Scientific Committee on Consumer Safety

SCCS

OPINION ON

Parabens

Updated request for a scientific opinion on propyl- and butylparaben

COLIPA n° P82

The SCCS adopted this opinion by written procedure on 3 May 2013

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
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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

"Parabens" are currently authorized as preservatives in entry 12 of Annex VI of the Cosmetics Directive at a maximum concentration of 0.4% when used individually or 0.8% when used as a mixture of esters. Different substances are covered by this entry, with the most commonly used being: methyl-, ethyl-, propyl-, butylparabens, isopropyl- and isobutylparabens.

Since 2005, these substances have been assessed by the subsequent Scientific Committees on a number of occasions. In March 2011, the Scientific Committee on Consumers Safety (SCCS/1348/10) considered that:

- Methylparaben and ethylparaben were safe, when used at the maximum authorized concentrations;

- Butylparaben and propylparaben were safe, if the sum of their individual concentrations did not exceed 0.19%.

- For isopropylparaben, isobutylparaben, phenylparaben, benzylparaben and pentylparaben, the human risk could not be evaluated for lack of data.

On 21 March 2011, Denmark notified the Commission that it had banned propyl- and butylparaben, the isoforms and salts in cosmetic products for children up to three years of age. On 10 October 2011, the SCCS adopted a clarification to its previous opinion in light of the Danish clause of safeguard. The Committee (SCCS/1446/11) concluded that:

- For general cosmetic products containing parabens, excluding specific products for the nappy area, there was no safety concern in children.

- For leave-on cosmetic products designed for application on the nappy area and in the case of children below the age of six month, a risk could not be excluded in the light of both the immature metabolism and the possibly damaged skin in this area.

In March 2012, a Member State presented the results of a study on the reproductive toxicity of propylparaben to the Working Group on Cosmetic Products. The study showed no effects on the reproductive parameters; therefore it did not confirm the conclusions of the previous studies that pointed towards negative effects on reproduction.

2. TERMS OF REFERENCE

1. *Taking into consideration recent data, does the SCCS consider that its opinions of 2010 (SCCS/1348) and 2011 (SCCS/1446) on propylparaben when it is used as preservative in cosmetics products, both intended for adults and young children, need to be updated?*

2. *Taking into consideration recent data, does the SCCS consider that its opinions of 2010 (SCCS/1348) and 2011 (SCCS/1446) on butylparaben when it is used as preservative in cosmetics products, both intended for adults and young children, need to be updated?*

3. Several Member States have highlighted that, despite the Commission's recommendation to avoid exposure to the sun of children below three years old, young children are exposed and they are protected from the harmful effects of the sunlight through the use of sunscreens. The SCCS is therefore asked to take into account in its assessment the information available about exposure to sunscreens, especially as far as children below three years old are concerned.

3. OPINION

3.1 Introduction

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 and potential endocrine related toxicity of *propylparaben* [1], *butylparaben* as well as 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 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 Opinion SCCS/1446/11).

In humans, on the other hand it is possible that parent (un-metabolized) 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; (Fisher et al. 1999),
- 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-, benzylparaben and pentylparaben.

[1] For reasons of clarity, in the context of this Opinion, the terms **propylparaben** and **butylparaben** refer to the linear-chained isomers **n-propylparaben** and **n-butylparaben**, respectively, unless otherwise specified.

In its last **Opinion SCCS/1446/11**, the SCCS responded 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 related (and continues to relate) to potentially increased susceptibility and exposure of children to certain potential endocrine disrupters such as propyl- and butylparaben compared to adults.

The SCCS considered the relevant age groups of children (from full-term newborns up to adolescents), their different stages of immaturity and maturation with age-dependent different susceptibilities and sensitivities compared to adults, in particular essential functional changes occurring in the period between the first week and the first few months after birth.

In this respect the SCCS extensively reviewed the following issues:

- The dermal exposure of the newborn and early infant, differences and risk factors that are different between adult and immature skin,
- The potential estrogenicity of p-hydroxybenzoic acid (PHBA, the common metabolite of parabens),
- The difference in metabolism of parabens in humans and in rodents,
- The immature metabolism of drug metabolizing enzymes converting parabens into inactive metabolites (PHBA or paraben conjugates) in newborns and in infants, and
- Recent biomonitoring data of parabens in humans.

The SCCS finally concluded (SCCS/1446/11):
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 confirmed and regarded to be very conservative. The view of the SCCS was additionally found to be supported 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. The current weight of evidence supports the view that the known metabolites of parabens, PHBA and conjugated parabens (glucuronides, sulfate esters), can be considered not to possess estrogenic potential, based on the outcome of experimental studies and SAR considerations. The conclusions continued:

"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.
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."

The previous opinions of the SCCP on the subject of parabens, which provide additional information, have been compiled in the list of references.

Sunscreens:

Finally, the SCCS recognised the Danish argument that high exposure to **sunscreens** for the age group of children up to 3 years can occur as a result of repeated use. However, the

SCCS stated that children of this age group should not be exposed to direct sunlight, and if exposed, should be covered by appropriate clothing [2]. Sunscreens then need only to be applied on those areas that are exposed to sun and that cannot be protected by clothing. The SCCS considered 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 Issues

3.2.1 Potential endocrine effects of parabens

Possible effects on the developing organism

After considering the main arguments of a recent review of Boberg et al. (2010), the SCCS stated in its Opinion (SCCS/1446/11): 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 (see Appendix 1). 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 the SCCP/SCCS was that of Fisher et al. (1999) with a NOEL of **2 mg/kg bw/day** for **butylparaben** (no other doses studied) in male juvenile rats after repeated subcutaneous application.

In other studies in female and male rodents, often (much) higher dose levels (several hundred up to 1200 mg/kg bw) were administered (see Appendix 1). In some of these studies, subcutaneous application of the test substance was chosen, which does not reflect human exposure. Dermal absorption and skin metabolism were, as such, not taken into consideration. Furthermore, when hormone levels or endocrine functions are found to be changed *in vitro* or *in vivo* it is often not clear whether the effects are adverse to the organism or not. These circumstances (and not the lack of any studies) make 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 considered 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).

In its Opinion (SCCS/1446/11), the SCCS responded in more detail on some particular aspects of the Boberg et al. (2010) review and the request of the Danish Authorities. These refer to the (non-)estrogenicity of the common metabolite PHBA and the paraben conjugates as well as the inhibition of sulfotransferases in human skin and liver by parabens, a mechanism that may contribute to the estrogenic effects of parabens.

3.2.2 Toxicokinetics and metabolism of parabens in humans and rodents

In its Opinion (SCCS/1446/11), the SCCS has re-assessed the role of metabolism of parabens, as there is increasing evidence that rats and humans markedly differ in this respect and that the rat appears to be a model of limited relevance when extrapolating the toxicokinetics of parabens to humans (reviewed by Boberg et al. 2009, 2010 and in the Opinions SCCS/1348/10 and SCCS/1446/11).

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

While parabens in rats are almost exclusively hydrolysed to PHBA in the skin after topical application and in the systemic circulation after oral or subcutaneous administration as well (Aubert 2009), free and predominantly conjugated parabens (glucuronides and sulfate esters) have been detected in biomonitoring studies in human serum or urine (reviewed in SCCS/1446/11, Annex 4; Buttke et al. 2012) and in experimental human studies after dermal application (Janjua et al. 2007 and 2008). These studies have been conducted in 26 young adult males with dermal repeated exposure to butylparaben at a daily dose of 10 mg/kg bw together with two phthalate esters each at the same dose for five days (for details see **Appendix 2**). The extent of hydrolysis to PHBA has not been quantified in the human studies. It is assumed that the parabens dermally taken up into the systemic circulation are in part further metabolized to PHBA and paraben 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 (reviewed in Annex 3 of the Opinion SCCS/1446/11).

The SCCS concluded with regards to the toxicokinetics and metabolism of parabens in humans and rodents:

The level of free parabens (free parabens are considered responsible for the toxicological effects) 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 the 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 newborns and infants up to 6 months of age when compared to adults after topical application of cosmetics containing parabens. In any case, the missing data regarding parabens metabolism in adult humans, neonates/newborns and early infants require particular consideration in the risk assessment.
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 has emphasized 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* or by an approach combining human *in vitro* data on the metabolism of parabens and toxicokinetic modelling. 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.

3.2.3. Dermal absorption and human exposures to parabens

(Text from SCCS 1348/10 and SCCS/1446/11, modified)
Dermal absorption studies and their shortcomings have been extensively reviewed and evaluated in previous opinions (summarized in SCCS 1348/10, section 3.3.1.) Until a properly conducted dermal absorption and toxicokinetic study in humans will allow the assignment of a more scientifically solid value, the SCCS will use a dermal absorption value of **3.7 %** in its MoS safety calculations.

Furthermore, in its previous opinions, the SCCS took the following parameters into account

for the final safety assessment of the parabens:

The SCCS could not determine an adequate NO(A)EL-value for the paraben esters under consideration from the studies in Appendix 1. Consequently, the NOEL value of 2 mg/kg bw/day, based on Fisher et al. (1999) remains the conservative choice for the calculation of the MoS of propyl- and butylparaben. The Committee acknowledged the fact that the Fisher et al. (1999) study involves subcutaneous instead of oral administration, but emphasized that 2 mg/kg bw/day clearly represents a NOEL instead of an NOAEL.

For the calculation of the SED the cumulative value of 17.4 g/day was used (SCCS Notes of Guidance, SCCS/1416/11), assuming that parabens were used as preservatives in all cosmetic products.

Thus, the following parameters for the final calculation of the MoS of butylparaben were used:

Dermal absorption:	3.7%
Intended concentration in finished product:	0.4%
Typical body weight:	60 kg
Cumulative exposure to preservatives:	17.4 g/day
NOEL (subcutaneous, rat, 17 days):	2.0 mg/kg bw/day

$$\text{SED} = \frac{17400 \text{ mg/day} * 0.4/100 * 3.7/100}{60 \text{ kg}} = 0.043 \text{ mg/kg bw/day}$$

MoS = NOEL / SED = 46.6

This means that, in order to obtain a MoS ≥ 100, the concentration of butylparaben in the finished cosmetic product would need to be reduced to **0.19%.**

Based on the exposure calculation made for adults in opinion SCCS/1348/10, an extrapolation has been 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)[3] 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 leave-on products: 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

MoS = NOEL / SED = 49

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

However, it is not realistic to assume that a child of three months is 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 [4] was requested to provide exposure data for children which might exist 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 this 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 of three months of age (5.3 kg bw):

Table 1

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)			

[4] European Cosmetics Association, now Cosmetics Europe

MOS	2393	112	275

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[5]; resulting in a daily applied amount of 123.2 x 5.3= 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.

In conclusion, 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 newborns 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, SCCS/1446/11, sections 3.2.1 and 3.3.3), 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 of 49 is not considered acceptable for this age group.

Rinse-off-products:

For rinse-off products, the MOS is considered sufficient both for body care products and for products for the nappy area (table 2).

Table 2

Rinse- off products		
	Body care products	**Products for buttock area**
Dermal absorption	3.7%	3.7%
concentration	0.19%	0.19%

[5] SCCS Notes of Guidance, § 4-2, Tab 3
http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_006.pdf

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

3.2.4 Biomonitoring studies: paraben levels in urine and plasma

Information on exposure to parabens can be derived from human biomonitoring studies. Concentrations in human biological fluids (e.g. urine, blood) 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.

