Scientific Committee on Consumer Safety

SCCS

# OPINION ON

# Parabens

COLIPA n° P82

The SCCS adopted this opinion at its 9th plenary on 14 December 2010

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.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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

SCCS

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

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## 1. BACKGROUND

4-Hydroxybenzoic acid, its salts and esters ("parabens") are currently authorised in Annex VI, entry 12 of the Cosmetics Directive (76/768/EEC) at a maximum use concentration of 0.4% (acid) for one ester and 0.8% for a mixture of esters.

Between January 2005 and June 2008, the Scientific Committee on Consumer Products (SCCP) adopted four opinions on parabens:

- The first opinion (SCCP/0874/05) addressed parabens and breast cancer: "*Extended Opinion on parabens, underarm cosmetics and breast cancer"* and concluded that *according to the current knowledge, there is no evidence of a demonstrable risk for the development of breast cancer caused by the use of underarm cosmetics*.
- The second opinion "(SCCP/0873/05) was "*An extended opinion on the Safety Evaluation of parabens*" with the following conclusions:

  "***Methyl- and ethylparaben***

  *For the methyl and ethyl p-hydroxybenzoic acid esters, the maximum authorised concentrations remain unchanged.*

  ***Propyl-, isopropyl-, butyl- and isobutylparaben***

  *As the present discussion is based solely upon data in the literature, it is the SCCP's opinion that more information is needed in order to formulate a final statement on the maximum concentration of propyl-, isopropyl-, butyl- and isobutylparaben allowed in cosmetic products. More specifically, the following data are requested before end of March 2005:*

  *- full descriptions of available in vitro percutaneous absorption studies;*

  *- a complete dossier with regard to the reproductive and developmental toxicity of propyl, isopropyl, butyl and isobutylparaben, with special focus on the male reproductive system."*
- The third opinion (SCCP/1017/06) was adopted by the SCCP in October 2006 and concluded that the tests provided in Submission I of February 2006 contained too many shortcomings in order to be considered as scientifically valid and that the conclusion of opinion SCCP/0873/05 remained unchanged.
- After consultation of the SCCP, new data were submitted by Colipa, leading in June 2008 to adoption of the fourth SCCP opinion (SCCP/1183/08) concluding: "*As already concluded in earlier opinions, methylparaben and ethylparaben are not subject of concern.*

  *The SCCP is of the opinion that, based upon the available data, the safety assessment of propyl- and butylparaben cannot be finalised yet. Parabens are important cosmetic preservatives and they have wide use in multiple product types.*

  *Since no unequivocal conclusion can be drawn with regard to the contradictory reproductive toxicity studies available, of which none appears to be scientifically acceptable, the SCCP welcomes the proposal made by industry to conduct further work in the field of skin penetration/metabolism and pharmacokinetics to further support existing data. It is, however, recommended to supplement the envisaged studies in the rat with toxicokinetic studies in human volunteers after dermal application of representative cosmetic products containing propyl- and butylparaben, since these may deliver essential information.*

*In case significant systemic exposure to propyl- and/or butylparaben is measured in the requested human volunteer study, a rodent 2-generation toxicity study may be unavoidable, although it is the opinion of the SCCP that this should only be performed as a last resort.*

*Safety data need to be provided for all authorised parabens, including iso-alkyl- and phenylparabens."*

- In November 2009 Denmark submitted the report "Survey and Health Assessment of the exposure of 2-year-olds to chemical substances in Consumer Products" published by the Danish EPA (2009) for evaluation by the SCCS together with the expected new data from Colipa.
- In December 2009 Colipa submitted a pharmacokinetic study on methyl-, propyl- and butylparaben (Aubert 2009) together with the justification of the decision not to conduct a study on human volunteers. No data for other 4-hydroxybenzoic acid, its salts and esters ("parabens") such as iso-alkyl- or benzylparaben were submitted.
- In February 2010 the Danish Authorities submitted a report by the Danish National Food Institute, DTU: *Update on uptake, distribution, metabolism and excretion (ADME) and endocrine disrupting activity of parabens 2009*. In the meantime it has been published as an article of Boberg et al. (2010).

## 2. TERMS OF REFERENCE

1. *Does the SCCS consider the continued use of propyl- and butylparaben in a concentration up to 0.4% for one ester or 0.8% when used in combination in cosmetic products safe for the consumer taken into consideration the provided scientific data?*
2. *Does the SCCS consider the continued use of methyl- and ethylparaben in a concentration up to 0.4% for one ester or 0.8% when used in combination in cosmetic products is influenced in anyway taken into consideration the new provided scientific data?*
3. *Does the SCCS consider the continued use of isopropyl-, isobutyl- and phenylparaben in a concentration up to the existing 0.4% for one ester or 0.8% when used in combination in cosmetic products safe for the consumer taken into consideration that no scientific data has been provided?*

**This opinion has been subject to a commenting period of four weeks after its initial publication. During this period, information was received from the Norwegian Scientific Committee for Food Safety (VKM) that the evaluation of parabens in cosmetic products by the Norwegian Institute of Public Health in 2003 (Paulsen and Alexander, 2003) was not considered valid anymore due to a misinterpretation of dermal absorption data contained in the applicant's dossier which had impacted the dermal absorption estimation. The evaluation has been superseded by a risk assessment carried out by VKM in 2006.**

## 3. ISSUES

Considering the questions raised during the last six years on the safety evaluation of parabens, three separate issues need to be considered:

1) The relationship between the use of parabens in deodorants and the development of breast cancer.
2) The potential *in vitro* and *in vivo* endocrine modifying effects of parabens, in particular estrogenic/anti-androgenic activities and the NO(A)EL value to be used for the calculation of the MoS for the different paraben esters.
3) The toxicokinetics (dermal absorption and biotransformation) of the different paraben esters (in humans and rodents).

Each issue has been previously discussed and described in a number of publications and/or official reports. The following sections summarise the available data per issue with special emphasis on the remaining problem points.

The previous opinions of the SCCP on the subject of parabens, which provide additional information, can be found at:

SCCP/0873/05:
http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_019.pdf

SCCP/0874/05:
http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_00d.pdf

SCCP/1017/06:
http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_074.pdf

SCCP/1183/08:
http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_138.pdf

### 3.1 THE RELATIONSHIP BETWEEN THE USE OF PARABENS AND THE DEVELOPMENT OF BREAST CANCER

With regard to their general toxicological profile, acute, subacute and chronic toxicity studies in rats, dogs and mice have proven parabens to be practically non-toxic, not carcinogenic, not genotoxic or co-carcinogenic, and not teratogenic (SCF 1994). Nevertheless, in 2004 a possible link between the use of underarm cosmetics and breast cancer was claimed in a number of scientific publications.
After thorough study of the available knowledge, the SCCP concluded that there was insufficient data to establish a link between the use of underarm cosmetics and breast cancer (SCCP/0874/05). Meanwhile, no additional data providing evidence to the contrary were encountered.

A more recent review article (Darbre and Harvey 2008) repeats the arguments that have all been refuted in SCCP/0874/05. It does not add new data nor adds any conclusive evidence. Therefore, this issue will not be reconsidered in the present opinion.

## 3.2 THE ESTROGENIC / ANDROGENIC PROPERTIES OF PARABENS

### 3.2.1 Data described in previous SCCP opinions

Two previous SCCP opinions (SCCP/0873/05, SCCP/0874/05) describe and discuss a number of *in vitro* and *in vivo* studies. A recombinant yeast estrogen screen showed parabens to be able to bind to the estrogen receptor, to activate genes controlled by these receptors, to stimulate cell growth and to increase the level of estrogen receptor protein. The estrogenic potency *in vitro* was shown to increase with increasing length of the linear alkyl chain and with increased branching of the alkyl chains, resulting in the following potency ranking order: methyl- < ethyl- < propyl- < butyl- < isobutylparaben. The potency, however, remained at all times 1,000 to 1,000,000 times below the potency of 17β-estradiol. p-Hydroxybenzoic acid (PHBA), the common metabolite of all parabens, was inactive in the *in vitro* assays presented in the 2005 opinion.

The *in vivo* estrogenic activities of parabens have been tested in uterotrophic assays employing female rodents, either immature or adult ovariectomised, after oral, subcutaneous or dermal administration. Butylparaben appeared to be more potent than propyl-, ethyl- and methylparaben, and again the values remained several magnitudes of order below the potency of 17β-estradiol.
Conflicting results, however, were reported for PHBA tested *in vivo*. One study claimed that it had no estrogenic effect, whereas another study gave potency values 1000-fold below the 17β-estradiol level (EFSA 2004, Anonymous 2004, Paulsen and Alexander 2003).

In summary, the *in vitro* data and *in vivo* rodent test results up to 2005 indicated that parabens can exert estrogenic activity, but with potency values that are 3 to 6 orders of magnitude lower than the potency of the positive control 17β-estradiol. The estrogenic activity of parabens appears to increase with increasing chain length.

### 3.2.2 Update on the hormonal (estrogenic / anti-androgenic) properties of parabens

Table 1 in the appendix to this opinion provides an overview of the most relevant studies, covering *in vitro* and *in vivo* assays with the linear paraben esters methylparaben (MePB), ethylparaben (EtPB), propylparaben (PrPB) and butylparaben (BuPB), but also with the branched esters isopropylparaben (IsoPrPB) and isobutylparaben (IsoBuPB), and with the less commonly used benzylparaben (BzPB, phenylmethyl 4-hydroxybenzoic acid). In some cases, the major metabolite p-hydroxybenzoic acid (PHBA) was also tested. For phenylparaben (PhPB, phenyl 4-hydroxybenzoic acid), no data are available.

