenols of different structures (Lindsay et al 2008). In tissues from adults, SULT1A3 is present as a major SULT isoenzyme in small intestine but could not be detected in liver whereas human foetal liver and small intestine contain SULT1A3 in appreciable amounts (Riches et al. 2009; Stanley et al. 2005). SULT1B1 consisting of two isoenzymes, 1B1_a and 1B1_b, is predominantly expressed in small intestine and kidney but also found in liver. The role of SULT1B1 in drug metabolism is unclear so far: Although it has a broad spectrum of substrates similar to SULT1A1, the substrate affinities are in general much lower.

SULT isoenzymes conjugating parabens in human skin

No information on sulfate ester formation of parabens in the skin of neonates and young infants is available in the published literature. Sulfate ester formation of parabens in human skin *in vitro* has not been investigated in detail so far. Prusakiewicz et al. (2007) reported that butylparaben sulfate was generated in small amounts in human skin cytosol (about 10% compared to human liver). On the other hand, they found that butylparaben along with other parabens inhibits the sulfonation of estradiol (with an IC_{50} of 37 µM).

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In human skin and keratinocytes cultures, the following SULT isoenzymes have been detected or investigated: SULT1A1, 1A3, 1E1, and 2B1 (Falany et al. 2006; Svensson et al. 2009).

Apart from SULT1A1, from these isoenzyme forms, only SULT 1A3 can currently be assumed to conjugate parabens although it cannot be excluded that SULT1E1 and/or SULT2B1 isoenzymes are also capable of conjugating parabens in human skin. It is concluded that sulfation of parabens in human skin from adults in vitro occurs albeit to a much lower extent than in human liver. The contribution of sulfation to the inactivation of parabens in human skin after dermal exposure remains to be determined.

Ontogeny of SULT isoenzymes in human development

The ontogeny and development of SULT isoenzymes has been investigated and compared in human tissues of foetal origin, from neonates, infants and adults (reviewed by Hines 2008). This compilation is restricted to SULT1A1, SULT1A3, SULT1B1 and SULT1C2. SULT1A1 is expressed in human liver of all age groups investigated (including foetuses, neonates and infants) in substantial and comparable amounts whereas different trends were observed in extrahepatic tissues. Hepatic SULT1A3 was expressed at high levels in foetal tissue of about the second trimester, but substantially decreased in neonatal or infant liver to about 10% or less of the foetal level and was essentially absent in the adult liver. Similar trends of SULT1A3 activities were observed with lung and kidney tissues. SULT1A3 levels in foetal gut tissue were highest and essentially not different from adults (Adjej et al. 2008; Richard et al. 2001; Stanley et al. 2005). SULT1B1 was only found in foetal small intestine. The protein expression of SULT1C2 was much higher in foetal small intestine than in foetal kidney or liver and was found to be barely expressed in human colon or liver of adults suggesting that SULT1C2 is more widely expressed in the foetus than in the adult (Stanley et al. 2005). Although these three SULT isoenzymes discussed appear to tend towards higher levels in the human foetus than in adults, activities or protein levels also tend to decrease in neonates and infants. The decrease of SULT1A1 enzyme activity is less marked than the interindividual differences in foetal, early postnatal and adult liver samples and may be considered slight. Nevertheless, the data on the sulfation of parabens in neonates and young infants are poor so that no firm conclusions regarding the role of sulfate ester formation of parabens can be drawn in these age groups.

Conclusions

In biomonitoring studies, only small proportions of free parabens were detected whereas conjugates of parabens consisting of glucuronides and sulfate esters predominated both in serum and urinary samples of adults. Higher proportions of free parabens were determined in urinary spot samples from preterm infants compared to adults. In contrast, in rats, only p-hydroxybenzoic acid and no free or conjugated parabens were found after dermal or oral exposure due to rapid hydrolysis of parabens to p-hydroxybenzoic acid. Thus, the rat is a model of limited value when predicting the toxicokinetics of parabens in humans. Because of proven internal exposure of humans to free parabens, the question has to be solved whether neonates and infants when dermally exposed are at higher internal exposure of free parabens than adults given the immature and thus reduced functions of UDP-glucuronosyltransferases (UGTs). A related issue is whether sulfate ester formation of parabens by sulfotransferases (SULTs) can counterbalance the reduced glucuronidation in neonates and infants.