The results of these studies (see SCCS/1446/11, Annex 4 for details and references) indicate that the (average) systemic exposure dose is considerably lower than estimated in the previous paraben opinion (SCCS/1348/10) 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.

3.3 The recent study on reproductive toxicity and toxicokinetics of propylparaben in juvenile male Wistar rats

Propylparaben has been described as having effects on sperm parameters and plasma testosterone concentrations of male rats following juvenile exposure (Oishi 2002a). In order to confirm and further characterize these effects, *in vivo* studies on the toxicokinetics and reproductive toxicity of propylparaben in male juvenile Wistar rats starting from PND 21 were conducted in 2010-2012 (Ricerca Biosciences 2011, 2012a, 2012b, 2012c, 2012d).

The project was initiated with regard to the safety assessment of marketed pharmaceutical products containing parabens and sponsored by the French Medicines Agency (AFSSAPS). An industry consortium of marketing authorization holders was associated with the project. The main study (Ricerca Biosciences, 2012d) and two analytical method validation studies were conducted under GLP in general compliance with FDA (2006) and EMA (2008) guidelines on reproductive toxicity testing and ICH guideline S3A (1994) on toxicokinetics. A pilot toxicokinetic study (Ricerca Biosciences SAS 2011) and a subsequent preliminary toxicokinetic study (Ricerca Biosciences SAS 2012a) lack GLP status, but were conducted according to the SOPs of the testing facility.

The **preliminary toxicokinetic study** was conducted in July 2010. The objectives of the study were to provide preliminary toxicokinetic data of propylparaben in the juvenile male rat (Wistar Crj: WI (Han) in order to define the optimal sampling time-points for a toxicokinetic investigation in a subsequent post-weaning juvenile toxicity study. The study was conducted according to the following design: Four dose levels for oral administration were selected (3, 10, 100, 1000 mg/kg bw, gavage). Group 1 animals (control) received the vehicle alone (1 % (w/v) hydroxyethylcellulose. Blood samples for the toxicokinetic evaluation were taken pre-dose, 5, 15 and 30 minutes, and 1, 2, 4, 8 and 24 hours after a single administration on post-natal day 31 (PND 31). Serum samples were acidified with 0.1 M formic acid and propylparaben analysed according to a validated method using an LC-MS/MS system and deuterated (ring-D4)-propylparaben as an internal standard. The toxicokinetic parameters were determined from the mean plasma concentrations by non-compartmental analysis. Linearity was assessed from AUC_{0-4h} and dose-proportionality was assessed from C_{max} and AUC_{0-4h}. Pharmacokinetic parameters for total (free and conjugated) propylparaben from treated groups were as follows:

Table 3

Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-4h} (ng.h/mL)
3	872	0.25	559
10	3135	0.25	2342
100	8664	0.0833	14172
1000	17183	0.5	39999

Total propylparaben appeared to be eliminated very rapidly following oral administration as suggested by the half-life values observed at 10 and 100 mg/kg which were 0.789 and 0.970 hours, respectively. The half-life for total parabens at the dose of 1000 mg/kg was not reported in the study but could be assessed to be about 3.5 hours from the individual data in Addendum 4 of the study. The increase of C_{max} was non-linear above 10 mg/kg and markedly less than dose-proportional at 100 and 1000 mg/kg. AUC_{0-4h} values were linear with dose up to 100 mg/kg bw whereas AUC_{0-4h} for the highest dose was too short for assessing linearity with dose because of the longer half-life at this dose. The conclusion was that plasma samples should be obtained around T_{max} (0.25 to 0.5 hours after dosing) and up to at least 8 hours after dosing.

Comment
It is not clear to which extent hydrolysis of esters and of conjugates occur under these conditions.

The objectives of the main **reproductive toxicity study** (Ricerca Biosciences, 2012d) were to determine the toxicity of the test item, propylparaben, following daily oral administration to the juvenile male Wistar rat from the age of weaning on post-natal day (PND) 21 through sexual maturation and up to 11 weeks of age (8-week treatment period) and to assess systemic exposure under the defined experimental conditions. The selected treatment period covers the juvenile (PND 21-35), peri-pubertal (PND 35-55), pubertal (55-70) and early adult stages in the male rat.

As in the Oishi (2002a) study, the study was performed in the same strain of juvenile male rat (Wistar Crj: WI (Han) and treatment started on PND 21. However, the duration of exposure was extended from 4 to 8 weeks (PND 77) and gavage (once daily) was used

instead of dietary admixture. Furthermore, a fourth dose level-group (low dose) was included in an attempt to determine a NOAEL. Additional animals were included to evaluate the reversibility of any toxic signs during a 26-week treatment-free period (to cover 3 spermatogenic cycles). Toxicokinetic groups were also included to assess systemic exposure under the defined experimental conditions. Additional endpoints such as histopathology and serum LH and FSH levels were included in order to determine the mechanisms of the awaited testicular and epididymal effects. The pathology data and evaluation were subjected to an external review.

Table 4

Group/Treatment	Nominal dose level (mg/kg/day)	Dose volume (mL/kg/day)	Nominal dose concentration (mg/mL)	Number of animals		
				Main group animals		Satellite animals for toxicokinetics
				Sub-group 1	Sub-group 2	
1. Control	0	10	0	10	10	9
2. Low dose	3	10	0.3	10	10	17
3. Low-mid dose	10	10	1	10	10	17
4. High-mid dose	100	10	10	10	10	17
5. High dose	1000	10	100	10	10	17

Sub-group 1 animals (see table) were necropsied at the end of the 8-week treatment period, sub-group 2 animals at the end of the 26-week treatment-free period.

Study specific precautions were taken in order to prevent contamination by parabens from products used by personnel such as cleaning liquids, shampoos, moisturisers, topical pharmaceuticals etc. The vehicle was 1 % (w/v) hydroxyethylcellulose 80-125 centipoises at 2 % in water for injection. Purity of the test substance, stability in the vehicle and homogeneity of the test suspension were controlled. The test item was applied once daily by gavage and Group 1 animals (controls) received the vehicle alone. For the analysis of testosterone, LH and FSH, blood samples of about 2 ml were taken from the retro-orbital sinus of all animals under isoflurane anaesthesia from the animals fasted for at least 14 hours in the morning of PND 78 and PND 79.

Study results:
No unscheduled deaths were observed. Clinical signs were restricted to transient post-dose hyper-salivation of animals of the high dose group, first noted on study day 9 (PND 30) and thereafter until the end of the treatment period, occasionally together with abnormal foraging. There was no influence of treatment on mean body weight gain in any group through to the end of the treatment period (study day 56) or treatment-free period (study day 237). Terminal mean body weight at the end of the treatment and treatment-free period was comparable with that in the concurrent control in all treated groups.
There was no influence of treatment on time of sexual maturation of the males in any group. Mean body weights on the day of occurrence of balano preputial skinfold cleavage (in average on PND 43-44) were comparable in all groups.
No influence of treatment on the levels of the measured hormones (LH, FSH and testosterone) was observed in any group. Isolated deviating findings were not dose-related and considered to be incidental.
There were no effects of treatment on mean sperm counts and motility parameters at terminal sacrifice and sacrifice after the treatment-free period, apart from one single finding in the low-mid (10 mg/kg) dose group after the treatment period and one in the high dose

recovery group. Both were associated with severe macroscopic and microscopic findings in testes or epididymes but were considered incidental because of the isolated occurrence. There were no body or organ weight differences that might indicate a treatment related effect. Occasional weight differences, including those with statistical significance between controls and treated animals were not dose-related and hence considered to be incidental or only to reflect normal individual variation.
At the end of the treatment period, the only effects of note were limited to minimal tubular atrophy/hypoplasia recorded in the right testis of three animals from the low dose group as well as in one animal from the high dose group. Severe tubular atrophy/hypoplasia of the right testis was sporadically recorded in one animal in the mid-low dose group, in correlation with soft testes in addition to small epididymides correlated with atrophy and aspermia.
At the end of the period free of treatment (26-weeks), findings of note were limited to occasional organ weight differences. One animal from the high dose group had small testes in correlation with severe hypo-spermatogenesis in the right testis.
In summary of the pathology investigations, daily oral administration of propylparaben in post-weaning juvenile male Wistar rats for 8 weeks followed by a 26-week treatment-free period did not result in test item-related macroscopic or microscopic changes in the testes and epididymides. There was no evidence of any treatment-related effect on testicular and epididymal weights or on sperm count and motility data in any of the treated groups.

In conclusion, the **NOAEL** of the study is **1000 mg/kg bw/day** for the treatment period of 8 weeks. The present study did not confirm the effects on the reproductive functions reported by Oishi (2002a).

The **satellite toxicokinetic study** by the oral route (gavage) in the juvenile rats was performed as follows (Ricerca Biosciences, 2012d):
The satellite animals were subjected to the same dosing regime as the main groups from day 0 (PND 21) to day 56 (PND 77). After the first dosing day 0 (PND 21), blood samples of approximately 0.4 mL (day 0) or approximately 1 mL (day 56) were withdrawn from a retro-orbital sinus under isoflurane anaesthesia. The animals were not fasted before sampling. Samples were taken as follows:

The blood samples were collected in tubes containing K_3-EDTA as anticoagulant and centrifuged at 4 °C. Plasma samples were stored deep-frozen (between -90° and -70 °C) until analysis. The satellite animals were killed and discarded without further examinations after the last blood sampling occasion.

Table 5

Time after dosing (hours)	0.25	0.5	1	4	8	24
First 3 animals/group[(1)]	+					
Second 3 animals/group[(1)]		+				
Third 3 animals/group[(1)]			+			
Fourth 3 animals/group				+		
Fifth 3 animals/group					+	
Last 2 animals/group						+

+: animals sampled.

(1): The control animals were sampled only at the 0.25, 0.5 and 1 hour time-points.

Samples were analysed according to a validated method using an LC-MS/MS system and deuterated (ring-D4)-propylparaben as an internal standard. Toxicokinetic parameters (at least the maximum observed concentration (C_{max}), time to reach C_{max} (T_{max}), area under the concentration-time curve (AUC), accumulation ratio and dose proportionality) were determined for total propylparaben (free and sulphate metabolite after enzymatic conversion by sulfatase from *Helix pomatia*, Sigma-Aldrich No. S9626 [6]) using a non-compartmental pharmacokinetic methodology.

Results:
No free or conjugated propylparaben was found in plasma from the control group. Toxicokinetic parameters from treated groups were as shown in the table below (table 6). Three out of 8 doses in the satellite toxicokinetic study were much lower than the nominal doses (see table 6) and were explained by the study authors due to homogeneity problems of the test substance in the vehicle suspensions. Toxicokinetic data in table 6 are related to actual doses.

Propylparaben was rapidly absorbed and plasmatic peaks rapidly appeared. For **total propylparaben** (free and conjugated), the maximum plasma concentrations were generally observed 0.25-0.5 hours after dosing. Total propylparaben plasma concentrations were quantifiable at least up to 8 hours at 100 and 1000 mg/kg/day.

On both PND 21 and PND 77, C_{max} values increased markedly less than dose-proportional between 100 mg/kg and the highest dose. On PND 21, the increase of AUC_{0-8h} values of total propylparaben between 3 and 1000 mg/kg/day can be considered dose-proportional. Corresponding values on PND 77 increased less than dose-proportional at the highest dose. The study authors explained this difference by maturation of the carboxylesterase(s) in the juvenile rats during adolescence (De Zwart et al 2008, Karanth and Pope 2000).