#### 3.2.2.1 *In vitro* experiments

In the ***in vitro* assays**, different hormonal-related mechanisms are examined:

- *Effects of 4 parabens and PHBA on the estrogen sulfotransferase (SULT) activity in cytosol from **human** skin and liver*:
  SULT activity appeared to be inhibited to various degrees by methylparaben, ethylparaben, propylparaben and butylparaben at micromolar concentrations, but not by PHBA. The potency and extent of SULT inhibition increased with increasing paraben ester chain length (Prusakiewicz et al. 2007).
- *The anti-androgenic potential of 3 parabens and PHBA by measuring inhibition of testosterone-induced transcriptional activity in a **human** embryonic kidney cell line:*
  Methylparaben, propylparaben and butylparaben inhibited an 0.1 nM testosterone-induced transcriptional activity at concentrations above 10 µM (max. 40% inhibition), whereas flutamide and vinclozolin (pos. controls) inhibited transcriptional activity induced

by a tenfold higher testosterone concentration at 10 to 100-fold lower levels. PHBA showed no effects (Chen et al. 2007).

- *The potential of 7 parabens and PHBA to induce proliferation in MCF-7 cells, a human breast cancer-derived cell line shown to be estrogen-responsive:*
  A weak potential was noted for all tested parabens (potency 5 to 6 orders of magnitude below that of 17β-estradiol) and PHBA was negative (van Meeuwen et al. 2008).
- *The ability of 7 parabens and PHBA to inhibit aromatase (enzyme converting androgens into estrogens) activity in human MCF-7 cells (indirect anti-estrogenic potential):*
  All parabens were capable of inhibiting aromatase *in vitro*, although effective concentrations ($IC_{50}$ values) were far above the paraben levels detected in human samples. There was no link between aromatase inhibition and chain length. PHBA was negative (van Meeuwen et al. 2008).
- *The ability of ethylparaben or eutylparaben to interfere with steroidogenesis in a human adrenocortical carcinoma cell line:*
  Ethylparaben and butylparaben increased progesterone production at 30 µM, but had no effect on testosterone or estradiol production (Taxvig et al. 2008). No positive control was included.
- *The potential of butylparaben to act as a thyroid receptor agonist/antagonist in a rat pituitary cell line:*
  Butylparaben was considered a potential weak thyroid receptor agonist based upon increased cell proliferation at 3 µM. The effect was slightly more pronounced in the presence of triiodothyronine ($T_3$). No positive control was included (Taxvig et al. 2008).
- *The estrogenic potential of the ethylparaben and propylparaben based upon human MCF-7 gene expression related to estrogenic responses, making use of DNA microarray analysis:*
  A clear difference was noted in the expression profiles after treatment with ethylparaben and propylparaben. The activity showed a positive correlation with the chain length of esters. Gene expression profiles of propylparaben and butylparaben treated cells were, however, closer to each other than the profile of estrogen treated cells was to any of them (Terasaka et al. 2006).

**Sub conclusion 1:**

***In vitro* studies show the potential of endocrine modifying effects of parabens, with estrogenic activity as a function of chain length. PHBA, the common metabolite does not seem to exhibit endocrine modifying effects.**

#### 3.2.2.2 *In vivo* experiments

The ***in vivo* experiments** cover different potential estrogenic/anti-androgenic mechanisms and involve oral or subcutaneous administration of sets of parabens to immature or pregnant **rats** and **mice**. Over the years, two important sets of *in vivo* studies were submitted to the SCCP/SCCS.

A first series of studies is described in four publications of the Tokyo Metropolitan Institute of Public Health. They contain the results of *in vivo* assays studying the effects on the male reproductive system of methylparaben, ethylparaben (Oishi 2004) and propylparaben (Oishi 2002a) in rats and of butylparaben in rats (Oishi 2001) and mice (Oishi 2002b). The author of these studies comes to the conclusion that exposure of post-weaning rats and/or mice to butylparaben at dosage levels down to about 10 mg/kg bw/day adversely affected the secretion of testosterone and the function of the male reproductive system. Combined with an earlier uterotrophic assay showing that dosage levels of 200 mg butylparaben/kg bw/day and higher, significantly increased the uterus wet weights in the female rats (Routledge et al. 1998), Oishi concluded that more research into the effects of parabens on the reproductive system was needed (Oishi 2004). For propylparaben, only minor effects were

noted at the 10 mg/kg bw/day level, which was further considered the NOAEL value for that paraben ester.

Methylparaben and ethylparaben were shown not to adversely affect the secretion of sex hormones or male reproductive function, up to dose levels of about 1000 mg/kg bw/day (Oishi 2004).

At the time of the 2005 SCCP opinion, the only *in vivo* study in which the lowest (and only) dosage level of butylparaben did not cause any adverse effect on the male reproductive parameters measured, was a rat assay in which the ester was subcutaneously administered to neonatal rats for 17 consecutive days. Out of this study a NOEL of 2 mg/kg bw/day could be extracted for butylparaben (Fisher et al. 1999).

Since Industry considered both the NOEL of 2 mg/kg bw/day for butylparaben (Fisher et al. 1999) and the NO(A)EL of 10 mg/kg bw/day for propylparaben (Oishi 2002) an overestimation of the reproductive hazard of the parabens under study, the applicant decided to repeat the Oishi assay in male rats with a more robust study design. Butylparaben and methylparaben were chosen as test compounds as they were considered to bracket the chain lengths of all parabens used and to allow interpolation of the results for ethylparaben and propylparaben. The full study report was submitted to the SCCP in 2006 and was later published (Hoberman et al. 2008).
After thorough examination, the SCCS identified some important shortcomings and concluded that the repeat studies were not scientifically acceptable (SCCP/1017/06 and SCCP/1183/08). The major comments are summarized below:

1) *Both repeat "reproduction studies" did not follow a well-established scientific protocol (e.g. OECD guideline, EC Regulation No 440/2008 standardised testing method).*

   The applicant argued that as the intention was to refute the results of Oishi, the same protocol was used instead of any officially issued OECD guideline.

   The SCCP accepted this argumentation.

2) *The raw data provided were considered to be insufficient. The study report mentioned that the 64 animals of the repeat assay were from 10 dams, but failed to provide further details (e.g. which pups came from the same dam).*

   Industry argued that cross-fostering at breeding increases diversity. Estimating that a minimum of 13 litters is represented, this is considered to be a large number for a study with 64 animals.

   The SCCP remark, however, was not focused on the number of dams, but specifically on the fact that the test description did not allow to determine which pups could be associated with the same dam. Viewing the suspected illness of the animals, the Committee thought that it could be possible that only a restricted number of dams was involved. By excluding these from the study, the results could have improved.

3) *The body weights of the animals varied considerably. Usually a variation of 20% in body weight is acceptable. The assays under consideration displayed deviations up to 48% within one dosage group.*

   The applicant explained that body weight variations of the laboratory animals were typical for this species strain and age. The animals were younger than those in traditional toxicity studies. The primary selection criterion for the study was for age, not for body weight.

   Independently of the fact that the age of the animals (22 days) and not their body weight was the selection criterion for the tests, a large variation range in body weight leads to a large variation range in the final dosages given to the animals (factor of at least 2). In the Oishi studies, for example, the animals were aged 19-21 days and showed much lower weight variation. It was further recognised, however, that the lack of raw data in these studies seriously hampered analysis of the data provided.

4) *In the methylparaben study protocol it was mentioned that testosterone, follicle-stimulating hormone (FSH) and luteinising hormone (LH) were measured in the blood. These values were not present in the raw data provided.*

   Industry explained that LH and FSH samples were only taken as a back-up in case the main sperm parameters would have shown an effect. Given that no effect was seen for methylparaben, these samples were not further processed.

   The Committee was of the opinion that, since the blood was collected and available, hormone levels should have been measured as it was done for butylparaben, which did not show reproductive effects either.

5) *Standard deviations of the hormone levels measured after butylparaben administration were large and exact sampling times for blood collection were not included in the raw data. This information was considered important as diurnal variations affect hormone levels.*

   Industry responded that standard deviations for hormone levels were typical and that the sampling period was within a specific 2-hour interval in the morning.

6) *26% of the animals displayed unexpected clinical signs such as chromorhinorrhea, chromodacryorrhea, etc., which raised questions about general animal husbandry.*

   Industry explained that the clinical signs were the result of frequent retro-orbital blood sampling for hormone determinations and that the symptoms observed were the typical result of careful, daily, cage side observations made in good laboratories.

   Blood sampling in experimental animals using retro-orbital bleeding, however, is no longer considered a humane method (Hui et al. 2007). In the hands of unskilled operators, side effects typically include blindness, ocular ulcerations, puncture wounds, loss of vitrous humor, infection or keratitis (Hoff 2000). In addition, increases in blood parameters (hormones, glucose, catecholamines) are described to be directly related to stressful methods of blood collection (Hoff 2000, Grouzmann et al. 2003). In case the animals are anesthetised before blood sampling, the interaction with the anesthetic needs to be documented (Hui et al. 2007). Therefore, the SCCP not only considered the observed chromorhinorrhea and chromodacryorrhea as insufficiently explained, but also expressed additional doubts on the relevance of the obtained hormone levels.