It has been shown in vitro that several UDP-glucuronosyltransferase (UGT) isoenzymes are capable of glucuronidation of parabens in the liver of adult humans. Although glucuronidation of parabens in human skin appears possible, the contribution of glucuronidation to the inactivation of parabens in adults, neonates and infants remains to be elucidated. In addition, there is only little information available on the ontogeny and development of the UGT isoenzymes conjugating parabens in neonates and young infants up to six months.

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Of the sulfotransferase (SULT) isoenzymes accepting exogenous phenols as substrates, SULT1A1 is the only SULT enzyme form with proven (although low or moderate) catalytic activity towards one of the parabens, namely butylparaben, and is considered so far as the only established defence among the SULT isoenzymes against this member of the parabens in adults, neonates and infants. SULT1A1 and the three SULT isoenzymes potentially forming sulfate esters from parabens (SULT1A3, SULT1B1, and SULT1C2) are differentially expressed in foetal tissues and show different expression profiles in human development and in adults. The role of sulfation of parabens in human skin and systemic circulation remains to be elucidated.

Taken together, in humans including neonates and infants, glucuronidation and sulfate ester formation play a critical role of in the inactivation and elimination of free parabens in skin and systemic circulation, different from rats. Existing data suggest that the glucuronidation of parabens is reduced in neonates and infants at least up to six months of age. Of the sulfotransferases, only SULT1A1 has been shown to convert parabens to sulfate esters *in vitro* so far. Because of patchy data in neonates and infants, it is questionable whether sulfate ester formation of parabens by SULT isoenzymes can counterbalance the reduced glucuronidation. Thus, for neonates and infants up to 6 months of age, it cannot be excluded that the internal dose and the half-life of the unmetabolised parabens may be higher than in adults after topical application of cosmetics containing parabens. However, based on the limited information available, no quantitative conclusions can be drawn for differences in glucuronidation and sulfation between adults and children.

The SCCS emphasizes in the Opinion of March 2011 that relevant human data regarding metabolism of parabens is missing so far, which is required for reducing uncertainties and for a sound risk assessment. This data can be gained by either a human toxicokinetic study in vivo (e.g., by use of deuterated parabens) or by an approach combining *in vitro* data on the metabolism of parabens and toxicokinetic modelling, similar as in the case of bisphenol A (Mielke and Gundert-Remy 2009; Mielke et al. 2011). However, relevant in vitro data regarding hydrolysis and phase II metabolism of parabens in human skin and liver is missing; these data is a prerequisite for toxicokinetic modelling of parabens metabolism in newborns, infants and adults.

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ANNEX 4 - Biomonitoring of parabens in humans

The main measurements of parabens[11] in human sera/plasma, seminal sera, and urine presented in the papers discussed below are presented in Table **A4-1**.

Serum/plasma

Ye et al. (2008) measured methyl-, ethyl- and propylparaben in 15 commercially available serum samples collected between 1998 and 2003 from 4 male and 11 female donors. The serum samples were frozen on dry ice and shipped to the laboratory, where upon receipt the samples were stored at −70 °C. Both free and total parabens (sum of unconjugated, deglucuronidated and desulfated parabens) were measured in serum. Free PP was detected in 47% of the samples. The median level of free PP was below the limit of detection (<LOD) and the maximum level was 2.3 ng/ml. Total PP was measured to 1.4 ng/ml (median) with a maximum level of 67.4 ng/ml. For free MP the median value was 0.2 ng/ml with a maximum value of 9.8 ng/ml.