Plasma concentrations of **free propylparaben** were quantifiable only at 100 and 1000 mg/kg/day (LLOQ = 20 ng/mL). At 1000 mg/kg, they could be determined up to 8 hours after dosing on PND 21 and up to 1 hour after dosing on PND 77.

[6] This type of sulfatase also contains some ß-glucuronidase activity. Probably the metabolite propylparaben ß-glucuronide was also partly or completely hydrolysed under the conditions used.

On PND 21, at the highest dose applied, C_{max} was 1727 ng/ml and the concentration values for 4 and 8 h were 207 and 70.7 ng/mL. At this dose, no AUC value for free propylparaben on PND 21 was derived in the study report because the concentrations for the 0.5 h and 1 h samples were found outside the range of the validation criteria (both values reported between 200 and 1000 ng/ml). Despite these missing data in the study report, the AUC_{0-8h} for free propylparaben has been roughly estimated by the SCCS to be about 2600 ng x h/ml.
Whereas AUC values of total propylparaben apparently increased with dose in a proportional manner on PND 21, the increase in systemic exposure of free propylparaben was higher than dose-proportional between 47.0 (actual dose) and 1000 mg/kg/day: The AUC value increased by a factor of about 100 (compared to an increase in dose of about 20) suggesting beginning saturation of inactivating enzymes towards propylparaben at the highest dose on PND 21.
Also for free propylparaben, a decrease in systemic exposure was noted between PND 21 and PND 77 which was already seen for total propylparaben.

In conclusion, an accumulation of propylparaben during repeated dosing over 8 weeks could not be observed. In contrast, the systemic exposure to total and free propylparaben decreased between PND 21 and PND 77. The lower systemic exposure to total and free propylparaben observed on PND 77 may be attributable to an increase in carboxylesterase activity.

Comments

- It is not clear whether the glucuronide conjugate is completely hydrolysed under the conditions used (see footnote 6)
- Values outside the validation criteria (+/- 15%) are not available in the report of the satellite toxicokinetic study. This concerns some of the actual doses and several concentrations in the plasma.
- Analytical data on individual animals are not available in the satellite toxicokinetic study.
- The percentage of conjugates has not sufficiently been considered regarding the inactivation of propylparaben.
- The decrease of both total and free propylparaben between PND 21 and PND 77 underlines the predominating role of enzymatic hydrolysis of propylparaben by carboxylesterases on PND 77 compared to the conjugating enzymes.

Table 6

Occasion	Compound(s)	Nominal dose (Actual dose*) (mg/kg bw/day	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-8h} (ng*h/mL)	AUC_{0-24h} (ng*h/mL)
PND 21	Total propylparaben (free and conjugated)	3	786	0.25	408**	NC
		10 (5.71)	1,971	0.25	NC#	NC
		100 (47.0)	7,246	0.25	14,613	NC
		1000	25,003	0.5	148,840	243,348
PND 21	Free propylparaben	100 (47.0)	54.5	0.25	NC <30***	NC
		1000	1,727	0.25	NC# *2,600****	NC
PND 77	Total propylparaben (free and conjugated)	3	500	0.25	538	NC
		10 (7.80)	1,458	0.25	2,020	NC
		100	5,610	0.25	12,707	13,224
		1000	12,030	0.25	47,760	NC
PND 77	Free propylparaben	100	22.7	0.25	NC	NC
		1000	1,021	0.25	342	NC

NC not calculated in the study
NC# not calculated in the study since the 0.5 and 1 h value were outside the validated range.
*) Actual dose presented when it was outside +/-15% of the nominal dose
**) AUC_{0-1h} instead of AUC_{0-8h}
***) value assessed by the SCCS from data available in Addendum 7 of the study (see text)

General comments on

1) **the toxicokinetic studies**
- Urinary excretion of propylparaben and its metabolites was not investigated.
- A mass balance cannot be performed since the main metabolite PHBA was not determined.

2) **the reproductive toxicity study**

The GLP study on reproductive toxicity has been well conducted and is considered appropriate to refute the study of Oishi (2002a) which reported reproductive toxicity in juvenile male rats. The toxicokinetic data indicate a rapid and effective metabolism of

propylparaben after oral exposure due to rapid and effective hydrolysis of the substance by carboxylesterases. Inactivation of propylparaben by conjugating enzymes plays a minor role. This new data supplement previous data on the toxicokinetics of parabens in rats (e.g., Aubert 2009) and support the view that the metabolism in rats is obviously in a quantitative manner different from the available toxicokinetic data in humans. These toxicokinetic differences reinforce the previous concern of the SCCS on the use and relevance of the oral rat model with regards to the risk assessment of propyl- and butylparaben (see Discussion and Appendix 2). The study does not cover the potentially sensitive period after birth until PND 21.

3.4 Safety evaluation

As in its previous opinions, the SCCS takes the following parameters into account for the final safety assessment of the parabens:

Until a properly conducted dermal absorption and toxicokinetic study in humans will allow the assignment of a more scientifically solid value, the SCCS will use a dermal absorption value of 3.7% in its MoS safety calculations.

The SCCS could not determine an adequate NO(A)EL-value for the paraben esters under consideration from the studies in Appendix 1. Consequently, the NOEL value of 2 mg/kg bw/day, based on Fisher et al. (1999) remains the conservative choice for the calculation of the MoS of propyl- and butylparaben. The Committee acknowledged the fact that the Fisher et al. (1999) study involves subcutaneous instead of oral administration, but emphasized that **2 mg/kg bw/day** clearly represents a NOEL instead of an NOAEL For the calculation of the SED.

The cumulative value of **17.4 g/day** was used (SCCS Notes of Guidance, SCCS/1416/11), assuming that parabens were used as preservatives in all cosmetic products.

Thus, the following parameters for the final calculation of the MoS of butylparaben were used:

Dermal absorption:	3.7%
Intended concentration in finished product:	0.4%
Typical body weight:	60 kg
Cumulative exposure to preservatives:	17.4 g/day
NOEL (subcutaneous, rat, 17 days):	2.0 mg/kg bw/day

$$\text{SED} = \frac{17400 \text{ mg/day} * 0.4/100 * 3.7/100}{60 \text{ kg}} = 0.043 \text{ mg/kg bw/day}$$

MoS = NOEL / SED = 46.6

This means that, in order to obtain a MoS ≥ 100, the concentration of butylparaben in the finished cosmetic product would need to be reduced to **0.19%.**

3.5 Discussion

3.5.1 Evaluation of the recent study on the reproductive toxicity of propylparaben and its toxicokinetics in male juvenile Wistar rats

The reproductive toxicity study (Ricerca Biosciences (2012d) was conducted under GLP with the aim to confirm the study results of Oishi (2002a)[7] who observed effects on sperm parameters and plasma testosterone concentrations of juvenile male Wistar rats when exposing the rats for 4 weeks to **propylparaben** in doses of 12.4, 125 and 1290 mg/kg bw per day in food. Therefore, a similar study design including the use of the same rat strain was chosen with some modifications (gavage instead of application by food) and additional testing, e.g., some additional hormonal parameters described in Section 3.3. However, virtually no effects on the endocrine or reproductive functions of the rats were found, hence the effects observed in the Oishi study (2002a) could not be confirmed and the NOAEL has been set at 1000 mg/kg bw/day. Although not a guideline study, in agreement with the study objectives, the study can be considered valid with regards to the investigation of reproductive toxicity. However, the *relevance* [8] of the study for human risk assessment is limited because of the rapid and effective metabolism in rats unlike to humans (for details see Appendix 2 and discussion below).

Similar results have been obtained in a previous study with **butylparaben** (Charles River 2005; later published as **Hoberman et al., 2008**) also attempting to confirm the data of an Oishi study (Oishi 2001). However, the study has been considered having severe shortcomings which raised doubts on the *reliability* [9] of the study (SCCS/1348/10 and previous Opinions).

In addition to the reproductive toxicity part of the recent study, accompanying toxicokinetic studies and data provide additional information on the systemic fate of the parent compound **propylparaben** after oral exposure of rats. After oral application by gavage, propylparaben was rapidly and efficiently metabolized by the rats: In both toxicokinetic studies (Ricerca Biosciences 2012a, 2012d), T_{max} values of 0.5 h or less were observed for total parabens (free and conjugated) and 0.25 h for free propylparaben, respectively.
On PND 21, the first day of exposure, the AUC_{0-8h} value for free propylparaben at 100 mg/kg bw. on PND 21 has been estimated to be below 30 ng*h/ml which is considered a very low value (<0.08% of the dose orally absorbed) given the high oral bioavailability of the compound at this dose (about 85% determined by Aubert 2009 in a study with SD rats).
Likewise, at the highest dose, the AUC_{0-8h} value of about 2600 ng * h/ml for free propylparaben is also considered very low (about 0.3% of the dose orally absorbed). Even markedly lower C_{max} and AUC values of free propylparaben in rat plasma were found on PND 77 after an exposure of the rats to the highest dose of propylparaben for 8 weeks (AUC_{0-8h} 342 ng x h /ml corresponding to 0.04% of the dose orally absorbed). This even more effective metabolism of propylparaben after repeated exposure can be explained by maturation of rat carboxylesterases or another adaptive stimulation of enzymatic hydrolysis of propylparaben.
Total propylparaben accounted for approximately 15-21% of the dose orally absorbed both on PND 21 and PND 77 with the exception of the highest dose on PND 77 where only about 6% total propylparaben was determined.
The main metabolite PHBA was not determined in this study as PHBA formed from parabens probably as it cannot be distinguished from other sources of exposure such as food where it may be found as a natural component.

In conclusion, this data indicate that propylparaben is rapidly and very efficiently metabolized in rats after single or repeated oral exposure. Depending on the oral dose, about 80-94% of propylparaben was inactivated by enzymatic hydrolysis and about 15-20%

[7] The Commission could not retrieve the original data of the Oishi studies.

[8] According to KLIMISCH criteria

[9] According to KLIMISCH criteria

by conjugating enzymes. This data is useful, as it consistently supplements previous data on the toxicokinetics of propyl- and butylparaben in rats which is discussed in the next section.

3.5.2 Other data on toxicokinetics and metabolism of parabens in rats

In this section, additional information is given on toxicokinetics focusing on metabolism of parabens in rats *in vivo* and in rat tissues *in vitro*. Furthermore, in **Appendix 2**, available data *in vivo and in vitro* is evaluated whether a read-across of the toxicokinetics of propyl- and butylparaben in rats is possible and whether a comparison of rat data with propylparaben/butylparaben and human toxicokinetic data with butylparaben can be made.

Rapid and efficient metabolism of methyl- propyl- and butylparaben has been observed in a toxicokinetic study using dermal, oral or subcutaneous (only butylparaben) administration in SD rats (Aubert 2009). Ring-^{14}C labelled parabens were used. Independent from the paraben and the way of application, the only metabolite detected in plasma and urine was ^{14}C-PHBA. As shown in Appendix 2 in detail, the toxicokinetic data of propyl- and butylparaben were similar and comparable irrespective of the route (dermal or oral). A major difference between the Aubert (2009) study and the recent study is the determination of free and total propylparaben in the recent study (Ricerca 2012d), as free and total propylparaben have not been analysed in the Aubert study. This difference may be due to different methodological approaches and sensitivities/specificities of analytical tools.

Harville et al. (2007) have shown that propyl- and butylparaben in rat skin fractions are both hydrolyzed at similar rates [10] and three orders of magnitude more rapidly than in human skin fractions. Propyl- and butylparaben were also hydrolysed at a about 10-fold higher rates in rat liver fractions compared to human liver. Independent on the tissue fraction studied, similar rates of hydrolysis have been found with both propyl- and butylparaben. In another study it was shown that kinetic characteristics of the esterases in rat skin S9 fraction suggest that even high concentrations of butyl paraben applied to the skin are unlikely to saturate metabolism (Leazer, 2004; Hoberman et al. 2008).