7) *Too many adverse effects with statistical significance were dismissed due to the lack of dose-dependency, abnormal high values in control animals, etc.*

   Industry emphasised that, although sporadic statistical changes were observed in their studies with methylparaben and butylparaben, none were dose-responsive, none were consistent over time, and none were corroborated by accompanying effects. One would expect a biologically significant reduction in testosterone concentration to be accompanied by a decrease in weight of testosterone-dependent tissues, or a perturbation in sperm parameters to be accompanied by a change in weight or presence of histopathology in the testis or epididymides. All effects seen were isolated and not dose dependent. They reflected normal variability in the parameters assessed.

   The SCCP, however, considered the numerous parameters affected a significant limitation of the reliability and relevance of the conclusions drawn from the study.

The Industry applicant stressed that there were indications that the Oishi laboratory lacked the expertise to appropriately evaluate the parameters being measured. More specifically, (i) the mean values for some parameters fell far outside the accepted historical control ranges and (ii) the standard deviations in the data were far less than the normal biological variability that has been observed by other groups (details can be found in SCCP/1183/08).

These doubts were shared by the SCCP. Unfortunately, although a formal request was made by the European Commission on behalf of the SCCP, the full protocols and raw data of the Oishi publications were not available.

The SCCP concluded that a) the quality of the Oishi studies could not be properly assessed as the full test description and the complete raw data packages were not available, b) with regard to the Industry repeat studies, although the full descriptions and raw data were available and although some of the questions raised by the SCCP were addressed during an Industry hearing, the remaining issues hampered their acceptance as unarguable refutation of the Oishi findings. This also meant that the NOEL of 2 mg/kg bw/day of butylparaben, obtained in the Fisher et al. (1999) study, was still considered as the NOEL to be used in further calculations.

Between 2008 and 2010, additional *in vivo* data on parabens were published. An overview of the most pertinent ones is given below:

- *Effects of ethylparaben and butylparaben on steroidogenesis in parental rats and offspring after subcutaneous administration to pregnant rats*:
  Ethylparaben and butylparaben (up to 400 mg/kg/day) showed no treatment-related effects on testosterone production, anogenital distance, or testicular histopathology. Butylparaben decreased ERβ mRNA expression in fetal ovaries, and mRNA expression of steroidogenic acute regulatory protein and peripheral benzodiazepine receptor in adrenal glands. However, these effects show no dose-dependency (Taxvig et al. 2008).
- *Effects of isobutylparaben on reproductive parameters and hormone levels after subcutaneous administration to pregnant rats:*
  Isobutylparaben decreased the plasma corticosterone concentration and increased the uterus weight in dams as well as the uterine sensitivity to estrogen in adult female offspring (Kawaguchi et al. 2009a). No dosage level was stated and no positive control was included.
- *Effects of isobutylparaben on emotional behaviour and learning performance in mature offspring after subcutaneous administration to pregnant rats:*
  Subcutaneous administration of isobutylparaben to dams increased anxiety, and specifically disturbed passive avoidance performance of offspring, although the effects were male-specific (Kawaguchi et al. 2009b). No exact dosage level was stated and no positive control was included.
- *Estrogenic effects of butylparaben, isobutylparaben and isopropylparaben measured through the uterotrophic assay (subcutaneous injection in immature female rats and Calbindin-D9-k (CaBP-9k) used as biomarker for estrogenic effects):*
  Butylparaben, isobutylparaben and isopropylparaben induced increased uterine wet weight at 1000 mg/kg/day, at dosage level 1000-fold higher than positive control effect level. The assay gives indication of estrogen-receptor and progesterone-receptor mediated pathways (Vo et al. 2009).
- *Effects of propylparaben and butylparaben on reproductive parameters and hormone levels after subcutaneous administration to pregnant mice:*
  Subcutaneous injection of dosages up to 950 mg/kg/day of propylparaben and butylparaben failed to affect number of pups born, litter weights, individual pup weight and pup survival, whereas 17β-estradiol terminated all pregnancies (Shaw and de Catanzaro 2009).
- *Uterotrophic assay with butylparaben through subcutaneous administration in two different mice strains:*
  Butylparaben does not affect uterine wet or dry mass at any dose in either strain. 17β-estradiol consistently increased uterine mass in both strains (Shaw and de Catanzaro 2009).
- *Studies on suppressive effects of 6 parabens on reproductive organs in female rats during the critical developmental stage:*
  At the highest dosage level (1000 mg/kg/day), each of the tested parabens (methylparaben, ethylparaben, propylparaben, butylparaben, isopropylparaben, isobutylparaben) induces one or more of the following effects: decreased ovary/kidney weight, increased thyroid gland/adrenal weight, reduced serum estradiol levels, decrease

of corporea lutea, increase in number of cystic follicles, myometrial hypertrophy. At lower dosage levels, no dose-dependent effects were noted. $IC_{50}$ values for binding ERα and ERβ receptors are at least 3 orders of magnitude below the ones for 17β-estradiol and as far as their potencies are concerned, the parabens can be ranked as follows: isobutylparaben > butylparaben > isopropylparaben = propylparaben > ethylparaben > methylparaben (Vo et al. 2010).

**Sub conclusion 2:**

***In vivo* studies on parabens published between 2008-2010 showed effects with relatively high dosage levels (mainly about 1000 mg/kg bw/day) of paraben esters. The recent findings do not clarify the diverging results between the Oishi and Hoberman studies in male rats. The shortcomings of the Hoberman study prevent its acceptance. It cannot be used to refute the Oishi findings; these, in turn, cannot be properly assessed due to the unavailability of raw data.**

**This means that the NOEL of 2 mg/kg bw/day for butylparaben, derived from the Fisher et al. (1999) study in the rat, is still considered as the NOEL to be used in further calculations.**

**For the iso-derivatives of butyl- and propylparaben, and for benzyl- or phenylparaben no suitable data are present.**

## 3.3 DERMAL ABSORPTION AND OTHER TOXICOKINETIC DATA

### 3.3.1 Dermal absorption

#### 3.3.1.1 Dermal absorption *in vitro*

The Norwegian Institute of Public Health published in 2003 a report (Paulsen and Alexander, 2003), briefly summarising the toxicity of the parabens and using in their calculation of the MoS a value of 3.5% dermal absorption, based on *in vitro* studies with human skin (Cross and Roberts 2000): This document was taken up in the 2005 SCCP opinion on parabens (SCCP/0873/05) and was considered to give a realistic value for dermal absorption. During the commenting period of the present opinion (SCCS/1348/10) however, the SCCS was informed that the value of 3.5% dermal absorption was based on a misinterpretation of the original study results contained in the applicant's dossier and should therefore not be used.

As discussed in SCCP/1017/06, four *in vitro* dermal absorption studies were submitted, one with methylparaben and butylparaben on split-thickness rat and human skin (Fasano 2004b), and three with butylparaben on full thickness **human** or **pig** skin (Fasano 2004a, 2005; Diembeck and Duesing 2005). These studies are summarised in Table 2 in the appendix to this opinion. The SCCP concluded that the studies displayed a number of shortcomings and that they appeared to show a significant dermal absorption of butylparaben in human skin.

The Fasano 2004b study with split-thickness skin indicated there was a higher level of absorption of parabens through **human** skin than through **rat** skin. The generated dermal absorption values were at the level of about 50% for methylparaben and 37% for butylparaben. The metabolism into PHBA more easily occurred in rat skin. This is not in line with the applicant's argument that all esters are quickly metabolised into PHBA in human skin. The cause for this apparent discrepancy may be the fact that the study was not performed with full thickness skin, but with dermatomed skin in which the metabolizing capacity is compromised. The latter view is supported by the findings in Fasano 2004a and 2005, where butylparaben appeared to be largely metabolised in the full thickness **human** skin samples, as mainly PHBA was measured in the receptor fluid. Taking both studies together, 0.23 to 0.67% butylparaben was measured in the receptor compartments of 6 out of the 16 skin samples (for the remaining 10 cells, the butylparaben concentration was below the detection limit). However, in these studies the metabolite distribution in the

different skin compartments and the solubility of both parabens in the receptor fluid was not determined.

Based upon a combination of the three Fasano (2004a,b and 2005) studies, the SCCS derived the value of 3.7% as a worst case assumption for the dermal absorption of unmetabolised butylparaben. This percentage originated from the mean dermal absorption of 37% measured in split-thickness skin (Fasano 2004b), using a correction factor of 10 to account for skin metabolism as seen in the full thickness skin experiments (Fasano 2004a, 2005). The factor of 10 is considered a conservative value as in these studies the measured butylparaben concentration in the receptor fluid was not 10, but 65 to 150 times lower than the metabolite (PHBA)concentration, meaning that butylparaben undergoes extensive metabolism in human skin.

Pape and Schepky (2009) recently re-analysed some existing 'preliminary' dermal absorption results (presumably the Diembeck and Duesing 2005 data) dealing with the penetration of butylparaben through 3 full thickness pig skin samples. The study is only briefly described and appears to show that in the epidermis, butylparaben was found unmetabolised, whereas in the dermis, 50% unmetabolised butylparaben and 50% PHBA were found. In the receptor fluid, mainly PHBA and less than 1% butylparaben were measured. Stability of butylparaben in the receptor fluid was not documented. The report is confusing, mixing percentages with amounts per $cm^2$, and results from a preliminary study. Finally, the authors mention that other paraben esters (methylparaben, ethylparaben, propylparaben) were also tested under the same conditions, but detailed data were not available to the SCCP/SCCS.