Frederiksen et al. (2011) measured parabens in urine, blood and semen samples obtained from 60 young and healthy Danish men (average age 19.7 years, samples collected 2006). Urine, serum and seminal plasma were analyzed and the total levels of parabens were determined. It is noted that the paraben levels, with exception of the maximum level of EP, were considerably lower than in the study of Ye et al. (2008). The difference is probably due to the fact that the study of Frederiksen et al. (2011) was performed on young men, while Ye et al. (2008) studied commercial sera from 11 women and 4 men.

Sandanger et al. (2011) measured parabens (methyl-, ethyl-, propyl-, butyl-, and benzylparabens) in plasma from 322 women (blood drawn in 2005). All blood samples were frozen within 3 days of collection. Butyl- and benzylparabens were not detected. PP was detected in 29% of the group (median < 2 ng/ml). It is stated in the report that PP "was only detected above MDL (method detection limit) in women who used body lotion "once a day" (2.2 ng/ml) and "twice or more per day" (4.0 ng/ml. The maximum level of PP measured was 43.9 ng/ml.

The authors have not hydrolyzed any of the plasma samples and state: "The high concentration of native parabens identified in this study is not likely caused by hydrolysis of conjugates as paraben conjugates in human serum have been shown to be stable over 30 days when stored at 37 °C (Ye et al. 2009). The contribution of conjugate hydrolysis is therefore considered negligible to the values reported." SCCS does not find this argumentation convincing, as Ye et al. (2009) studied the stability of paraben conjugates in serum that had been prepared and frozen at -70° C before it was thawed and used for stability studies. Sandanger et al. (2011) used blood samples that had been kept for up to 3 days (temperature not given) before being frozen. The stability of the conjugated parabens may obviously differ in full blood and sera that have been frozen.

The medium level for free MP reported by Sandanger et al. (2011) was nearly as high as found by Ye et al. (2008) for total MP and the maximum level of free MP found by Sandanger et al. (2011) was nearly 15 times higher than the corresponding level found by Ye et al. (2008). The medium level of free PP was below LOD both in the study of Sandanger et al. (2011) and Ye et al. (2008), while the maximum value was found by Sandanger et al. (2011) was nearly 20 times that reported by Ye et all. (2007) and nearly as high as they reported for total PP.

Janjua et al. (2007) studied the systemic uptake of some phthalates and butylparaben following whole-body topical application. Twenty-six healthy male volunteers (mean age 26

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[11] Abbreviations used: MP = methylparaben, EP = ethylparaben, PP = propylparaben, BP = butylparaben

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years old) participated in the study. The subjects were only allowed to use a phthalate and BP free moisturizer and deodorant supplied by us one week before the study and during the study. The study lasted two consecutive weeks: a control week followed by a treatment week. A cream containing 2% (800 mg/person, 10 mg/kg bw based on measured body weights) of BP (together with 2% diethylphthalate and 2% diethylmethylphthalate) was applied every day for 5 days. The test persons waited 20 min to let the cream absorb into the skin before dressing. Blood samples were centrifuged and aliquots for chemical analysis were acidified with to inhibit endogenous enzyme activity. The aliquots were stored at -20 C until analysis. It is not stated if free or total BP was studied; however as no use of enzymes were reported and the sera were acidified it is assumed that the authors analyzed free BP. The level of BP increased to about 100 ng/ml after 1 hour. A maximum (mean) of 135 ng/ml BP was found 3 hours after applying the cream. Subsequently the level decreased. At 24 hours after the first application the level of BP was 18 ng/ml. The level was then constant for the next 4 days. The authors estimated that (0.135 mg/l x 6 l) 0.8 mg of butylparaben was in circulation at the time of peak concentration corresponding to 0.1% of parent compound. It is not clear if the reduction in the level of BP observed at 24 hours was due to further distribution in the body, enzymatic conjugation or hydrolysis to p-hydroxybenzoic acid.

SCCS notes that the doses applied are much higher than the worst-case doses that the consumer receives and that the study clearly demonstrates that BP does not accumulate.