Taken together, despite the marked differences of enzymatic hydrolysis between rat and human tissue fractions observed, *in vitro* enzyme kinetics in skin and liver fractions of rats and humans suggest that propyl- and butylparaben are both hydrolysed at similar rates in each of the fractions and in the respective species. *In vitro* and *in vivo* data in rats consistently suggest that, with respect to toxicokinetics read-across between propyl- and butylparaben can be justified.
Furthermore, the toxicokinetic data of the recent study is consistent with previous toxicokinetic data in rats and provide additional data on the occurrence of free and total propylparaben which both have not been detected in the previous study of Aubert (2009).

In addition to the previous data, the recent toxicokinetic data support and confirm earlier concerns of the SCCS on the limited relevance of the oral rat model because of the rapid metabolism of propyl- and butylparabens in rats compared to humans.

3.5.3 Evaluation of toxicity studies in rodents in the light of the recent study data

Available studies have been compiled and summarized in Appendix 1.
Experimental studies of basic research on endocrine effects or mode of action of a substance *in vivo* often use i.p., i.v. or s.c. administrations aiming to achieve rapid and effective systemic exposure of the organism to the substance. For instance, such studies

[10] "Similar" means in this context that the hydrolysis rates *in vitro* differed by less than 20% between propyl- and butylparaben.

using s.c. administrations have been conducted with parabens to elucidate the endocrine potentials or mode(s) of action of the substances (see Appendix 1). However, such administrations imply the circumvention of physiological barriers and with regards to parabens do not represent the normal ways of human exposure considered in this Opinion.

Several studies using subcutaneous exposure of rodents to parabens have clearly shown estrogenic effects on reproductive organs or functions of rodents (see Appendix 1). Mostly, high doses based on mg/kg bw/day were applied which lead to much higher systemic exposures when compared with oral exposures on a mg/kg bw/day basis. Therefore, although studies using subcutaneous exposure may be in principle valuable means for determining inherent toxic potentials (hazards) or modes of actions of chemical substances, these studies are not per se considered as suited for quantitative risk assessment (unless the systemic exposure under s.c. conditions has been determined). Usually, subcutaneous studies are not the best choice for performing risk assessment and should be avoided when more adequate data are available. However, in the absence of more adequate data, as in the case for parabens, the NOAEL derived from such subcutaneous studies may be used as it is very conservative.

Some previous oral studies with propyl- or butylparaben in rodents were reported to show endocrine potential or reproductive toxicity effects at low doses, in particular those of Oishi (2001, 2002a , 2002b). These studies are considered not *reliable*, as raw data are not available and some studies conducted under similar experimental conditions and under GLP with oral application even at high doses up to 1000 mg/kg bw/day were without effects (Charles River 2005, later published as Hoberman et al. 2008; Ricerca Biosciences 2012a-d).
As discussed above, metabolic inactivation of parabens in rats is rapid and effective. The resulting low systemic exposures to free parabens after oral exposure may protect the rats from potential adverse effects of parabens.
In conclusion, the oral rat model is of limited *relevance* for human risk assessment. Moreover, the oral rat model may be misleading when applied to human risk assessment; the available oral rat studies on potential endocrine/oestrogenic effects cannot be used to demonstrate that dermal exposure to parabens does **not** pose a risk to humans.

3.5.4 Comparison of rat and human data on propyl- and butylparaben

Parabens topically applied to the human skin are absorbed, partly/predominantly metabolized in the skin and during systemic circulation (mainly in the liver) and rapidly excreted into the urine, presumably largely as p-hydroxybenzoic acid (PHBA, the non-oestrogenic metabolite) and probably also as glucuronides and sulfate esters. The interplay between the three main metabolic inactivation pathways (ester hydrolysis, glucuronidation and sulfonation of the parent parabens), determines the level of free parabens in the body. It is expected that the level of systemic exposure to free parabens determines the potential endocrine modulating activity of these compounds. Insofar, the main inactivating metabolic pathways play a critical role in the availability of free parabens in the body of adults. With respect to inactivating metabolic pathways, age differences between neonates/newborns, infants, and adults need to be evaluated.

A comparison of rat and human data is difficult, as adequate data on metabolism and toxicokinetics of parabens in humans is insufficient.

Uncertainties relate to data gaps and questionable data on

- dermal uptake/absorption of parabens by human skin *in vivo and in vitro*,
- dermal and systemic metabolism of parabens in humans, in particular in neonates/newborns and early infants,
- systemic exposure to free parabens as seen in biomonitoring studies, in particular

the contribution of carboxylesterases to the inactivation of parabens and
- human exposure to parabens in cosmetic products,

The **dermal uptake/absorption** by human skin and related studies *in vitro* and *in vivo* have been extensively discussed in previous Opinions of the SCCP/SCCS. As before and as a layer of conservatism, the SCCS will use the value of **3.7 %** for dermal uptake/absorption.

Whereas the **metabolism** of parabens in rats after dermal or oral uptake is well known, data from humans is scarce (reviewed in SCCS/1446/11). As discussed above, *in vitro* kinetic data in skin fractions from rats and humans suggest that parabens in rat skin are much more rapidly hydrolysed by carboxylesterases than in human skin. Whereas the proportion of PHBA formation by enzymatic hydrolysis of absorbed parabens in humans is unknown, oral toxicokinetic studies in rats have shown that parabens are predominantly and very efficiently hydrolysed to the main metabolite PHBA. It is unknown to what extent other inactivating enzymes such as UDP-glucuronosyltransferases (UGTs) and sulfotransferases (STs) can compensate for presumed lower activities of carboxylesterases in humans. This concern relates in particular to neonates/newborns and early infants due to their immature carboxylesterases below 1 year of age and some of their immature UGT or ST enzyme forms at least below 6 months of age.

A **human toxicokinetic study** has been conducted in 26 young adult males with dermal repeated exposure to butylparaben at a daily dose of 10 mg/kg bw together with two phthalate esters each at the same dose for five days (Janjua et al. 2007, 2008). An attempt has been made by the SCCS to compare the toxicokinetic data of this study with those from the toxicokinetic oral studies with propylparaben in juvenile rats described above (Ricerca Biosciences 2012a and d) (discussed in more detail in **Appendix 2**) as read-across between the two substances is considered justified. The comparison of the AUC values in blood reveals that the systemic exposure to free butylparaben in human males at a dermal dose of 10 mg/kg/day is similar to that in juvenile male rats at a 100-fold higher oral dose of 1000 mg/kg bw propylparaben (about 1600 ng*h/ml in humans versus about 2600 ng*h/ml in juvenile rats).It seems likely that rats metabolise propyl- and butylparaben in a much more rapid and effective way than humans. However, the comparison of both the human and rat study is difficult for several reasons and the differences and uncertainties should be carefully discussed; the question is whether the surprisingly similar systemic exposures of rats and humans to free paraben at 100-fold different external doses can be explained by the following identified differences of the study conditions:

- Dermal exposure in humans is compared with oral exposure of rats
- Butylparaben was used in the human study versus propylparaben in the rat study
- Concomitant dermal application of two phthalate esters at high doses together with butylparaben in the human study.

As discussed in Appendix 2, only the latter may contribute to an meaningful higher internal dose to the paraben and only in case of a high inhibition of inactivating enzymes (>80%) by the two phthalate esters in human skin. Although such high inhibition would be not be expected this cannot be excluded.

Another uncertainty to be mentioned is the unrealistic high dose of butylparaben in the *in vivo* dermal absorption study in humans. The external dose was 10 mg/kg bw/d whereas the external dose from a a concentration of 0.19% (concentration recommended by the SCCS) resembles only 0.55 mg/kg bw/d (factor 18 lower) [11]. Compared to this worst case

[11] 17.4 g cosmetic products applied/day x 0.19% parabens = 33 mg/day = 551 µg/kg bw/day. The corresponding daily dose of maximally permitted parabens in cosmetic products (0.4%) would be about 70 mg/day or 1.2 mg/kg bw/day.

exposure assessment by the SCCS a refined aggregate exposure assessment yielded in part considerably lower estimates (Cowan-Ellsberry and Robison 2009). As discussed in section 3.2.3 and Appendix 2, adequate data on the range and average dermal exposure of consumers to propyl- and butylparaben using typical concentrations in cosmetic products is missing.

As discussed in **Appendix 2**, the similar systemic exposures of rats and humans to free paraben at 100-fold different external doses can be explained by markedly different toxicokinetics between the species. Hence, a MoS derived on a toxicokinetic basis would be more adequate than the derivation of a conventional MoS which could even be misleading. A MoS based on toxicokinetic data from the human and the recent rat study would be far below 25. Due to missing human exposure data on parabens in cosmetic products it is uncertain whether a MoS of 25 can be achieved. However, it should be taken into account that the range and average dermal exposure of consumers to propyl and butylparaben is much lower than the exposure used in the study of Janjua et al. For these reasons, uncertainties of risk assessment remain, which at present cannot be resolved.

In **biomonitoring** studies, free parabens and their conjugates have been detected in human serum/plasma and urine (reviewed in SCCS/1446/2011). Concentrations in human biological fluids account for both dietary intake (e.g. from foods with paraben preservatives) and dermal applications of products with parabens; according to Soni et al. (2005) the latter is considered to be the major contributor. As there is evidence that parabens do not accumulate in humans (Janjua 2007, 2008) the sum of free and conjugated parabens in urine may provide hints on human exposure to parabens. However, it should be noted that the amount of p-hydroxybenzoic acid (PHBA) formed in the systemic circulation from the fraction of parabens absorbed from human skin is unknown and yet remains to be determined. Therefore, any calculations considering only free and conjugated parabens do not take into account the amount of parabens hydrolyzed to their common (assumed major) metabolite p-hydroxybenzoic acid (PHBA) after reaching the systemic circulation. This may lead to an underestimation of the internal exposure of humans to free parabens absorbed from human skin. Moreover, the proportion of parabens (and PHBA in food) taken up by the oral route is unknown.

There is evidence that paraben exposure is much higher among women than among men in studies that are probably representative for the US (Calafat et al. 2010). Recent data on girls aged 12-16 years suggest a similar or even higher exposure to methyl- and propylparaben compared to adult woman in the US (Buttke et al. 2012). For this female age group an average daily exposure of about 20 µg/kg bw for the sum of methyl- and propylparaben (both total, i.e. free and conjugated) can be derived. Other parabens may also be taken up but their amounts are normally much lower that that of methylparaben which has been found the predominating paraben in urine samples from the US and Europe. Thus, the results of the biomonitoring studies support the view that the worst case exposure calculation made in the Opinion SCCS/1446/11 (see footnote 11 and section 3.2.3) overestimates consumer exposure even if PHBA as a major metabolite formed from parabens absorbed from human skin would be taken into account. It has also to be noted, that the use levels of parabens in the USA are not regulated and might be higher than in Europe.

Taken together, although the biomonitoring data suggest a sufficient margin compared to the calculated worse case exposure, uncertainties remain with regard to the amount of parabens absorbed from human skin because the extent of PHBA formed from parabens in the systemic circulation is unknown and yet remains to be determined.

In conclusion, all the above data including the recent data confirms and reinforces previous doubts of the SCCS whether the rat is a *relevant* model for testing effects of parabens after oral exposure because of marked species differences in metabolism.