**Sub conclusion 3:**
**The *in vitro* dermal absorption studies point towards a potential difference in dermal absorption and metabolism of higher chain parabens between rodents and humans. Studies with full thickness human skin showed that unmetabolised methylparaben and butylparaben were barely detectable in receptor fluid, whereas studies with split-thickness human skin reveal higher *in vitro* dermal absorption values for unmetabolised butylparaben. Unfortunately, none of the provided dermal absorption assays were of satisfying scientific quality. However, In the absence of new human dermal absorption data, as previously requested by the SCCP, and in the light of the fact that over the last years the weight of evidence approach in risk assessment is given more importance, the available *in vitro* dermal absorption studies on butylparaben were used to derive the value of 3.7%, which is considered to be a conservative estimate. Indeed, both in full thickness and, to a lesser extent, in split thickness human skin studies, a high level of biotransformation of butylparaben was observed although both *in vitro* models are not designed to obtain optimal biotransformation as is the case for freshly isolated human skin.**

#### 3.3.1.2 Dermal absorption *in vivo*

**In human** volunteers exposed for one week to a cosmetic formulation containing 2% of butylparaben, 2% of diethyl phthalate and 2% of dibutyl phthalate, serum measurements revealed that butylparaben was detectable. No effect was noticed on a number of relevant hormone levels: thyroid-stimulating hormone (TSH), luteinising hormone (LH), estradiol, Inhibin B, thyroxine ($T_4$) and free thyroxine ($FT_4$) (Janjua et al. 2007). Although these results are supportive for the safety of butylparaben, they do not exclude the possibility of endocrine effects for propylparaben.

Serum analysis showed the presence of unmetabolized butylparaben in the exposed human volunteers. The results were obtained from a combined test of butylparaben with two phthalates, which does not represent ideal test conditions to investigate the specific parabens concerned.

In the current submission, Industry acknowledges that the co-application of high concentrations in the Janjua 2007 study may have saturated skin esterases and produced an increased absorption of intact esters.

**Sub conclusion 4:**

**One study with some shortcomings provides evidence for *in vivo* dermal absorption of butylparaben in the absence of notable effects on hormone levels. No data is available for the other parabens.**

### 3.3.2 Additional toxicokinetic data

#### 3.3.2.1 *In vivo* pharmacokinetic study in the **rat**

Industry proposed to perform an *in vivo* pharmacokinetic rat study through the oral, dermal and subcutaneous route with methyl- , propyl- and butylparaben and requested the approval of the SCCP. The SCCP declared that this study was welcomed, but that it should be supplemented with toxicokinetic studies in human volunteers after dermal application of representative cosmetic products containing propylparaben and butylparaben, since these could deliver essential information (SCCP/1183/08).

The current submission contains the *in vivo* pharmacokinetic rat study, investigating the absorption, plasma kinetics, body distribution, metabolism (determination of plasma metabolites) and excretion of [$^{14}$C]-labelled short-chain (methyl), medium-chain (propyl) and long-chain (butyl) parabens (Aubert 2009).

Dosage groups consisted of 12 male and 12 female Sprague Dawley rats who received single doses of 100 mg/kg $^{14}$C methylparaben, propylparaben or butylparaben via the oral or dermal routes. An additional group of 12 male and 12 female rats were administered a single dose of 100 mg/kg [$^{14}$C]-butylparaben via the subcutaneous route.

Blood samples were collected from alternating 3 animals per sex and administration route at pre-dose, 0.5, 1, 2, 4, 8, 12, 22 and 24 hours after dosing or the start of dermal exposure, respectively. Blood/plasma samples were analysed for total [$^{14}$C]-radioactivity by liquid scintillation counting. After the last blood sample, the animals of the kinetic groups were sacrificed.

Plasma metabolic profiling was conducted in pooled samples per group that were collected between 0.5 and 8 hours for the orally, and between 0.5 and 4 hours for the subcutaneously treated groups. For the dermal route, samples were collected at $t_{max}$. Samples were analysed using a HPLC/UV/radioactivity monitoring system.

For the groups assigned to excretion balance determination, urine, faeces and cage washes were collected up to 168 hours. After this period, the animals were sacrificed, weighed, major organs and tissues were collected and stored frozen up to determination of radioactivity. The results of the study are summarised as follows:

(i) Pharmacokinetics:

Oral administration of methylparaben, propylparaben and butylparaben at 100 mg/kg resulted in high systemic uptake (based on radioactivity) with $C_{max}$ values that generally occurred at 0.5 hrs ($t_{max}$) and tended to be higher in females than males, ranging from 11.4 (propylparaben, males) to 42.3 (propylparaben, females) µg-equivalents/ml. Corresponding plasma $AUC_{0-t}$ values ranged from 58.3 (propylparaben, males) to 143.6 (methylparaben, females) µg-eq x hrs/ml. Blood levels declined rapidly and reached the limit of quantification at 8 to 22 hours.

Dermal administration of methylparaben, propylparaben and butylparaben at 100 mg/kg resulted in relatively low $C_{max}$ values relative to those measured after oral administration, which ranged from 0.6 µg-eq/ml (propylparaben, males) to 3.1 µg-eq/ml (methylparaben, males) which occurred generally at 8 hrs ($t_{max}$). Corresponding

plasma $AUC_{0-t}$ values ranged from 5.4 (propylparaben, males) to 20.4 (methylparaben, males) µg-eq x hrs / ml. A small, initial (1 hour time point) peak in the plasma levels in males was attributed to oral uptake, secondary to cage contamination, fur contact and oral uptake. This is, according to the authors, a common observation after open dermal treatment of rats, even in the presence of Elizabethan collars. Blood levels declined rapidly and reached the limit of quantification at 12 or 22 hours.

Subcutaneous administration of butylparaben at 100 mg/kg produced $C_{max}$ values of 6.5 (males) or 12.2 µg-eq/ml (females) with corresponding plasma $AUC_{0-t}$ values of 52.0 (males) or 88.9 (females) µg-eq x hrs/ml, respectively. $C_{max}$ occurred after 2 and 4 hours after injection in males and females, respectively. Blood levels declined rapidly and reached the limit of quantification at 12 to 22 hours.

(ii) Plasma metabolite characterisation

Pooled plasma samples were collected and analysed by HPLC/[$^{14}$C]-detection. For the dermal route, only samples collected at $t_{max}$ were analysed as other time points provided insufficient concentrations for analysis. In all plasma samples, independent of time of collection, paraben type and route of administration, only a single peak was found, which corresponded to PHBA. No evidence for the presence of parent parabens or other parabens-related metabolites was found. These results suggest that, in rats, after oral, dermal or subcutaneous administration of parabens, the principal systemic exposure agent is PHBA.

(iii) Excretion balance – oral administration

Following oral administration, the mean recovery of [$^{14}$C] in rats treated with methylparaben, propylparaben or butylparaben ranged from 89 to 95% of the applied [$^{14}$C], suggesting an adequate mass balance. Urinary excretion was the major pathway of elimination (range: 71 to 84% of the administered [$^{14}$C], suggesting similar bioavailability for all parabens, whereas faecal excretion was low to negligible, i.e. in the range of 1% of the administered [$^{14}$C]. The elimination of [$^{14}$C] via the urine was rapid and occurred mainly during the first 24 hours after administration. After sacrifice (168 hours), a very small amount of [$^{14}$C] was retained in the tissues and ranged from non-detectable to 2% of the administered dose. These data suggest rapid clearance of a single dose from the organism and absence of selective storage in organs or tissues.

(iv) Excretion balance – dermal administration

Following dermal administration, the mean recovery of [$^{14}$C] in rats treated with methylparaben, propylparaben or butylparaben ranged from 104 to 116% of the applied [$^{14}$C], suggesting an adequate mass balance. Most of the radioactivity was recovered in the swaps used for treated skin area and cage cleaning (upper part) at the end of the exposure period (range: 46 to 58% of the applied radioactivity).
Urinary excretion was the major pathway of elimination (range: 14.5 to 27.1% of the administered [$^{14}$C]) suggesting significant skin penetration and similar systemic availability for all parabens, whereas faecal excretion was negligible. The elimination of [$^{14}$C] via the urine was rapid and occurred mainly during the first 48 hours after administration. After sacrifice (168 hours), a very small amount of [$^{14}$C] was retained in the organs or the treated skin sites and ranged from non-detectable to 2% of the administered dose. The remainder of radioactivity was recovered in the carcasses (range: 21 to 37% of total radioactivity).
In the absence of significant skin or organ residues, these residues were attributed to the fur, muzzle and paws secondary to the open administration and subsequent cage contamination. Overall, these data suggest rapid clearance of a single dose from the organism and absence of selective storage in organs or tissues.

(v) Excretion balance – subcutaneous administration (butylparaben only)

Following subcutaneous injection, the mean recovery of [$^{14}$C] in rats treated with butylparaben was 84.0 and 82.7% of the administered [$^{14}$C] for males and females

respectively, suggesting an almost complete mass balance. Urinary excretion was the major pathway of elimination (range: 67 to 76% of the administered [$^{14}$C]), suggesting similar bioavailability, whereas faecal excretion was negligible. The elimination of [$^{14}$C] via the urine was rapid and occurred mainly during the first 24 hours after administration. After sacrifice (168 hours), a very small amount of [$^{14}$C] was retained in the tissues and ranged from non-detectable to 2% of the administered dose; a single carcass contained 2.3% of the applied radioactivity.
These data suggest rapid absorption and clearance of a single subcutaneous dose of butylparaben from the organism and absence of selective storage in organs or tissues.