Seminal plasma

The presence of parabens in the seminal plasma found by *Frederiksen et al. (2011)* is of considerable interest. The authors point out that they cannot say whether the parabens measured in seminal plasma are derived from fluids coming from the testis together with the spermatozoa and thus reflect a direct exposure of the testis or whether they derived from seminal fluid coming from the accessory glands. But irrespective of the route, the authors consider it may be of concern that the medium level of PP in seminal plasma (0.68 ng/ml) is 3 times higher than measured in serum (0.32 ng/ml).

Urine

Ye et al. (2006) measured the urinary concentrations of methyl-, ethyl-, propyl-, butyl (n- and iso-)-, and benzylparabens in a demographically diverse group of 100 anonymous adults with unknown exposure to parabens. The samples were collected from 2003 to 2005 at different times throughout the day. The authors detected MP and PP in > 96% of the samples. The other parabens were detected in more than half of the samples. It was found that parabens in urine appear predominantly in their conjugated forms. The high correlation (Pearson correlation coefficient r = 0.92, p < 0.0001) between total urinary concentrations of MP and PP suggests that human exposures to MP and PP most likely share common sources. The authors did not differentiate between samples from men and women. *Calafat et al. (2009)* measured the concentrations of free and total (free plus conjugated) MP and PP in urine collected from 42 premature infants in two neonatal intensive care units in the Boston area in 2003. The parabens were detected in all of the samples. The authors point out that their findings suggest that infants may be exposed during critical periods of their development to several potential reproductive and developmental toxicants at levels higher than those reported for the general population.

Calafat et al. (2010) report concentrations of parabens measured in 2548 urine samples from the US National Health and Nutrition Examination Survey (NHANES) 2005-2006 study. The urine specimens were collected from a one-third subset of participants > 6 years of age. Participants provided one spot urine sample during one of three daily examination sessions. The samples were shipped on dry ice to CDC's National Center for Environmental Health and stored at temperatures below -20 °C until analyzed. The samples were analyzed for total parabens. MP and PB in urine were detected in > 92% of the samples examined; EP and BP

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in about 50%. The high frequency of detection of MP and PP most likely resulted from their wide use in food products and in common personal care products (e.g., lotions, cosmetics, hair preparations). The range of urinary concentrations spanning up to three orders of magnitude may be related to lifestyle factors, including diet, that result in exposure differences and/or to individual variations in bioavailability, distribution kinetics, or metabolism of the parabens. The concentrations of parabens were higher in women than in men.*Meeker et al. (2011)* collected urine samples from males attending an infertility clinic in USA between 2000 and 2004. The study involved 194 men between 18 and 55 years of age. The urine was analyzed for total (free plus conjugated) MP, PP, BP, and bisphenol A (BPA). Associations with serum hormone levels (n = 167), semen quality parameters (n = 190), and sperm DNA damage measures (n = 132) were assessed using multivariable linear regression. The urine samples were divided in aliquots and frozen at -80 °C. Detection rates in urine were 100% for MP, 92% for PP, and 32% for BP. Paraben exposures in the present study were likely representative of those found among men in the U.S. general population as the numbers were similar to those reported in males participating in the NHANES for 2005-2006 (Calafat et al. 2010). It should be noted that paraben exposure was much higher among women than among men in NHANES.

With the exception of a suggestive inverse association between MP and TSH (thyroid-stimulating hormone), and a suggestive positive association between BP and FAI (free androgen index), no evidence for a relationship between MP, PP, or BP and altered hormone levels or conventional semen quality parameters was found. For sperm DNA damage, a suggestive inverse association between PP and TDM (tail distributed moment), a suggestive positive association between MP and Tail%, and a statistically significant positive association between BP and Tail% was found. *Frederiksen et al. (2011)* measured parabens in urine samples obtained from 60 young and healthy Danish men (average age 19.7 years, samples collected 2006). Urine was analyzed and the total levels of parabens (sum of unconjugated, deglucuronidated and desulfated parabens and metabolites) were determined. The authors point out that compared with previous studies of urinary concentration of parabens in US male and female adults (Ye et al. 2006) the median urinary concentration of the parabens were in general about 2.5-fold lower in Danish men, with the exception of EP, which was twice as high in the Danish men. This may represent a country difference in use of parabens between the USA and Denmark. However, the US study also included women, whereas the Danish study did not, and thus the difference in excretion pattern may also reflect a difference in exposure between women and men.