The study which is at the origin of this new SCCS review is an oral rat study concerned with reproductive toxicity of propyl paraben. It shows no effects on the reproductive parameters in rats. This study does not add nor takes away the previous concerns expressed by the SCCS with respect to the lack of scientific sound data on the pivotal link between dermal exposure to rats and humans, in particular in relation to the metabolism of the parent compounds in the skin. The latter can only be addressed through the generation of human data.
As the conclusions, drawn in both previous opinions, were made with a conservative approach, and relevant age groups from full-term newborns up to adolescents were considered, there is no new argument to change these.

3.6 Comments on the use of sunscreen

Exposure to sunlight is correlated with the occurrence of skin cancer. Consequently, it is important to protect our skin from childhood onwards and educational programs with respect to correct sunscreen use can play an important role to prevent over-exposure to sunlight which increases the risk of skin cancers (Sancho-Garnier et al. 2012). Sunscreen use can indeed reduce the occurrence of solar keratoses and of squamous cell carcinoma. Its effect, however, on basal cell carcinoma is not clear. A number of studies have shown that sunscreen use can even be associated with a higher risk of nevus, melanoma and basal cell carcinoma (Autier et al. 2007). This occurred when sun exposure was intentional, namely with the desire to acquire a tan and to spend as long as possible time in the sun with as much skin exposed as possible (Autier 2009, Autier et al. 1997, 2000, 2007).

The Australasian College of Dermatologists recommended that children up to 6 months of age should not be exposed to direct sunlight. However, the use of sunscreens in small children is advised when sun exposure cannot be avoided by other means, including shade, adequate clothing and wide-brimmed hats which are the best measures to protect small children. Sunscreens are then applied in skin areas which are not protected by the clothes (Balk 2006). The American Academy of Pediatrics also recommended the use of sunscreens on children of less than 6 months on small areas of skin, if adequate clothing and shade are not available (Balk 2006). These are conclusions provided in a recent review of the most relevant articles indexed between 1999-2012 in Medline/PubMed on photoprotection in childhood (Criado et al. 2012). It was further said that for children up to 2 years of age, the use of physical sunscreens is preferable since they are less allergenic in comparison with chemical screens (Criado et al. 2012).

Sunscreen should be applied before the skin is exposed to the sun and reapplied every 4 hours or earlier in case of excessive sweating or if intense contact with water occurs. The recommended amount of sunscreens was 2mg/cm², stating that one can expect that in reality less than half of the recommended amount will be applied (Criado et al. 2012). This is in line with the amounts mentioned in the SCCS Notes of Guidance, 8th revision, in which whole body values between 0.5 and 1.3 mg/cm² were reported (p. 72). Gottlieb et al (1997) have found average amounts of 1.3 mg/cm² for various body regions and using different galenic formulations, applied under controlled conditions. They also mention that in routine use, lower amounts are to be expected. Of particular interest, with respect to sun protection is, that they could not detect a change in measured SPF when different amounts of sunscreens were applied on human volunteers. They applied 1.0, 1.3, and 2.0 mg/cm² of 5 different sunscreens with SPFs of 4, 8, 10, 15 and 29, respectively (Gottlieb et al. 1997). No significant difference was observed in comparison with the manufacturer-determined SPFs. These results suggest that sunscreens can offer maximal protection even if applied on skin in less than the quantities that have been used during the experimental setting (2mg/cm²) to determine the SPF for labeling of the product (Gottlieb et al. 1997).

Studies carried out with sunscreen with SPF 15 and using effectively 2mg/cm² showed that the synthesis of active vitamine D was reduced in 98% of the cases studied (Sambandan and Ratner 2011), leading to a debate with respect to potential vitamine D3 deficiency and the importance of acquiring the necessary vitamine D through diet.

In the SCCS Notes of Guidance, 18g sunscreen is recommended as an average value to be used per day/ per person during periods of sun exposure. This value is only indicative and not absolute as one has to consider that sun protection of the skin will depend on many variables such as the SPF of the product, galenic formulation, its chemical composition, spreading of the product, skin penetration, location on the body, skin temperature, age, gender, phototype, presence of skin hair, previous sun exposure, genetic predisposition, etc It is up to the Responsible Person to bring cosmetic products, in this case sunscreens, on the EU market that are safe for the consumers and to take care of special groups such as children (Regulation N°1223/2009).

- In the case of an adult person, 18 g is recommended in the Notes of Guidance on a surface of 17500 cm², thus per day for the whole body;

- For a 3 month old child with a mean body surface of 3100 cm², 18 g would be an excessive amount. If one uses indeed 2 mg/cm² over the whole body (which is not recommended over the whole body surface, see above), 6.2 g is needed per application;

- For children up to 2 years old a maximum body surface of 5000 cm² is present. Use of 2 mg/cm² over the whole body would result in 10g product per application. As the napkin zone usually is still protected by napkins and not exposed to sun light, the amount needed would be much less.

- Children of 9 to 10 years have a skin surface of about 11000 cm². They will already want to apply sun products themselves on sun exposed parts. In a recent German study it was shown that children's own sun protection knowledge increases with age, while their sun protection behaviour develops the opposite way, already significantly visible at younger age (6 years) (Li et al. 2011). Therefore, when 3/5 of the surface is covered with the measured amount of 1.3 mg/cm²(Gottlieb et al. 1997), twice a day would need 11.4 g sunscreen.

Seen the above, the SCCS is of the opinion that the use of 18 g sunscreen per day/person during the limited periods per year of intended sun exposure represents a realistic amount which is protective as well for babies, children and adults.

4. CONCLUSION

1. Taking into consideration recent data, does the SCCS consider that its opinions of 2010 (SCCS/1348) and 2011 (SCCS/1446) on propylparaben when it is used as preservative in cosmetics products, both intended for adults and young children, need to be updated?

2. Taking into consideration recent data, does the SCCS consider that its opinions of 2010 (SCCS/1348) and 2011 (SCCS/1446) on butylparaben when it is used as preservative in cosmetics products, both intended for adults and young children, need to be updated?

Recent data confirms that the toxicokinetics of parabens in rats and humans differ considerably. The concerns of the SCCP/SCCS expressed previously and reiterated in recent Opinions remain unchanged and reinforced after the evaluation of both the reproductive toxicity and the toxicokinetic studies on propylparaben recently submitted to the SCCS. The same data were extrapolated for the evaluation of the risk by butylparaben exposure.

The additional submitted data does not remove the concern expressed in the previous opinions on the relevance of the rat model for the risk assessment of parabens. Although much toxicological data on parabens in rodents exists, adequate evidence has not been provided for the safe use of propyl- or butylparaben in cosmetics. For these reasons, the SCCS reiterates its previous conclusions and requests regarding an improvement of the data, in particular

a) on the exposure of humans including children to propyl- and butylparaben in cosmetic products and
b) the toxicokinetics of propyl- and butylparaben in humans.

3. Several Member States have highlighted that, despite the Commission's recommendation to avoid exposure to the sun of children below three years old, young children are exposed and they are protected from the harmful effects of the sunlight through the use of sunscreens. The SCCS is therefore asked to take into account in its assessment the information available about exposure to sunscreens, especially as far as children below three years old are concerned.

The SCCS has reviewed the available data on human exposure to sunscreens for: infants 3 month old, other groups of children up to the age of 10 years as well as adults. The SCCS is of the opinion that the use of 18 g sunscreen per day/person during the limited periods per year of intended sun exposure represents a realistic amount which is protective as well for babies, children and adults. The SCCS emphazises the need that children up to 6 months of age should not be exposed to direct sunlight but should be protected from sunlight by use of appropriate means such as adequate clothing, shade etc. If these measures are followed, sunscreens are then applied only in skin areas which are not protected by the clothes.

5. MINORITY OPINION

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APPENDIX 1

Table 1: Data on estrogenicity-related properties and toxicity of parabens

Test substances	Test system	Test principle(s)	Result(s) and major SCCP/SCCS comments	Reference
***In vitro* assays**				
MePB EtPB PrPB BuPB	MCF-7 cells (**human**-breast cancer derived cell line shown to be estrogen responsive)	Principle of gene expression profiling based on DNA microarray analysis with 120 genes selected as showing greater statistical reliability for estrogen-responses.	Clear difference in expression profile between EtPB and PrPB. The activity showed a positive correlation with the chain length of esters. Clear correlation between profiles of PrPB and BuPB. Nevertheless, profiles of PrPB and BuPB were closer to each other than the estrogen profile was to any of them.	Terasaka et al. 2006
MePB EtPB PrPB BuPB PHBA	Skin and liver cytosol and **human** epidermal keratinocytes	Parabens elevate estrogen levels by inhibiting estrogen sulfotransferases (SULT) in skin	SULT activity was inhibited in skin cytosol by MePB, EtPB, PrPB, BuPB, **not** by PHBA. Potency increased with chain length (IC_{50} BuPB = 37 µM). No inhibition of androgen sulfation. In the human epidermal keratinocytes, BuPB displayed an IC_{50} of 12 µM. No positive control was included.	Prusakiewicz et al. 2007
MePB PrPB BuPB PHBA flutamide vinclozolin	a stably transfected **human** embryonic kidney cell line that lacks critical steroid metabolizing enzymes	Investigate anti-androgenic activity by measuring inhibition of 0.1 nM testosterone (T)-induced transcriptional activity	MePB, PrPB, BuPB inhibited 0.1 nM T-induced transcriptional activity at concentrations above 10 µM (max. 40% inhibition). PHBA was negative. Pos. controls (flutamide and vinclozolin) inhibited 1nM T-induced signal at concentrations of 0.1 to 10 µM (11 to 90% inhibition).	Chen et al. 2007

Test substances	Test system	Test principle(s)	Result(s) and major SCCP/SCCS comments	Reference
MePB EtPB PrPB BuPB IsoPrPB IsoBuPB BzPB PHBA 17β-oestradiol	MCF-7 cells (**human**-breast cancer derived cell line shown to be estrogen responsive)	Investigate estrogenic effects of mixtures of parabens on cell proliferation; investigate anti-estrogenic effect through inhibition of aromatase, the enzyme that converts androgens into estrogens	EtPB, PrPB, BuPB, IsoPrPB, IsoBuPB and BzPB induced cell proliferation with EC_{50} values between 0.5 and 10 µM. PHBA was negative. Assays with mixtures of PB showed an additive effect. Potency of PB remains 5 to 6 orders of magnitude below that of 17β-oestradiol. Parabens inhibited aromatase with IC_{50} values between 3.5 and 26.4 µM, but there was no link between chain length and IC_{50}. PHBA was negative. Authors note that typical human PB concentrations (10-80nM) are much lower than EC_{50} and IC_{50} values encountered here.	van Meeuwen et al. 2008