Blood plasma analysis in all parabens-treated groups following all exposure routes showed only the presence of PHBA. For the dermal route, only samples collected at $t_{max}$ were analysed as other time points provided insufficient concentrations for analysis. In all plasma samples, independent of time of collection, paraben type and route of administration, only a single peak was found, which corresponded to PHBA. No evidence for the presence of parent parabens or other parabens-related metabolites was found.
Plasma data after oral or subcutaneous, but not after dermal administration showed a trend towards higher systemic exposure values in females when compared with those in males. Overall, oral administration produced plasma values suggesting high systemic uptake for all parabens; after dermal administration, the systemic exposure was approximately an order of magnitude lower than that after oral dosing, whereas subcutaneous injection of butylparaben produced exposure patterns that resembled that of oral (similar $C_{max}$ and AUC values) as well as dermal (delayed $t_{max}$ values) administration.

Pharmacokinetic results showed plasma patterns typical for the different routes of administration: high $C_{max}$ and AUC values were observed after oral dosing, after dermal administration the respective values were approximately one order of magnitude lower, whereas subcutaneous dosing produced similar, but somewhat lower values relative to those seen after oral administration. The principal route of excretion was via the urine and no selective organ / tissue storage was observed.

**Sub conclusion 5:**
**The toxicokinetic study confirms that, in rats, short-, mid- and long-chain parabens are rapidly absorbed and eliminated after single oral or subcutaneous administration. After dermal administration, they are partly (15 to 27%) absorbed and rapidly eliminated. Blood analysis only showed the presence of PHBA.**

#### 3.3.2.2 Requested *in vivo* pharmacokinetic study in **human** volunteers

Although this study was requested, Industry chose not to perform it. The following argumentation was given:

*The design of a comprehensive and relevant human clinical study would encounter significant problems. The choice of a relevant dose and vehicle would have to be carefully assessed. Trying to mimic a real life exposure dose from cosmetic products would probably produce very low plasma levels necessitating the use of extremely sensitive analytical equipment (LC/MS/MS). In order to show skin metabolism one would have to quantitatively characterise systemic metabolites. The principal metabolite of parabens, PHBA, is ubiquitous in plants and human nutrition and expected to naturally occur in humans. In addition, PHBA is a widely used preservative in consumer care products and food. Therefore, in order to distinguish systemic levels of PHBA resulting from topical exposure to parabens in cosmetics from those that result from food or other sources, such a study would require skin application of [$^{14}$C]-labelled parabens. However, ethical constraints limit the amount of [$^{14}$C] that may be applied to human skin.*
*The results of the rat study showed that, after dermal administration of high doses of [$^{14}$C]-parabens to rat skin, resulting plasma levels of [$^{14}$C]-PHBA were relatively low. Taking into account the sensitivity of [$^{14}$C]-detection and metabolite characterisation in rat plasma, the method permitted to track the major metabolite PHBA, but was not sufficiently sensitive to identify trace amounts of intact parabens.*

*Given that a human study would have to apply lower amounts of radioactivity and taking into account that human skin is less permeable than rat skin, a human study with [$^{14}$C]-parabens is expected to have even lower sensitivity and would not address the question of the ratio of parabens that reach the systemic circulation intact.*
*As a last resort, a human study would have to be conducted under total dietary control and analysis excluding food and other products that contain PHBA.*
*Theoretically, such data could be generated with large efforts in time and resources. However, considering the limited actual human exposure to long-chain parabens (Cowan-Ellsberry and Robison, 2009) and the current state of knowledge as well as the weight-of-evidence with regard to skin penetration/metabolism of parabens, and weighing it against the relatively limited new information that could be obtained in a new human PK study, the available information appears to sufficient for a human risk assessment.*

**Sub conclusion 6:**

**The requested *in vivo* pharmacokinetic data in human volunteers after exposure to paraben-containing cosmetic products are not available.**

#### 3.3.2.3 Paraben exposure in humans: additional data

As noted above, butylparaben has been detected in serum of volunteers who had been exposed to a cosmetic formulation containing 2% of butylparaben and 2% each of two different phthalates (Janjua et al. 2007). In a follow-up analysis, the authors analyzed also urinary concentrations of butylparaben and metabolites by LC-MS/MS in 24h urine collected before and after topical application (Janjua et al. 2008). All subjects showed increased levels in urine during treatment: butylparaben excretion was 2.6 ±1.1 mg/24h which corresponds to 0.32 % of the applied dose. This indicates that part of the dermally applied butylparaben is not hydrolyzed to PHBA.

A biomonitoring study examined urinary concentrations of free and conjugated methylparaben, ethylparaben, propylparaben, butylparaben (n- and iso-), and benzylparaben in a demographically diverse group of 100 adults in the US (Ye et al. 2006). Methylparaben and propylparaben were detected at the highest median concentrations (43.9 ng/ml and 9.05 ng/ml, respectively) in nearly all (> 96%) of the samples. The other parabens were detected in more than half of the samples (ethylparaben 58%; butylparaben 69%), and at much lower levels (1.0 ng/ml and 0.5 ng/ml, respectively). Although parabens in urine appear predominantly in their conjugated form (glucuronides, sulfates), free parent compounds were also detected. Similar median urinary levels of methylparaben and propylparaben seen in the Ye et al. (2006) study were reported in a recent biomonitoring study of methylparaben and propylparaben in 77 Harvard students (Carwile et al. 2009).

The concentration of five parabens, methylparaben, ethylparaben, propylparaben, butylparaben and benzylparaben in urine, serum and seminal plasma samples from 60 healthy Danish men were examined, using a sensitive and specific LC-MS/MS method for simultaneous determination of the five parabens in the three different matrices (Frederiksen et al. 2010). Highest concentrations of the parabens were found in urine, wherein methylparaben, ethylparaben, propylparaben and butylparaben were measurable in 98%, 80%, 98% and 83% of the men, respectively. Benzylparaben was only measurable in urine from 7% of the men. Serum and seminal plasma samples revealed the presence of mainly methylparaben and propylparaben, although in seminal plasma, butylparaben was also detected. Overall, urinary paraben concentrations correlated to the paraben concentrations in both serum and seminal plasma (Frederiksen et al. 2010).

**Sub conclusion 7:**

**Human biomonitoring studies show the presence of parabens (free and conjugated species) in urine and/or serum and seminal plasma. Although these biomonitoring studies can neither discriminate between paraben exposure from oral intake or dermal application, nor between sources of exposure (medicinal**

**products, cosmetics, etc.), the presence of free and conjugated parabens in urine and/or serum and seminal plasma clearly indicates that –in contrast to the situation in rat– the compounds are not completely hydrolysed into the metabolite PHBA.**

## 3.4 SUMMARY OF CONCLUSIONS RELATED TO ISSUES 3.1-3.3

1) Human-based *in vitro* data show an increasing potential for **endocrine modifying effects** with **increasing chain length**. **PHBA**, a common metabolite of all paraben esters, however, appears to exhibit **no endocrine modifying effects**.

2) The major repeated dose studies in rat (Oishi 2001 and 2002, Hoberman et al. 2008) are controversial and provide very **divergent critical effect levels** for butylparaben ranging from a **LOAEL** of **10 mg/kg bw/day** to a **NOAEL 1000 mg/kg bw/day**, respectively. Older data on butylparaben revealed a reproductive **NOEL of 2 mg/kg bw/day** in the rat (Fisher et al. 1999). The latter will be used as a conservative value in further calculations.

3) The presented in vivo pharmacokinetic studies on methylparaben, propylparaben and butylparaben in the rat (oral, dermal, subcutaneous administration) show that these parabens are rapidly absorbed and eliminated in this species.
   Available in vitro dermal absorption study results point towards a **potential difference** not only in dermal absorption (Fasano 2004b, Pape and Skepky 2009) but also **in metabolism** of higher chain parabens (Ye et al. 2006, Janjua et al. 2007) **between rat and man**. Consequently the rat data as such cannot be simply extrapolated to the human situation without additional supportive data.
   To this respect, no human study results for the parabens under discussion (with the exception of butylparaben) are available that show unchanged levels of hormones which are of importance for the ongoing discussion.
   Furthermore, no metabolism studies have been submitted that clearly prove that no difference in metabolism exists between the rat and man. Such studies are needed to show that the higher chain parabens are completely metabolised into PHBA as claimed by industry. The biomonitoring studies presented in Section 3.3.2.3 indicate that in the **human body**, **parabens** may **not be completely hydrolysed into PHBA**. This means that the **necessary data needed to demonstrate that the available results for rats are also valid for humans are still missing**.