Table A4-1: Levels of parabens measured in human serum/plasma, seminal plasma, and urine.

Paraben	Study (parabens analyzed as free and/or total)	Medium (ng/ml)	95 percentile (ng/ml)	Maximum (ng/ml)
Serum/plasma				
Methylparaben	Ye et al. 2008 (free/total); adults	0.2/10.9 (2%)*		9.8/301 (3%)
	Frederiksen et al. 2011 (total); male	1.53		59.6
	Sandanger et al. 2011; female	9.4		142.9
Ethylparaben	Ye et al 2008 (free/total)	<LOD**/0.2		<LOD/5.4
	Frederiksen et al. 2011 (total); male	<LOD		20.8
	Sandanger et al. 2011; female	<0.3		45.9
Propylparaben	Ye et al 2008 (free/total)	<LOD/1.4		2.3/67.4(3%)

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	Frederiksen et al. 2011 (total); male	0.32		5.50
	Sandanger et al. 2011; female	<0.2		43.9
Butylparaben	Frederiksen et al. 2011 (total); male	<LOD		0.87
	Sandanger et al. 2011; female	<LOD		<LOD
Benzylparaben	Frederiksen et al. 2011 (total); male	<LOD		0.29
Seminal plasma				
Methylparaben	Frederiksen et al. 2011 (total); male	0.99		180
Ethylparaben		0.14		5.65
Propylparaben		0.68		35.5
Butylparaben		0.06		1.73
Benzylparaben		<LOD		1.48
Urine				
Methylparaben	Ye et al. 2006 (free/total); adults	0.8/43.9 (2%)	27.8/680 (4%)	
	Calafat et al. 2009 (free/total); premature neonates	23/243 (9%)		515/4010 (13%)
	Calafat et al. 2010 (total); 6-11 year	25	125	
	Calafat et al. 2010 (total); female	137	1110	
	Calafat et al. 2010 (total); male	23.7	491	
	Meeker et al. 2011 (total); male	32.6	340	1037
	Frederiksen et al 2011 (total); male	17.7		2002
Ethylparaben				
	Ye et al. 2006 (free/total); adults	<LOD/1.0	1.5/47.5 (3%)	
	Calafat et al. 2010 (total); 6-11 year	<LOD	9.90	
	Calafat et al. 2010 (total); female	1.30 (<LOD-2.20)	98.7	
	Calafat et al. 2010 (total); male	<LOD	25.2	
	Frederiksen et al 2011 (total); male	1.98		564

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Propylparaben				
	Ye et al. 2006 (free/total); adults	<LOD/9.1	3.4/279 (1%)	
	Calafat et al. 2009 (free/total); premature infants	1.7/17.0 (10%)		171/1360 (13%)
	Calafat et al. 2010 (total); 6-11 year	2.50	125	
	Calafat et al. 2010 (total); female	29.1	357	
	Calafat et al. 2010 (total); male	2.30	306	
	Meeker et al. 2011 (total); male	4.45	107	229
	Frederiksen et al 2011. (total); male	3.60		256
Butylparaben				
	Ye et al. 2006 (free/total); adults	<LOD/0.5	0.3/29.5 (1%)	
	Calafat et al. 2010 (total); 6-11 year	<LOD	7.50	
	Calafat et al. 2010 (total); female	0.50	34.9	
	Calafat et al. 2010 (total); male	<LOD	3.20	
	Meeker et al. 2011 (total); male	<LOD	3.73	32
	Frederiksen et al. 2011 (total); male	0.19		67.6
Benzylparaben				
	Ye et al. 2006 (free/total); adults	<LOD/<LOD	<LOD/0.5	
	Frederiksen et al. 2011 (total); male	<LOD		2.06

*Percentage of free paraben in relation to the total paraben concentration
**Limit of detection

Discussion

Parabens are used as antimicrobial preservatives in cosmetics and food. The estrogenic activities of parabens have been associated with the free parabens and it is unlikely the conjugated parabens have estrogenic activity (see chapter 3.3.1.). Most of the biomonitoring studies discussed above have only measured the total concentration (free plus conjugated) of the paraben and only a few studies have measured both free and total parabens.