EtPB BuPB	Human adrenocortical carcinoma cell line **rat** pituitary GH3 cell line	H295R assay evaluating the ability to interfere with steroid hormone biosynthesis and T-screen assay to define whether the compound is either a thyroid hormone receptor agonist or antagonist by investigating binding and activation of the thyroid receptor (TR), resulting in GH3 cell proliferation	Progesterone production was increased in H295R assay at 30 µM EtPB and BuPB. No effect on testosterone or oestradiol production. No positive control included. BuPB increased cell proliferation in GH3 rat cells at 3 µM; considered potential weak TR-agonist. No positive control included.	Taxvig et al. 2008
***In vivo* experiments: female rodents**				
MePB BuPB	Alpk:AP **rats**	Uterotrophic assay with immature rats. MePB and BuPB were administered on PND 21-22 once daily for 3 consecutive days at the following dosage levels: - MePB orally at 40, 400 and 800 mg/kg/day - MePB subcutaneously (sc) at 40 and 80 mg/kg/day - BuPB orally at 4, 40, 400, 800 and 1200 mg/kg/day - BuPB subcutaneously at 40, 200, 400, 600, 800, 1000 and 1200 mg/kg/day Uterotrophic assay with ovariectomized (OVX)rats (8-10 weeks old): - MePB subcutaneously (sc) at 800 mg/kg/day - BuPB subcutaneously at 800, 1000 and 1200 mg/kg/day	Immature rat model: MePB administered sc or orally failed to increase uterus weights up to 80 and 800 mg MePB/kg/day, resp.. BuP given orally failed to increase uterus wet and dry weights at dose levels up to 1200 mg BuPB/kg/day, whereas subcutaneous administration increased uterus wet weights at dosages ≥ 400 mg/kg/day. The lowest dosage level inducing any uterotrophic response was 200 mg BuPB/kg/day (sc) (increase of dry weight). OVX rat model: increased uterus weights only at ≥ 800 mg/kg BuPB (sc). The positive control oestradiol exerted its effects at an oral dose of 0.4 mg/kg or 0.04 mg/kg/day (sc). SCCS comment: No guideline study. Effects observed only after s.c. application. See discussion, section 3.5.3.	Routledge et al. 1998
IsoBuPB	CD1 **mice**	Uterotrophic assay with IsoBuPB in the mouse at following subcutaneous dosage levels (supposing a mouse of 18 days old weighs about 30g) of: - 40 mg/kg/day (1.2 mg/mouse) - 400 mg/kg/day (12 mg/mouse)	Wet uterine weight was increased at both dosage levels. Positive control 17β-oestradiol exerted comparable effects at 167 ng/kg/day (5 ng/mouse). SCCS comment: No guideline study. Effects observed after s.c. application. See discussion, section 3.5.3.	Darbre et al. 2002
MePB EtPB PrPB BuPB 17β-oestradiol (E2)	CD1 **mice** Wistar **rats**	Uterotrophic assay with both immature and ovariectomized adult mice and immature rats. Animals were subcutaneously (sc) treated for three consecutive days with different molar equivalent doses ranging from 3.62 to 1086 micromol/kg body weight of parabens (PBs) or E2 (0.036 micromol/kg). Estrogen receptor binding affinities of PBs relative to E2 were determined.	In mice, ED50 of E2 for increase in uterine weight was 7 µg/kg bw, ED50 of PBs were from 18 to 74 mg/kg bw. In rats, ED50 of PBs were from 33 to 338 mg/kg bw. NOELs for uterotrophic activity of PBs in immature mice were 0.6-6.5, in ovariectomized mice 6-55, and in immature rats 16.5-70 mg/kg bw, respectively. In the estrogen receptor binding assay, PBs except MePB competed with E2 and Ki values correlated to their estrogenic activity SCCS comment: No guideline study. Effects observed after s.c. application. See discussion, section 3.5.3.	Lemini et al., 2003

BuPB	Sprague Dawley **rats**	Developmental study according to OECD guideline. Oral gavage, 0, 10, 100 and 1000 mg/kg bw/day on gestation days 6-19. Foetuses examination on gestational day 20, developmental parameters measured	At the highest dose, maternal food consumption reduced during exposure time, weight gain reduced on days 18-20. No developmental parameters changed. Developmental NOEL: 1000 mg/kg/day. Maternal NOAEL: 100 mg/kg/day SCCS comment: Guideline study. Study valid for risk assessment of developmental effects. Recent toxicokinetic data indicate low systemic exposure to BuPB even at high doses and raise doubt about the study.	Daston ert al. 2004
EtPB BuPB	Wistar **rats**	Study of the effect of parabens on the steroidogenesis in rats and their offspring when dams are subcutaneously exposed to either: - 400 mg EtPB/kg/day; or - 200 - 400 mg BuPB/kg/day from gestation day 7 to 21.	Neither EtPB nor BuPB showed any treatment-related effects on testosterone production, anogenital distance, or testicular histopathology. BuPB caused a significant decrease as well in the mRNA β-ER expression level in fetal ovaries, as in mRNA expression of steroidogenic acute regulatory protein and peripheral benzodiazepine receptor in the adrenal glands. However, these effects show no dose-dependency. SCCS comment: No guideline study. Effects observed after s.c. application. See discussion, section 3.5.3	Taxvig et al. 2008
IsoBuPB	Sprague Dawley **rats**	Study designed to clarify the estrogenic effects during gestation and lactation on the endocrine systems of dams and offspring by measuring - in dams: plasma hormone concentrations and organ weights - in offspring: ratio of male pups, anogenital distance, organ weights and plasma hormone concentrations, puberty, estrous cycle and response of organ weight and plasma hormone concentrations to estrogen in adult females, and reproductive and adrenal function in adult males. Exposure occurred via silastic capsule implanted subcutaneously. No dosage level(s) stated.	Maternal exposure to IsoBuPB showed to decrease the plasma corticosterone concentration and to increase the uterus weight in dams as well as the uterine sensitivity to estrogen in adult female offspring. All other indices examined were unaffected by the treatment. No positive control was included. SCCS comment: No guideline study. Effects observed after s.c. application. See discussion, section 3.5.3	Kawaguchi et al. 2009
IsoBuPB	Sprague Dawley **rats**	Study designed to analyze the effects of maternal IsoBuPB treatment on the emotional behavior and learning performance in mature offspring. Exposure occurred via silastic capsule implanted subcutaneously. No dosage level(s) stated. 'Estimated dose' is 4.36 mg/kg bw/day	Early exposure to IsoBuPB may increase anxiety, and specifically disturb passive avoidance performance, although the effects are male-specific. Other parameters were unaffected and no signs of overt toxicity were noted. SCCS comment: No guideline study. Effects observed after s.c. application. See discussion, section 3.5.3	Kawaguchi et al. 2009b

PrPB BuPB IsoPrPB IsoBuPB 17α-ethinyl oestradiol	Sprague Dawley immature female **rats**	Uterotrophic assay. Subcutaneous injection of 62.5-250-1000 mg/kg bw/day of paraben for 3 days. Investigation of Calbindin-D9-k (CaBP-9k), biomarker for estrogenic effects.	Sc injection of 1000 mg/kg/day induced increased uterine wet weight for BuPB, IsoBuPB and IsoPrPB (also for pos. control at 1 mg/kg/day). The effect was blocked by addition of anti-estrogen fulvestrant, indicating estrogen receptor-dependent pathway. At the highest dosage level, parabens also increased the expression levels of uterine CaBP-9k through progesterone-receptor involved pathways. SCCS comment: No guideline study. Effects observed after s.c. application. See discussion, section 3.5.3	Vo and Jeung 2009
BuPB PrPB 17β-oestradiol	CF-1 and CD-1 female **mice**	Subcutaneous injection of 0-1.4-14-271-407-542-813-949 mg BuPB/kg/day, of 0-949-1084 mg PrPB/kg bw/day on day 1 to 4 of gestation. Additional uterotrophic assay with BuPB at 0-20-200-949 mg/kg/day in two different mice strains. 14 mg/kg/day 17β-oestradiol was administered as positive control in both assays.	Sc injection of BuPB did not affect any of the measured parameters, such as the number of pups born, litter weights, individual pup weight and pup survival. Sc injection of PrPB did not affect any of the measured parameters, including the number of intrauterine blastocyst implantation sites. 17β-oestradiol terminated all pregnancies. The uterotrophic assay revealed that BuPB did not affect uterine wet or dry mass at any dose in either strain. 17β-oestradiol consistently increased uterine mass in both strains. SCCS comment: No guideline study. Effects observed after s.c. application. See discussion, section 3.5.3	Shaw and de Catanzaro 2009

MePB EtPB PrPB BuPB IsoPrPB IsoBuPB 17α-ethinyl oestradiol	Mated Sprague Dawley female **rats**	*In vivo* assay to investigate whether oral-subacute exposure to PB may induce suppressive effects on reproductive organs in female rats during the critical juvenile-peri-pubertal stage. Oral-subacute administration by gavage of 62.5-250-1000 mg/kg bw/day of paraben from postnatal day 21 to 40. Investigation of Calbindin-D9-k (CaBP-9k), biomarker for estrogenic effects.	<u>1000 mg/kg/day:</u> MePB, IsoPrPB: decreased ovary weight MePB, EtPB, PrPB: increased adrenal weight EtPB, IsoPrPB: decreased kidney weight, reduced serum oestradiol levels MePB, BuPB: increased thyroid gland weight IsoBuPB: decrease of corpora lutea, increase in no. of cystic follicles, myometrial hypertrophy PrPB: myometrial hypertrophy <u>All dosage levels:</u> BuPB: increased liver weight (no dose-response relationship) BuPB, IsoBuPB: decrease of corpora lutea, increase in no. of cystic follicles, myometrial hypertrophy (no dose-response relationship) All PB: changes in T_4 serum levels (no dose-response relationship) The SCCS observed that the responses are not dose related. A LOAEL cannot be derived. <u>IC_{50} values for binding ERα and ERβ receptors:</u> 17β-estradiol: 3.10^{-9} M IsoBuPB: 2.10^{-6} M BuPB: 5.10^{-6} M IsoPrPB: 2.10^{-5} M PrPB: 2.10^{-5} M EtPB: 5.10^{-5} M MePB: too low to be calculated SCCS comment: No guideline study. Recent toxicokinetic data indicate low systemic exposure to BuPB even at high doses and raise doubt on the relevance of the study.	Vo et al. 2010

MePB PrPB BuPB 17ß-oestradiol (E2)	Neonatal Sprague Dawley female **rats**	Effects of neonatal exposure to PBs on development of early follicle stages and ovarian factors regulating follicular development and steroidogenesis after subcutaneous administration of MePB, PrPB or BuPB at doses of 62.5, 250 or 1000 mg/kg bw/day or 17ß-oestradiol (40 µg/kg/day) once daily on PND 1-7. Ovaries were excised on PND 8 and prepared for histopathology. Follicles were counted and classified regarding their developmental stages. Relative mRNA expression of the following proteins was determined by quantitative real-time PCR: calbindin-9k (CaBP-9k, indicator of estrogenic activity in rat uterus), ovarian anti-Mullerian hormone (AMH), kit ligand/stem cell factor (KITL) and forkhead box protein I2 transcription factor (FoxI2), all three associated with follicle development in rat as well as the steroidogenic enzymes steroidogenic acute regulatory transport protein (StAR) and CYP11a1.	<u>Effects at 62.5 mg/kg/day and above:</u> MePB, PrPB: mRNA levels of StAR decreased (dose-response relationships) <u>Effects at 250 and 1000 mg/kg/day:</u> PrPB, BuPB: CaBP-9k (dose-response relationship) PrPB, BuPB: decreased numbers of early primary follicles (dose response relationship) MePB: increased numbers of primary follicles (no dose response relationship) PrPB, BuPB: mRNA levels of AMH and FoxI2 increased (both not affected by E2) (no dose response relationship) BuPB: mRNA level of KITL enhanced (dose response relationship) BuPB: mRNA levels of StAR decreased (dose-response relationships) MePB: mRNA levels of CYP11a1 decreased (dose-response relationships) PrPB, BuPB: mRNA levels of CYP11a1, mid-dose increased, high dose decreased (no dose-response relationships) <u>Effects only at 1000 mg/kg/day:</u> BuPB: increased ovary weight PrPB, BuPB: increased numbers of primordial follicles <u>SCCS comments:</u> LO(A)EL (sc) for MePB, PrPB: 62.5 mg/kg bw/day LO(A)EL (sc) for BuPB: 250 mg/kg bw/day Not all data appear consistent. Comment: No guideline study. Effects observed after s.c. application. See discussion, section 3.5.3	Ahn et al. 2012
***In vivo* experiments: male rodents**				