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 value of 3.7% used in this opinion originates from a pragmatic approach combining three *in vitro* dermal absorption studies. The first one is a split-thickness *in vitro* study (i.e. a study lacking major skin metabolism), which shows a dermal absorption of butylparaben of 37% (Fasano 2004b). Two other studies were performed with full-thickness skin, which is better equipped for biotransformation. These studies show that butylparaben can be measured in the receptor fluid at concentrations which are 65 to 140 times lower than the metabolite (PHBA) concentrations, meaning that butylparaben undergoes extensive metabolism in human skin. Nevertheless, as the study does not provide individual butylparaben/PHBA concentration levels in the different skin compartments, the SCCS prefers to follow a conservative approach by applying a correction factor of 10 to the dermal absorption value obtained with butylparaben in the split-thickness skin study. The SCCS considers this corrected value to be a realistic high end value, which is more conservative than the value of 1% proposed by the Industry and the 2% value proposed by the Danish DTU (2010).

# 4. DISCUSSION

Not only Industry and the SCCS, but also other stakeholders expressed their views on the safe use of parabens in cosmetic products. In order to provide an as complete as possible picture on all the available information, the individual points of view of all parties are also summarized below.

## 4.1 VIEW OF THE INDUSTRY

The current Industry submission uses the following argumentation to declare all parabens safe for use:

1. The choice of the reproduction NO(A)EL value:
   Industry emphasizes that the Oishi (2001) study is not reliable and that the CTFA/Colipa study (Hoberman et al. 2008) is well performed. One of their arguments is that the SCCP (2008) acknowledged the scientific value of the new study.
2. Toxicokinetic aspects related to the risk assessment of parabens:
   Industry presents a large pharmacokinetic study in the rat using different routes of exposure (Aubert 2009). A major conclusion is that in the rat, independent of the route of exposure, parabens are quickly hydrolysed and only occur in the systemic circulation in the form of the metabolite PHBA. In addition, excretion is rapid and mainly occurs via the urine. Total dermal absorption (parent compound + metabolites) in the rat is estimated to be around 27%.
3. With regard to the requested human toxicokinetic study:
   Industry decided not to perform it (arguments stated under 3.3.2.2).
4. For the final safety assessment of the parabens, the following parameters are taken into account:
   - The NO(A)EL used for all paraben esters is the Hoberman et al. (2008) value of 1000 mg/kg/day.
   - For the calculation of the SED, the cumulative value of 17.4 g/day is used (SCCS Notes of Guidance, SCCS/1416/11), assuming that parabens may be used as a preservative in all cosmetic products.
   - Only 1% of the paraben level is assumed to become systemically available, due to the hydrolysis of the parent compound into PHBA (based upon Schepky et al. 2009).

   The MoS values obtained are 83,300 for the individual paraben esters and 41,600 for the paraben mixture. An additional calculation takes into account aggregate exposure through non-cosmetic use of parabens as described by Cowan-Ellsberry and Robison (2009), but this does not add to the current discussion.

## 4.2 VIEW OF THE COSMETIC INGREDIENT REVIEW PANEL (CIR)

In 2008, the CIR Expert Panel reviewed the safety assessment of methyl-, ethyl-, propyl-, isopropyl-, butyl-, isobutyl- and benzylparaben in cosmetic products (CIR, 2008). For their MoS calculations for the whole range of parabens, they used the NOAEL of 1000 mg/kg/day of the Hoberman et al. (2008) study, which was considered as the "*most statistically powerful and well-conducted study on the effects of butylparaben on the male reproductive system*".

## 4.3 VIEW OF THE EUROPEAN FOOD SAFETY AUTHORITY (EFSA)

The EFSA review panel used the 1000 mg/kg/day level for methyl- and ethylparaben, but considered more data necessary to determine a NO(A)EL value for propylparaben (EFSA, 2004).

## 4.4 VIEW OF THE DANISH NATIONAL FOOD INSTITUTE

As supplementary information for the drawing up of the current opinion, the European Commission provided the SCCS with the 'Update on uptake, distribution, metabolism and excretion (ADME) and endocrine disrupting activity of parabens', a report by the Danish National Food Institute, Technical University of Denmark (DTU 2010), later published as an article of Boberg et al. (2010).

This report summarises all available scientific literature on the subject (including SCCP opinions and literature data stated in the current opinion) and comes to the following major conclusions:

- Adverse effects were noted on sperm production and testosterone levels in young male rats exposed to butylparaben, isobutylparaben and propylparaben (Oishi publications).
- Parabens have been shown to be estrogenic *in vitro* and in uterotrophic assays *in vivo*, and estrogenicity appears to increase with side chain length.
- The ability of parabens to activate the estrogen receptor may not be the only mechanism of action, as they also show anti-androgenic effects, mitochondrial toxicity and ability to elevate endogenous estrogen levels via SULT inhibition.
- The use of the 1000 mg/kg/day value used by the CIR-panel is not supported by the DTU since this value was derived from an animal study with many shortcomings, as already pointed out by the SCCP in 2006 (SCCP/1017/06). The DTU refers to the LO(A)EL value of 10 mg/kg/day derived from a published Japanese study (Oishi 2002) with propylparaben.
- The maximal dermal uptake of intact parabens is estimated to be 2% (conjugated and free), based on the results of Janjua et al. 2008.
- The total dermal uptake of parabens and metabolites amounts to 80%. Higher uptake and less metabolism were measured in human skin than in the applied rat models. However, more studies are needed to examine human levels of parabens and metabolites and to compare these levels to those obtained in experimental animal studies. It needs to be determined whether the endocrine disrupting effects seen in experimental animals are due to the (low) levels of intact parabens, or whether metabolites such as PHBA may play a role.

Finally, the DTU included a list of data gaps on parabens, among which reproduction studies on both long- and short-chain parabens, extended toxicokinetic studies (*in vitro* and *in vivo* combination assays) and studies exploring novel endpoints such as mammary development.

## 4.5 VIEW OF THE DANISH ENVIRONMENTAL PROTECTION AGENCY (EPA)

As supplementary information, DG SANCO provided the SCCS with the 'Survey and Health Assessment of the exposure of 2 year-olds to chemical substances in Consumer Products' by the Danish EPA (2009). The latter reports on a large-scale project investigating the exposure of 2 year-olds to chemical substances through contact with consumer products, carried out in Denmark from July 2008 to September 2009. A total of 12 product groups were included in the survey phase. Several substances were selected because of their endocrine modifying effects in animal studies. Among these chemicals were propylparaben, butylparaben and isobutylparaben.

For the individual risk assessments, however, the report refers to all SCCP opinions on parabens and the remaining uncertainties/open questions. In the report it is concluded that the amounts that 2 year-olds absorb from propylparaben and butylparaben can constitute a risk for estrogen-like modifications of the endocrine system. This contribution originates predominantly from cosmetic products such as oil-based creams/moisturising creams/lotions and sunscreens and was dealt with earlier (see Notes of Guidance).

## 4.6 VIEW OF THE SCCS

In light of the available data, including the latest Industry submission, the following conclusions can be made:

- The potential of butylparaben and propylparaben to modify the endocrine system is the major concern related to the use of parabens in cosmetics. Therefore, the availability of a sound *in vivo* reproductive toxicity study is essential in the hazard assessment of the different esters. However, no unequivocal conclusion can be drawn from the available male reproductive toxicity studies of Hoberman et al. (2008) and Oishi (2001; 2002a,b; 2004) with butylparaben and/or propylparaben. They deliver contradictory results and neither of them is considered to be scientifically acceptable. Therefore the SCCS cannot determine an adequate NO(A)EL-value for the paraben esters under consideration from these studies. Consequently, the **NOEL** value of **2 mg/kg bw/day**, based on Fisher et al. (1999) and also mentioned by Oishi (2001), remains the **conservative choice** for the calculation of the MoS of butyl- and propylparaben. The Committee acknowledges the fact that the Fisher et al. (1999) study involves subcutaneous instead of oral administration, but emphasizes that 2 mg/kg bw/day clearly represents a NOEL instead of an NOAEL and that another study shows butylparaben to cause similar effects at about the same dosage levels after subcutaneous or oral administration (Routledge et al. 1998).

- With regard to the toxicokinetic aspects related to parabens, the SCCP not only requested sound *in vitro* dermal absorption data, but also the performance of a human study in order to obtain adequate and detailed information on the absorption and metabolism of paraben esters in human skin. This request was based upon the fact that the observed *in vitro* (human and rat cell lines)/*in vivo* (rats and mice) endocrine modifying effects caused by parabens were attributed to the parent compounds and not to their common metabolite PHBA.

  Industry uses the argument that paraben esters are quickly and nearly completely hydrolyzed into PHBA after dermal application to human skin, so their systemic toxicity becomes negligible. The SCCS, however, is aware of studies indicating that the biotransformation of the different paraben esters into PHBA is not as efficient as claimed. The weight of evidence in this matter is described in Section 3.3.

  The available set of *in vitro* dermal absorption studies is considered of poor scientific quality and the results of biomonitoring studies show the presence of unmetabolised parabens in the plasma of human volunteers. This emphasizes the importance of sound *in vivo* human data, obtained by administration of parabens through the dermal route. To this respect, the applicant cites a human study (Janjua et al. 2007) in which three putative estrogens, among which butylparaben, were together in a cream applied to the skin of 26 volunteers. The fact that three substances were combined in this assay and that no metabolite measurements were performed decreases the scientific value of the results obtained for the present risk assessment.

  Considering these points together, the SCCS is of the opinion that the issues raised earlier by the SCCP (SCCP/1183/08) have not been sufficiently addressed. Although the provided data is quite informative, there still is the missing link between the rat and human dermal absorption, especially of the absorption and metabolism of the parent compound in the skin. According to the applicant, the metabolism of the absorbed parabens through the skin is complete, but no study performed on human volunteers provides conclusive results.