In one study on serum (Ye et al. 2008) and two studies on urine (Ye et al. 2006, Calafat et al. 2009) both free and total parabens were measured. Both in serum and urine from adults the amount of free parabens was in the range 1% to 4% of total paraben measured

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(Ye et al. 2006, 2008). In one study of urine from premature infants (Calafat et al. 2009) the amount of free parabens was between 9% and 13%. Thus, the relative amounts of free parabens in relation to conjugated parabens may be higher in premature infants than in adults.

In all studies methylparaben was present in the highest concentration followed by propylparaben. Ethylparaben, butylparaben, and benzylparaben were generally present in lower concentrations. Generally, the levels of parabens were higher in women than in men. The levels of parabens in the urine of children (6 – 11 years old) were similar to those in males. The medium and maximum levels of methylparaben and propylparaben in urine were higher in premature infants than in any of the other groups (Calafat et al. 2009).

Frederiksen et al. (2011) have studied the levels of parabens from the same persons in serum, seminal plasma and urine. Their results indicate that the concentrations of parabens were similar in serum and seminal plasma, but more than 10 times higher in urine than in serum. When all results are considered together it can be concluded that the concentration of parabens are generally much higher in urine than in serum.

Possible relationships between the parabens levels and adverse health effects were only considered by Meeker et al. (2011) in their studies of males attending an infertility clinic. Urinary BP concentration were not associated with hormone levels or conventional semen quality parameters, but they were positively associated with sperm DNA damage (measured as DNA tail% in a comet assay) (p for trend = 0.03).

Free and total MP and PP in urine from premature infants were studied by Calafat et al. (2009). The urinary concentrations of MP and PP were surprisingly high compared to that measured in urine from adults. The finding that the levels of MP and PP were highly correlated (Spearman r = 0.73, p < 0.0001), indicate that exposures to these parabens most likely share common pathways. As discussed above, the relative amounts of free parabens compared to the total amounts of parabens were significantly higher in the premature infants than in adults. The authors point out that their findings suggest that infants may be exposed during critical periods of their development to several potential reproductive and developmental toxicants at levels higher than those reported for the general population. Their study focused on biomarkers of exposure and they did not explore whether such exposures were associated with adverse health effects in the infants. No information was given about the possible source of the parabens especially whether the source(s) of exposure for this subpopulation is representative for "normal full-term babies" outside the hospital or whether the premature infants were exposed from medical or other specialised products not used otherwise.

Janjua et al. (2007) studied the systemic uptake of butylparaben (800 mg/person; 10 mg/kg bw) after whole-body topical application. 3 hours after application, 0.1% of the applied dose was found in the blood circulation. Under the assumption that the authors measured free BP, the SCCS has calculated the half life of free BP in serum to be about 7 hours. It is noted that the amount applied under estimated worst case conditions of PP and BP (with 0.19% PP in all cosmetic product is about ([17.400 mg x 0.0019] / 60 kg) 0.55 mg/kg bw). The amount applied in the study by Janjua et al. (2007) was thus nearly 20 times higher than the worst case exposure dose. The study by Janjua et al. (2007) clearly shows that the parabens do not accumulate in the body.

Since the parabens do not accumulate it is possible to calculate the systemic exposure dose (SED) on the basis of their urinary excretion. It should be noted, however, that the calculations do not take into account the amount of parabens hydrolyzed to p-hydroxybenzoic acid (PHBA) after reaching the systemic circulation. This may lead to an underestimation of the internal exposure of humans to free parabens. The systemic circulation of p-hydroxybenzoic acid is unknown and yet remains to be determined.