BuPB	Wistar **rat**	Effects of neonatal exposure to BuPB on development of rat testis after subcutaneous administration of 2 mg BuPB/kg/day for 17 days (postnatal days 2-18). Other substances tested were diethylstilbestrol (DES), ethinyloestradiol (EE), bisphenol A, genistein, octylphenol.	DES and 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. Only one dose of BuPB (2 mg/kg bw/day was tested with no detectable effect on any of the measured reproductive parameters (testis weight and histological examination). Comment: No guideline study. Effects observed after s.c. application. See discussion, section 3.5.3	Fisher et al. 1999
BuPB	Sprague Dawley **rats**	Study of the effect of BuPB on the development of the reproductive organs of F1 offspring when pregnant rats are subcutaneously injected with 100 or 200 mg BuPB/kg/day from gestation day 6 to postnatal day 20 (lactation period).	At both dosage levels, the weights of testes, seminal vesicles and prostate glands were decreased, together with the sperm count and the sperm motile activity in the epididymis. Testicular expression of estrogen receptor (ER)-α and ER-β mRNA was significantly increased at the highest dosage level. Comment: No guideline study. Effects observed only after s.c. application.	Kang et al. 2002
BuPB	Wistar **rat**	Study of the potential reproductive effects of BuPB on male rats (19-21 days old), receiving BuPB through the oral route for 8 weeks at dosage levels of 10.4, 103 and 1026 mg/kg/day.	There were no treatment-related effects on testes, ventral prostates and preputial glands in any of the groups. Decreases in cauda epididymal sperm reserve, sperm count, daily sperm production and in serum testosterone concentration were observed from 10.4 mg/kg/day onwards (LOAEL). Comment: No guideline study. Study refuted by Charles River (2005) study, later published as Hoberman et al. (2008). Recent toxicokinetic data indicate low systemic exposure to BuPB even at high doses and raise doubts on the methodology and the relevance of the study for risk assessment.	Oishi 2001
PrPB	Wistar **rat**	Study of the effects of PrPB on general function of the male rat reproductive system. Rats (19-21 days old) received PrPB through the oral route for 4 weeks at dosage levels of 12.4, 125 and 1290 mg/kg/day.	There were no treatment-related effects on testes, epididymides, ventral prostates, seminal vesicles and preputial glands in any of the groups. At all three dosage levels, however, a decrease in cauda epididymal sperm reserve, sperm count and daily sperm production was observed and from 125 mg/kg/day on, serum testosterone concentration was decreased. LOAEL: 12.4 mg/kg/day. Comment: No guideline study. Study refuted by Ricerca Biosciences (2012a-d) studies. Recent toxicokinetic data indicate low systemic exposure to PrPB even at high doses and raise doubt on the relevance of the study for risk assessment. .	Oishi 2002a

BuPB	CD-1 ICR **mice**	Study of the effects of BuPB on general function of the male mouse reproductive system. Mice (25-27 days old) received BuPB through the oral route for 10 weeks at dosage levels of 14.4, 146 and 1504 mg/kg/day.	Administration of BuPB at 146 and 1504 mg/kg/day caused an increase in epididymal weights, a decrease in testis spermatid count and in serum testosterone concentration. The NOAEL is stated to be 14.4 mg/kg/day. Comment: No guideline study. Refuted studies in rats raise doubts on the methodology of the study. No data on toxicokinetics of parabens in mice available.	Oishi 2002b
MePB EtPB	Wistar **rat**	Study of the effects of parabens on testosterone secretion and the function of the male reproductive system in rats receiving the test substances orally at dosage levels of ± 100 and 1000 mg/kg/day. Rats were 25-27 days old and received the parabens for 8 weeks.	MePB and EtPB did not affect the male reproductive system including anti-spermatogenic activity to about 1000 mg/kg/day (NOEL).	Oishi 2004
MePB BuPB	Wistar **rat**	Repetition of the Oishi study (2001) under GLP with MePB or BuPB using the same strain of rats but 16 instead of 8 animals per dose group, same dosage levels of 0, 100, 1000 and 10,000 ppm in food. In addition of the Oishi study, blood samples were weekly taken for the analysis of LH (luteinizing hormone), FSH (follicle-stimulating hormone) and testosterone	There were no treatment-related effects on testes, ventral prostates and preputial glands in any of the groups. Unlike Oishi (2001), sperm parameters were found unaffected. With both MePB and BuPB, the highest dose level in food corresponds to approximately 1100 mg/kg bw/day (NOEL). Comment: No guideline study but GLP. Recent toxicokinetic data indicate low systemic exposure to BuPB even at high doses and raise doubt on the relevance of the study for risk assessment	Charles River 2005; later published as Hoberman et al. 2008

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Table 2: Overview of dermal absorption studies with parabens submitted to the SCCP/SCCS

Test substances	Test system	Test principle(s)	Result(s) and major SCCP/SCCS comment(s)	Reference
***In vitro* assays**				
BuPB	Full thickness **human** skin (1000 µm) 6 samples	Measurement of dermal absorption through human skin of BuPB at 0.4% in an o/w emulsion, applied at 8-10 mg/cm² and left in contact with skin for 24h.	Absorbed dose (%): Receptor fluid: 21.01 ± 6.95 Receptor wash: 0.49 ± 0.16 Skin (excl. tape strips): 36.92 ± 4.97 TOTAL: 58.42 ± 10.39 The authors state that the principle metabolite, PHBA, was detected in de the receptor fluid and that unmetabolised BuPB could only be detected in 1 of the 6 samples at a concentration below 0.67%. SCCP major comments: - insufficient skin samples used - only one concentration tested - ratio metabolised / unmetabolised Butylparaben only measured in receptor fluid, not in skin compartments - solubility of BuPB in receptor fluid (HEPES buffer + 3.75% BSA) not demonstrated	Fasano 2004a
BuPB	Full thickness **human** skin (1587-1983 µm) 10 samples from 2 donors	Measurement of dermal absorption through human skin of BuPB at 0.4% in an o/w emulsion, applied at 8-10 mg/cm² and left in contact with skin for 24h.	Absorbed dose (%): Receptor fluid: 14.90 ± 3.73 Receptor wash: 0.32 ± 0.14 Skin (excl. tape strips): 14.80 ± 4.67 TOTAL: 30.10 ± 7.08 The authors state that the principle metabolite, PHBA, was detected in de the receptor fluid and that unmetabolised BuPB could only be detected in 5 of the 10 samples with a mean concentration of 0.225%. SCCP major comments: - insufficient skin samples used - ratio metabolised / unmetabolised Butylparaben only measured in receptor fluid, not in skin compartments - only one concentration tested - solubility of BuPB in receptor fluid (HEPES buffer + 3.75% BSA) not demonstrated	Fasano 2005

Test substances	Test system	Test principle(s)	Result(s) and major SCCP/SCCS comment(s)	Reference
BuPB MePB	**Rat** and **human** skin (450 µm) 10 samples from ≥ 3 donors	Measurement of dermal absorption through rat and human skin of MePB and BuPB in an o/w emulsion, at 0.8% and 0.4% respectively, applied at 8-10 mg/cm² and left in contact with skin for 24h.	<u>Absorbed dose rat skin (%):</u> MePB / BuPB Receptor fluid: 54.94 ± 5.92 / 54.23 ± 5.92 Receptor wash: 0.43 ± 0.20 / 0.44 ± 0.20 Skin (excl. tape strips): 12.23 ± 5.57 / 13.01 ± 5.57 TOTAL: 67.61 ± 6.06 / 67.69 ± 9.06 52-54% of penetrated amount accounted for PHBA, whereas 24% (MePB) or 5.5% (BuPB) accounted for the unmetabolised paraben. EtPB was, in both cases, also measured in the receptor fluid. <u>Absorbed dose human skin (%):</u> MePB / BuPB Receptor fluid: 79.36 ± 15.62 / 73.51 ± 10.34 Receptor wash: 0.46 ± 0.11 / 0.72 ± 0.21 Skin (excl. tape strips): 4.88 ± 2.01 / 6.92 ± 1.77 TOTAL: 84.69 ± 15.46 / 81.15 ± 10.65 33-35% of penetrated amount accounted for PHBA, whereas 60% (MePB) or 50% (BuPB) accounted for the unmetabolised paraben. EtPB was, in both cases, also measured in the receptor fluid. <u>SCCP major comments:</u> - insufficient skin samples used - only one concentration tested - solubility of BuPB in receptor fluid (HEPES buffer + 3.75% BSA) not demonstrated	Fasano 2004b
BuPB	Full thickness **pig** skin N° of skin samples not stated	Measurement of dermal absorption through pig skin of BuPB in an o/w lotion at 0.5%, applied at 8-10 mg/cm² and left in contact with skin for 24h.	Epidermis: unmetabolised BuPB measured Dermis: 50% unmetabolised BuPB + 50% PHBA Receptor fluid: only PHBA measured. <u>SCCS major comments:</u> - description of test is not detailed enough - only one concentration tested - no data on solubility of BuPB in receptor fluid - confusing report, mixing percentages with amounts/cm²	Pape and Schepky 2009

Test substances	Test system	Test principle(s)	Result(s) and major SCCP/SCCS comment(s)	Reference
***In vivo* experiments**				
BuPB, combined with diethyl and dibutyl phthalate	**Human** male volunteers	5 day daily whole body topical 2 mg/cm² application of a skin cream containing 2% BuPB, 2% DEP and 2% DBP. BuPB levels measured in serum, together with reproductive hormones: - follicle stimulating hormone (FSH) - lutenising hormone (LH) - testosterone - oestradiol - inhibin B And thyroid hormones: - thyroid stimulating hormone (TSH) - free thyroxine (FT_4) - total triiodothyroxine (T_3) - total thyroxine (T_4)	Free BuPB was detected in serum after 1 hour (rapid uptake with peak of 135 µg/l after 4h). AUC value of free BuPB for the first 24 h was about 1600 ng*h/ml. No effect was noticed on a number of relevant hormone levels, such as TSH, LH, oestradiol, Inhibin B, T_4 and FT_4. <u>SCCP major comment:</u> The results are obtained from a combined test of BuPB with two phthalates, which does not represent ideal test conditions to investigate the specific paraben concerned.	Janjua et al. 2007
BuPB, combined with diethyl and dibutyl phthalate	**Human** male volunteers	Exposure conditions see Janjua et al. 2007 (see just above). BuPB levels measured in urine. Twenty-four-hour urine samples were daily collected. Analysis of urinary total BuPB (free and conjugated) by LC MS/MS, apart from phthalatesters and their metabolites	Concentrations of total BuPB (free and conjugated) reached plateau values in urine about 24 h after application. Total BuPB excreted in urine in the treatment week was about 2.6 mg/24 h. On average 0.32% of the applied dose were recovered. <u>SCCP major comments:</u> The major metabolite p-hydroxybenzoic acid PHBA was not determined. Total BuPB may be underestimated as BuPB sulphate was not determined. The results are obtained from a combined test of BuPB with two phthalates, which does not represent ideal test conditions to investigate the specific paraben concerned.	Janjua et al. 2008