  As dermal absorption is prone to species variability (especially between humans and rats), the rat toxicokinetic study, as currently presented, does not provide a conclusive answer.

  Industry's argumentation that 'real life exposure would probably produce very low plasma levels necessitating the use of extremely sensitive analytical equipment

(LC/MS/MS) .....' is not considered valid, as such equipment is now state-of-the-art in all modern analytical laboratories, and it has been applied successfully in measuring numerous parabens in human urine and/or plasma samples (Ye et al. 2006, Janjua et al. 2007).

In addition, the applicant explains that 'the principal metabolite of parabens, PHBA, is ubiquitous in plants and human nutrition and expected to occur naturally in humans'. Therefore Industry considers that 'in order to distinguish systemic levels of PHBA resulting from topical exposure to parabens in cosmetics from those that result from food and other sources, such a study would require skin application of [$^{14}$C]-labelled parabens and raises ethical constraints'.

The SCCS is aware of the problem that non-cosmetic exposure to PHBA could invalidate an interpretation of results that are based on metabolite analysis. However, the main point of interest is dermal absorption of unmetabolised parabens after topical application, and this would not necessarily require the use of radiolabeled compounds. Parabens are apparently not completely hydrolyzed to PHBA as indicated in several human studies (Ye *et al.* 2006, Janjua *et al*. 2007, Janjua *et al*. 2008, Carwile *et al*. 2009, Frederiksen *et al*. 2010).

As long as properly conducted dermal absorption and/or toxicokinetic studies in humans are not available, the Committee chooses to use a pragmatic approach and to base its calculations on the 3.7% dermal absorption value derived from the results of three in vitro dermal absorption studies (full rationale under 3.4). The limited data available for human in vivo studies support the assumption of an absorption value for unmetabolised parabens in the lower one-digit percentage range. This value is a more conservative estimate than the 1% proposed by Industry and the 2% value proposed by the Danish Technical University (2010)

- The MoS calculation as proposed by Industry, based upon the Hoberman et al. (2008) NO(A)EL value and a 1% dermal absorption is not acceptable for the following reasons:
  - the 1% value of systemic availability results from a re-analysed 'preliminary' dermal absorption study (Pape and Schepky 2009), of poor quality. In case parabens are completely hydrolyzed into PHBA, the latter will become systemically available.
  - the reproductive toxicity NO(A)EL is based on a study with insufficient scientific reliability. Using the *in vivo* estrogenicity studies and applying additional safety factors is not feasible either, as all studies are performed either through subcutaneous or oral route, meaning that skin metabolism is avoided. Therefore, with the current level of knowledge, their relevance for this risk assessment is not clear.

  Of the three assumptions present in the MoS calculation proposed by the Industry, being the dermal absorption value, the NO(A)EL value and the finished product exposure level, only the latter seems acceptable.

As explained before, the SCCS uses the following parameters for the final calculation of the MoS of butylparaben:

| | |
|---|---|
| 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 |

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

**MoS = NOEL / SED = 46.6**

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

## 5. OPINION

With respect to the safe use of parabens as cosmetic ingredients, concern was expressed as to the potential endocrine modifying effects of parabens of higher chain length including propylparaben, butylparaben and related *iso* compounds. Benzylparaben was also of concern. Based upon the currently available *in vitro* data and *in vivo* rodent test results, the SCCS agrees that the estrogenic properties displayed by parabens appear to increase with increasing chain length. Nevertheless, the SCCS stresses that the displayed potency levels remain about 3 to 6 orders of magnitude lower than the potency of the positive controls.

It is difficult to determine an adequate NO(A)EL value for the observed reproductive effects of butylparaben or propylparaben in rodents, as each of the two available key (sets of) oral studies suffered serious shortcomings. Industry attempted to resolve this issue by providing data to suggest the complete skin metabolism of parabens into the non-endocrine modifying and non-reproductive toxic metabolite p-hydroxybenzoic acid (PHBA).

Unfortunately, this data consisted of pharmacokinetic results from rodent studies only, whereas other reports clearly pointed towards a potential difference in dermal absorption between rats and humans (Fasano 2004b, Pape and Schepky 2009) and to differences in metabolism of the compounds concerned. Substantial amounts of unmetabolised parabens were detected in human/pig skin samples (Janjua et al. 2007, Ye et al. 2006, Fasano 2004a) and in urine of exposed volunteers (Carwile et al. 2009). Thus, for human skin, no clear demonstration is given of fast and complete metabolism of higher chain length parabens into the common and inactive metabolite PHBA, as is the case in rats.

Therefore, the SCCS cannot ascertain that butylparaben and propylparaben are completely metabolised into PHBA after application to human skin, and still considers the parent compounds as potentially systemically available, however not to an unlimited extent. Due to the lack of properly conducted dermal absorption and/or toxicokinetic studies in humans, the SCCS derived the conservative value of 3.7% dermal absorption for butylparaben. This leads to a MoS of 47 for both butylparaben and propylparaben at the intended use concentration of 0.4% (applying a read-across approach for these two esters).

As the two male reproductive toxicity studies in rodents are of insufficient scientific quality, the NOEL of the Fisher 1999 study (2 mg/kg bw/day) is used as the most conservative value by the SCCS.

Based upon the above, the SCCS considers the use of butylparaben and propylparaben as preservatives in finished cosmetic products as safe to the consumer, as long as the sum of their individual concentrations does not exceed 0.19%. This conclusion is based on the lack of scientifically sound data on the pivotal link between dermal absorption in rats and humans, in particular with regard to the metabolism of the parent compound in the skin. The latter can only be addressed through additional human data.

With regard to methylparaben and ethylparaben, the previous opinion, stating that the use at the maximum authorized concentrations can be considered safe, remains unchanged.

Finally, the SCCS emphasizes that the studies submitted to the Committee primarily concerned propyl- and butylparaben. Limited to no information was submitted for the safety evaluation of isopropyl-, isobutyl-, and phenylparaben. Therefore, for these compounds, the human risk cannot be evaluated.

The same is true for benzylparaben and pentylparaben (the latter not mentioned earlier in SCC(NF)P/SCCS opinions), two esters for which there are indications that they might be used in cosmetic products for 'other purposes', e.g. for their anti-microbial activity. None of them is listed in Annex VI of the Cosmetics Directive, as they do not fall under the indicated 'esters of 4-hydroxybenzoic acid' of entry n°12. The SCCS wishes to draw the attention of the Commission services to this anomaly, which may have effects on consumer safety.

## 6. MINORITY OPINION

Not applicable

## 7. REFERENCES

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## APPENDIX

**Table 1:** Literature overview on estrogenicity-related properties of parabens

| Test substances | Test system | Test principle(s) | Result(s) | Reference |
|---|---|---|---|---|
| ***In vitro* assays** | | | | |
| MePB<br>EtPB<br>PrPB<br>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.<br>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<br>EtPB<br>PrPB<br>BuPB<br>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.<br>In the human epidermal keratinocytes, BuPB displayed an $IC_{50}$ of 12 µM.<br>No pos. control was included. | Prusakiewicz et al. 2007 |
| MePB<br>PrPB<br>BuPB<br>PHBA<br>flutamide<br>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.<br>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 |
| MePB<br>EtPB<br>PrPB<br>BuPB<br>IsoPrPB<br>IsoBuPB<br>BzPB<br>PHBA<br>17β-estradiol | MCF-7 cells (**human**-breast cancer derived cell line shown to be estrogen responsive) | Investigate estrogenic effects of mixtures of parabens on cell proliferation;<br>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.<br>Potency of PB remains 5 to 6 orders of magnitude below that of 17β-estradiol.<br>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.<br>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 |

| Test substances | Test system | Test principle(s) | Result(s) | Reference |
|---|---|---|---|---|
| EtPB<br>BuPB | Human adrenocortical carcinoma cell line **rat** pituitary GH3 cell line | H295R assay evaluating the ability to interfere with steroid hormone biosynthesis and<br>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 estradiol production. No positive control included.<br>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** | | | | |
| MePB<br>BuPB | Alpk:AP **rat** | Uterotrophic assay with both immature and ovariectomized rats. MePB and BuPB were administered at the following dosage levels:<br>- MePB orally at 40, 400 and 800 mg/kg/day<br>- MePB subcutaneously (sc) at 40 and 80 mg/kg/day<br>- BuPB orally at 4, 40, 400, 800 and 1200 mg/kg/day<br>- BuPB subcutaneously at 40, 200, 400, 600, 800, 1000 and 1200 mg/kg/day | MePB administered sc or orally failed to increase uterus weights up to 800 mg MePB/kg/day.<br>BuP given orally increased uterus wet and dry weights at dose levels ≥ 800 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.<br>The positive control estradiol exerted its adverse effects at 0.04 mg/kg/day (sc). | Routledge et al. 1998 |
| 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). | No detectable effect on any of the measured reproductive parameters (testis weight and histological examination). | Fisher et al. 1999 |
| 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. | Oishi 2001 |
| PrPB | Wistar **rat** | Study of the effects of PrPB on general function of the male rat reproductive system.<br>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. | 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. | Oishi 2002b |