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Moreover, the proportion of parabens (and PHBA in food) taken up by the oral route is unknown. Thus, the calculation below will represent the sum of dermal and oral exposure.

Table A4-2: Excretion of parabens in urine calculated as µg/kg bw/day.

Paraben	Study	Median ng/ml	95 percentile / Maximum ng/ml	Medium excretion µg/kg bw/d*	95 percentile / Maximum excretion µg/kg bw/d
Methylparaben	Ye et al. 2006; adults	43.9	680 (95)	1.46	22.7 (95)
	Calafat et al. 2010; 6-11 year	25	125 (95)	1.67	8.33 (95)
	Calafat et al. 2010; female	137	1110 (95)	4.56	37.0 (95)
	Calafat et al. 2010; male	23.7	491 (95)	0.79	16.4 (95)
	Meeker et al. 2011; male	32.6	340 (95); 1037	1.09	11.3(95); 34.6
	Frederiksen et al 2011; male	17.7	2002	0.59	66.7
Ethylparaben					
	Ye et al. 2006; adults	1.0	47.5 (95)	0.03	1.58 (95)
	Calafat et al. 2010; 6-11 year	<LOD**	9.90 (95)	--	0.66 (95)
	Calafat et al. 2010; female	1.30	98.7 (95)	0.04	3.29 (95)
	Calafat et al. 2010; male	<LOD	25.2 (95)	--	0.84 (95)
	Frederiksen et al. 2011;	1.98	564	0.07	18.8

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	male				
Propylparaben					
	Ye et al. 2006; adults	9.1	279 (95)	0.30	9.3 (95)
	Calafat et al. 2010; 6-11 year	2.50	125 (95)	0.17	8.3 (95)
	Calafat et al. 2010; female	29.1	357 (95)	0.97	11.9 (95)
	Calafat et al. 2010; male	2.30	306 (95)	0.08	10.2 (95)
	Meeker et al. 2011; male	4.45	107 (095); 226	0.15	3.57 (95); 7.53
	Frederiksen et al 2011; male	3.60	256	0.12	8.53
Butylparaben					
	Ye et al. 2006	0.5	29.5 (95)	0.02	0.98 (95)
	Calafat et al. 2010; 6-11 year	<LOD	7.50 (95)	--	0.50 (95)
	Calafat et al. 2010; female	0.50	34.9 (95)	0.02	1.16 (95)
	Calafat et al. 2010; male	<LOD	3.20 (95)	--	0.11 (95)
	Meeker et al. 2011; male	<LOD	3.73(95); 32	--	0.12(95); 1.07
	Frederiksen et al 2011; male	0.19	67.6	0.01	2.25

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Benzylparaben					
	Ye et al. 2006; adults	<LOD	0.5 (95)		0.02 (95)
	Frederiksen et al 2011; male	<LOD	2.06		0.07

*   For adults an average body weight of 60 kg was assumed. For the age group of children 6-11 years, an average body weight of 30 kg was assumed. For all groups, a daily urine volume of 2 liter was assumed

**   Limit of detection

All the calculations in Table **A4-2** are based on the concentrations of total parabens. The highest values for exposure for parabens were found for females in the study of Calafat et al. (2010). For methylparaben the SEDs were calculated to 4.56 µg/kg bw/d and 37.0 µg/kg bw/d for medium and 95 percentile, respectively. The corresponding values for propylparaben were 0.97 µg/kg bw/d and 11.9 µg/kg bw/d for medium and 95 percentile, respectively and in the case of butylparaben the values were 0.02 and 1.16 µg/kg bw/d, respectively. The results of the biomonitoring studies thus support that the exposure calculation made in opinion SCCS/1348/10 overestimates consumer exposure. It also has to be noted, that the use levels of parabens in the USA are not regulated and might be higher than in Europe.

References Annex 4

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