Test substances	Test system	Test principle(s)	Result(s) and major SCCP/SCCS comment(s)	Reference
MePB PrPB BuPB	Sprague Dawley **rats**	Study of the absorption, plasma kinetics, body distribution, metabolism (determination of plasma metabolites) and excretion of [^{14}C-ring]-MePB, -PrPB and –BuPB. Oral and dermal administration of 100 mg/kg of MePB, PrPB and BuPB and sc administration of 100 mg/kg of BuPB.	<u>Oral administration</u> High and rapid (C_{max} at 0.5 hrs) uptake of radioactivity in serum for all three parabens. Elimination after 8 to 22 hrs. <u>Dermal administration</u> Relatively low and slower (C_{max} at 8 hrs) uptake of radioactivity in serum for all three parabens. Elimination after 12 to 22 hrs. <u>Sc administration</u> (only BuPB) High and relatively rapid (C_{max} at 2-4 hrs) uptake of radioactivity in serum for all three parabens. Elimination after 12 to 22 hrs. Plasma metabolite characterisation revealed only one metabolite, namely PHBA, independent of time of collection, paraben type and route of administration. The study revealed that the principal route of excretion was via the urine and that no selective organ / tissue storage was observed.	Aubert 2009

APPENDIX 2

Comparison of data on toxicokinetics and metabolism of parabens in rats and humans

1. Comparison of data on toxicokinetics and metabolism of propyl- and butylparabens in rats

Rapid and efficient metabolism of **methyl- propyl- and butylparaben** has been observed in a toxicokinetic study using dermal, oral or subcutaneous (only butylparaben) administration in Sprague Dawley rats (Aubert 2009). Ring-^{14}C labelled parabens were used each at a dose of 100 mg/kg bw. Despite the paraben and the way of application, the only metabolite detected in plasma and urine was ^{14}C-PHBA.
In the following, this data is evaluated to see whether a read-across of the toxicokinetics of propyl- and butylparaben is possible. After oral exposure, elimination of propylparaben in urine and faeces accounted for 85% in males and 74% in females, respectively. Corresponding values for butylparaben were 82% in males and 74% in females, respectively. For both parabens, excretion in faeces accounted for 1-3% of the oral dose. The summary kinetic data in blood is depicted in table 1. The data suggest more a gender effect than major differences between propylparaben and butylparaben. The toxicokinetics of propyl- and butylparaben appear similar and the AUC values in males after oral administration only differ by about 25%. Unexpectedly, even the AUC values after dermal exposure do not much differ between propyl- and butylparaben.

In conclusion, the parabens investigated were rapidly metabolized to the common main metabolite PHBA and the toxicokinetics of propyl- and butylparaben in Sprague Dawley rats were similar irrespective the dermal or oral exposure.

Table 1
Summary of kinetic parameters of parabens in blood of rats (Aubert 2009)

Route	Group	Test item	Sex	C_{max} (ng-eq/g)	t_{max} (h)	$AUC_{(0-t)}$ (ng-eq.h/g)
Dermal	1	METHYL-PARABEN	M	3146	1	20452
			F	1707	8	20791
	2	PROPYL-PARABEN	M	693	8	5421
			F	1033	8	6390
	3	BUTYL-PARABEN	M	986	1	12216
			F	614	8	9760
Oral	7	METHYL-PARABEN	M	26592	1	82153
			F	38664	0.5	143630
	8	PROPYL-PARABEN	M	11432	0.5	58344
			F	42280	0.5	118154
	9	BUTYL-PARABEN	M	15229	0.5	73585
			F	21040	0.5	99336
Subcutaneous	13	BUTYL-PARABEN	M	6501	2	52033
			F	12189	4	88917

M: male; F: female.

Harville et al. (2007) have shown shown that propyl- and butylparaben in rat skin fractions are both hydrolyzed at similar rates [12] and three orders of magnitude more

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rapidly than in human skin fractions. Propyl- and butylparaben were also hydrolysed at about 10-fold higher rates in rat liver fractions compared to human liver. Independent on the tissue fraction studied, similar rates have been found with both propyl- and butylparaben.
In another study it was shown that kinetic characteristics of the esterases in rat skin S9 fraction suggest that even high concentrations of butyl paraben applied to the skin are unlikely to saturate metabolism (Leazer, 2004; Hoberman et al. 2008).

In conclusion, *in vitro* enzyme kinetics in skin and liver fractions of rats and humans suggest that propyl- and butylparaben are both hydrolysed at similar rates in each of the fractions, despite the marked differences of enzymatic hydrolysis between rat and human tissue fractions observed. *In vitro* and *in vivo* data consistently suggest that the toxicokinetic data of propyl- and butylparaben in rats are comparable in terms of a read-across.

2. Comparison of toxicokinetics and metabolism of parabens in rats and humans

A human toxicokinetic study has been conducted in 26 young adult males with dermal repeated exposure to butylparaben at a daily dose of 10 mg/kg bw together with two phthalate esters each at the same dose for five days (Janjua et al. 2007, 2008).
The young adult males in the human study were whole body exposed to a cream (in average 40 g per day, 20 mg/cm^2) containing butylparaben and two phthalate esters, each in a concentration of 2%, once per day for 5 days. The daily applied amount of butylparaben corresponds to about 0.8 g at an average body weight of 80 kg of the males. This exposure is considered an extreme exposure to paraben exceeding the worst case of normal use [13] by a factor of 8.6 in adults and 4.3 in a child of 3 months of age when based on body weight, respectively. In a more realistic manner, this experimental exposure is 10- to 20-fold higher than the worse case of daily exposure of early infants based on Colipa data (0.6-1.4 g leave-on products per day corresponding to 2.4 - 5.6 mg dermal paraben exposure or 0.5 - 1 mg/kg bw/day) considered in section 3.2.3 of the Opinion. In human serum, up to 4 hours after the dermal application, concentrations of butylparaben were in the range of 100-135 ng/ml and decreased to about 18 ng/ml after 24 h, just before the next dermal application occurred. It is assumed that free butylparaben has been determined. Under this assumption and under the experimental conditions used, the SCCS has determined the half-live of butylparaben in serum to be about 7 hours. The AUC_{0-24h} of free butylparaben in human serum on the first day of exposure has been estimated by the SCCS to be about 1600 ng x h/ml. During the consecutive exposure days 3 and 5, AUC_{0-24h} values of 500-600 ng x h/ml of free butylparaben were determined, probably due to an adaptive response of inactivating esterases or conjugating enzymes. No effects of butylparaben (or the two phthalate esters and their metabolites) on serum hormonal levels were observed during the exposure time of 5 days, although the exposure conditions are considered markedly exceeding a worst case of normal use.

"Similar" means in this context that the hydrolysis rates *in vitro* differed by less than 20% between propyl- and butylparaben.

[13] Given the cumulative exposure to preservatives used in all cosmetic product categories is considered to be 17.4 g/day for adults and the allowed concentration of parabens is 0.4% in all leave-on products (see section 3.2.3), then the amount of parabens that may be daily applied to skin of adults is about 0.07 g or 1.16 mg/kg bw. For a child of 3 months of age (5.3 kg and a surface area 0.31 m^2) the cumulative exposure to leave-on products would result in 17.4 *0.31/1.75= 3.08 g/day (see section 3.2.3) and 12.3 mg or 2.3 mg/kg bw paraben exposure per day, respectively.

A comparison of the above dermal exposure study to butylparaben (10 mg/kg bw/day) in human males with the toxicokinetic data of the recent study in juvenile male rats (Ricerca Biosciences 2012d) reveals that the systemic exposure to free paraben in human males is similar to that in juvenile male rats when the 100-fold higher oral dose of 1000 mg/kg bw in rats is considered: In the rats, at the highest dose, an AUC_{0-8h} value of about 2600 ng * h/ml for free propylparaben (about 0.3% of the dose orally absorbed) has been assessed by the SCCS (see sections 3.3 and 3.5.1 of the Opinion) whereas a corresponding AUC value of about 1600 ng * h/ml has been assessed in the above human study with butylparaben.

However, the comparison of both the human and rat study is difficult for several reasons and the differences and uncertainties should be carefully discussed; the question is whether the surprisingly similar systemic exposures of rats and humans to free paraben at 100-fold different external doses can be explained by the following identified differences of the study conditions:

1) Dermal exposure in humans is compared with oral exposure of rats:
It is not unusual to compare dermal human data with rat oral data as the latter model is a standard model for risk assessment of ingredients. Dermal absorption in humans occurs slowly resulting in lower C_{max} values and longer T_{max} values compared with oral exposure in rats. It is expected in case of parabens that the dermal absorption in humans is much lower (assumed 3.7% by the SCCS) than the oral absorption in rats which is about 80-85% for both propyl- and butylparaben (Aubert 2009).

2) Butylparaben in the human study versus propylparaben in the rat study:
In the human toxicokinetic study, butylparaben has been used whereas propylparaben has been used in the oral study with juvenile rats. However, the toxicokinetic data of propyl- and butylparaben in the rats do not much differ as shown above, be it after oral or after dermal application. Possible differences between the toxicokinetics of propylparaben in juvenile Wistar rats and SD rats (in the Aubert 2009 study) should also be taken into account including potential differences in the formation/detection of paraben conjugates found in the recent study but not in the Aubert study; however, these differences are considered less important.

3) Concomitant dermal application of two phthalate esters at high doses together with butylparaben:
It is conceivable that the phthalate esters a) hamper the dermal absorption of the paraben or b) inhibit the enzymatic hydrolysis and/or conjugation of the paraben. In the first case the systemic exposure to the paraben would be lower, in the second case higher than in absence of the phthalate esters. Thus, both mechanisms would act into different directions. Only in case the inhibition of inactivating enzymes was high (>80%) this could contribute to an enhanced systemic exposure to butylparaben in a quantitatively meaningful manner. Although such high inhibition would be not be expected, this cannot be excluded.

Another uncertainty to be mentioned is the unrealistic high dose of butylparaben in the *in vivo* dermal absorption study in humans. The external dose was 10 mg/kg bw/d whereas the external dose from a a concentration of 0.19% (concentration recommended by the SCCS) resembles only 0.55 mg/kg bw/d (factor 18 lower) [14].. Compared to this worst case exposure assessment by the SCCS a refined aggregate exposure assessment yielded considerably lower estimates (Cowan-Ellsberry CE and Robison SH 2009). As

[14] 17.4 g cosmetic products applied/day x 0.19% parabens = 33 mg/day = 551 µg/kg bw/day. The corresponding daily dose of maximally permitted parabens in cosmetic products (0.4%) would be about 70 mg/day or 1.2 mg/kg bw/day.

discussed in section 3.2.3 and Appendix 2, adequate data on the range and average dermal exposure of consumers to propyl- and butylparaben using typical concentrations in cosmetic products is missing.

Taken together, there is no convincing argument that can explain the similar systemic exposures of rats and humans to free paraben at 100-fold different external doses by the identified differences of the study conditions, either single or in combination. Rather, the available data is more compatible with the assumption that the difference is based on markedly different toxicokinetics in rats and humans. Hence, a MoS derived on a toxicokinetic basis would be more adequate than the derivation of a conventional MoS. A MoS based on toxicokinetic would be below 25. Due to missing human exposure data on parabens in cosmetic products it is uncertain whether a MoS of 25 can be achieved even if it was taken into account that the range and average dermal exposure of consumers to propyl and butylparaben is probably much lower than the dose used in the study of Janjua et al. For these reasons, uncertainties of risk assessment remain which presently cannot be resolved.