| Test substances | Test system | Test principle(s) | Result(s) | Reference |
|---|---|---|---|---|
| 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:<br>- 40 mg/kg/day (1.2 mg/mouse)<br>- 400 mg/kg/day (12 mg/mouse) | Wet uterine weight was increased at both dosage levels. Positive control 17β-estradiol exerted comparable effects at 167 ng/kg/day (5 ng/mouse). | Darbre et al. 2002 |
| 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. | Kang et al. 2002 |
| MePB<br>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. | Oishi 2004 |
| EtPB<br>BuPB | Wistar **rat** | Study of the effect of parabens on the steroidogenesis in rats and their offspring when dams are subcutaneously exposed to either:<br>- 400 mg EtPB/kg/day; or<br>- 200 - 400 mg BuPB/kg/day<br>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. | 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<br>- in dams: plasma hormone concentrations and organ weights<br>- 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.<br>Exposure occurred via silastic capsule implanted subcutaneously.<br>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.<br>All other indices examined were unaffected by the treatment.<br>No positive control was included. | Kawaguchi et al. 2009 |

| Test substances | Test system | Test principle(s) | Result(s) | Reference |
|---|---|---|---|---|
| IsoBuPB | Sprague Dawley **rats** | Study designed to analyze the effects of maternal IsoBuPB treatment on the emotional behavior and learning performance in mature offspring.<br>Exposure occurred via silastic capsule implanted subcutaneously.<br>No dosage level(s) stated. 'Estimated dose' is 4.36 mg/kg/day | Early exposure to IsoBuPB may increase anxiety, and specifically disturb passive avoidance performance, although the effects are male-specific.<br>Other parameters were unaffected and no signs of overt toxicity were noted. | Kawaguchi et al. 2009b |
| PrPB<br>BuPB<br>IsoPrPB<br>IsoBuPB<br>17α-ethinyl estradiol | Sprague Dawley immature female **rats** | Uterotrophic assay. Subcutaneous injection of 62.5-250-1000 mg/kg/day of paraben for 3 days.<br>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.<br>At the highest dosage level, parabens also increased the expression levels of uterine CaBP-9k through progesterone-receptor involved pathways. | Vo and Jeung 2009 |
| BuPB<br>PrPB<br>17β-estradiol | 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 mgPrPB/kg/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.<br>14 mg/kg/day 17β-estradiol 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.<br>17β-estradiol terminated all pregnancies.<br>The uterotrophic assay revealed that BuPB did not affect uterine wet or dry mass at any dose in either strain.<br>17β-estradiol consistently increased uterine mass in both strains. | Shaw and deCatanzaro 2009 |

| Test substances | Test system | Test principle(s) | Result(s) | Reference |
|---|---|---|---|---|
| MePB<br>EtPB<br>PrPB<br>BuPB<br>IsoPrPB<br>IsoBuPB<br>17α-ethinyl estradiol | Mated Sprague Dawley female **rats** | *In vivo* assay to investigate whether long-term exposure to PB may induce suppressive effects on reproductive organs in female rats during the critical developmental stage.<br>Oral administration of 62.5-250-1000 mg/kg/day of paraben from postnatal day 21 to 40.<br>Investigation of Calbindin-D9-k (CaBP-9k), biomarker for estrogenic effects. | <u>1000 mg/kg/day:</u><br>MePB, IsoPrPB: decreased ovary weight<br>MePB, EtPB, PrPB: increased adrenal weight<br>EtPB, IsoPrPB: decreased kidney weight, reduced serum estradiol levels<br>MePB, BuPB: increased thyroid gland weight<br>IsoBuPB: decrease of corporea lutea, increase in n° of cystic follicles, myometrial hypertrophy<br>PrPB: myometrial hypertrophy<br><br><u>All dosage levels:</u><br>BuPB: increased liver weight (no dose-response relationship)<br>BuPB, IsoBuPB: decrease of corporea lutea, increase in n° of cystic follicles, myometrial hypertrophy (no dose-response relationship)<br>All PB: changes in $T_4$ serum levels (no dose-response relationship)<br><br><u>$IC_{50}$ values for binding ERα and ERβ receptors:</u><br>17β-estradiol: $3.10^{-9}$ M<br>IsoBuPB: $2.10^{-6}$ M<br>BuPB: $5.10^{-6}$ M<br>IsoPrPB: $2.10^{-5}$ M<br>PrPB: $2.10^{-5}$ M<br>EtPB: $5.10^{-5}$ M<br>MePB: too low to be calculated | Vo et al. 2010 |

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

| 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)<br>6 samples | Measurement of dermal absorption through human skin of BuPB at 0.4% in an o/w emulsion,<br>applied at 8-10 mg/cm² and left in contact with skin for 24h. | Absorbed dose (%):<br>Receptor fluid: 21.01 ± 6.95<br>Receptor wash: 0.49 ± 0.16<br>Skin (excl. tape strips): 36.92 ± 4.97<br>TOTAL: 58.42 ± 10.39<br>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%.<br><br>SCCP major comments:<br>- insufficient skin samples used<br>- only one concentration tested<br>- ratio metabolised / unmetabolised Butylparaben only measured in receptor fluid, not in skin compartments<br>- solubility of BuPB in receptor fluid (HEPES buffer + 3.75% BSA) not demonstrated | Fasano 2004a |

| Test substances | Test system | Test principle(s) | Result(s) and major SCCP/SCCS comment(s) | Reference |
|---|---|---|---|---|
| BuPB | Full thickness **human** skin (1587-1983 µm)<br>10 samples from 2 donors | Measurement of dermal absorption through human skin of BuPB at 0.4% in an o/w emulsion,<br>applied at 8-10 mg/cm² and left in contact with skin for 24h. | <u>Absorbed dose (%):</u><br>Receptor fluid: 14.90 ± 3.73<br>Receptor wash: 0.32 ± 0.14<br>Skin (excl. tape strips): 14.80 ± 4.67<br>TOTAL: 30.10 ± 7.08<br>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%.<br><br><u>SCCP major comments:</u><br>- insufficient skin samples used<br>- ratio metabolised / unmetabolised Butylparaben only measured in receptor fluid, not in skin compartments<br>- only one concentration tested<br>- 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<br>MePB | **Rat** and **human** skin (450 µm)<br>10 samples from<br>≥ 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,<br>applied at 8-10 mg/cm² and left in contact with skin for 24h. | <u>Absorbed dose rat skin (%):</u><br>MePB / BuPB<br>Receptor fluid: 54.94 ± 5.92 / 54.23 ± 5.92<br>Receptor wash: 0.43 ± 0.20 / 0.44 ± 0.20<br>Skin (excl. tape strips): 12.23 ± 5.57 / 13.01 ± 5.57<br>TOTAL: 67.61 ± 6.06 / 67.69 ± 9.06<br>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.<br><br><u>Absorbed dose human skin (%):</u><br>MePB / BuPB<br>Receptor fluid: 79.36 ± 15.62 / 73.51 ± 10.34<br>Receptor wash: 0.46 ± 0.11 / 0.72 ± 0.21<br>Skin (excl. tape strips): 4.88 ± 2.01 / 6.92 ± 1.77<br>TOTAL: 84.69 ± 15.46 / 81.15 ± 10.65<br>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.<br><br><u>SCCP major comments:</u><br>- insufficient skin samples used<br>- only one concentration tested<br>- solubility of BuPB in receptor fluid (HEPES buffer + 3.75% BSA) not demonstrated | Fasano 2004b |
| BuPB | Full thickness **pig** skin<br>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<br>Dermis: 50% unmetabolised BuPB + 50% PHBA<br>Receptor fluid: only PHBA measured.<br><br><u>SCCS major comments:</u><br>- description of test is not detailed enough<br>- only one concentration tested<br>- no data on solubility of BuPB in receptor fluid<br>- 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 | 7 day daily whole body topical 2 mg/cm² application of a skin cream containing 2% BuPB, 2% DEP and 2% DBP.<br>BuPB levels measured in serum, together with reproductive hormones:<br>- follicle stimulating hormone (FSH)<br>- lutenising hormone (LH)<br>- testosterone<br>- estradiol<br>- inhibin B<br>And thyroid hormones:<br>- thyroid stimulating hormone (TSH)<br>- free thyroxine ($FT_4$)<br>- total triiodothyroxine ($T_3$)<br>- total thyroxine ($T_4$) | BuPB was detected in serum after 1 hour (rapid uptake with peak of 135 µg/l after 4h), but no effect was noticed on a number of relevant hormone levels, such as TSH, LH, estradiol, Inhibin B, $T_4$ and $FT_4$.<br><br>SCCP major comment:<br>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 |
| MePB<br>PrPB<br>BuPB | Sprague Dawley **rats** | Study of the absorption, plasma kinetics, body distribution, metabolism (determination of plasma metabolites) and excretion of [$^{14}$C]-MePB, -PrPB and –BuPB.<br>Oral and dermal administration of 100 mg/kg of MePB, PrPB and BuPB and sc administration of 100 mg/kg of BuPB. | Oral administration<br>High and rapid ($C_{max}$ at 0.5 hrs) uptake of radioactivity in serum for all three parabens. Elimination after 8 to 22 hrs.<br>Dermal administration<br>Relatively low and slower ($C_{max}$ at 8 hrs) uptake of radioactivity in serum for all three parabens. Elimination after 12 to 22 hrs.<br>Sc administration<br>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.<br><br>Plasma metabolite characterisation revealed only one metabolite, namely PHBA, independent of time of collection, paraben type and route of administration.<br>The study revealed that the principal route of excretion was via the urine and that no selective organ / tissue storage was observed. | Aubert 2009 |