EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

Parabens

COLIPA N° P82

Adopted by the SCCP
during the 9th plenary meeting of 10 October 2006

TABLE OF CONTENTS

1. BACKGROUND

The Scientific Committee on Consumer Product (SCCP**)** adopted in January 2005 two opinions on parabens. The first opinion (SCCP/0874/05) addressed parabens and breast cancer: "*Extended Opinion on Parabens, underarm cosmetics and breast cancer".*

The second opinion "(SCCP/0873/05) was "*An extended opinion on the Safety Evaluation of Parabens"* with the following conclusions:

"*Methyl and ethyl paraben*
For the methyl and ethyl p-hydroxybenzoic acid esters, the maximum authorized concentrations remain unchanged.

Propyl, isopropyl, butyl and isobutyl paraben
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 isobutyl paraben 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 isobutyl paraben, with special focus on the male reproductive system."*

The current submission I, submitted by COLIPA[1], is the response to the SCCP request for additional data.

2. TERMS OF REFERENCE

Does the SCCP consider the continued use of propyl, isopropyl, butyl and isobutylparaben in a concentration up to the existing 0.4% weight/weight as individuals or 0.8% when used in combination in cosmetic products safe for the consumer?

3. OPINION

3.1 Historical background

Parabens are the alkyl esters of p-hydroxybenzoic acid and are allowed as antimicrobial preservatives for use in food products, medicinal products and cosmetics.

In the opinion of January 2005 with regard to the safety evaluation of parabens, an overview of the general toxicological profile of the different p-hydroxybenzoic acid esters was provided, together with a discussion on the available literature with regard to the putative estrogenic effects of the esters and their alleged effects on the male reproductive system [SCCP/0873/05].

[1] COLIPA - The European Cosmetic Toiletry and Perfumery Association

A summary of the discussed publications is displayed in the following table:

Reference	Test species	Dosage level	Effect(s) noted
Butyl Paraben			
Fisher et al. 1999	Male neonatal Wistar rat of 2 days old	2 mg/kg/day for 16 days sc injected	No detectable effect on any reproductive parameter
Oishi 2001	Male post-weaning Crj:Wistar rat	10 mg/kg/day for 8 weeks oral administration	↓ cauda epididymal sperm reserve ↓ sperm count ↓ daily sperm production ↓ serum testosterone
Kang et al. 2002	Female pregnant Sprague Dawley rat	100 to 200mg/kg/day for 14 days sc injected	Offspring: ↓ sperm count ↓ sperm motile activity in epididymus
Oishi 2002a	Male Crj:CD-1 ICR mouse	14.4, 146 and 1504 mg/kg/day for 10 weeks oral administration	↑ epididymal weights ↓ testis spermatid count ↓ serum testosterone (NOAEL = 14.4 mg/kg/day)
Daston 2004	Female pregnant Sprague Dawley rat	0, 10, 100 and 1000 mg/kg/day for 14 days oral gavage	Foetuses examination on gestational day 20 : only developmental parameters measured, no changes versus controls (maternal NOAEL = 100 mg/kg/day)
Propyl Paraben			
Oishi 2002b	Male Crj:Wistar rat	0, 10, 100 and 1000 mg/kg/day for 4 weeks oral administration	At 100 mg/kg/day: ↓ cauda epididymal sperm reserve ↓ sperm count ↓ daily sperm production ↓ serum testosterone Only minor effects at 10 mg/kg/day
Ethyl and Methyl Paraben			
Oishi 2004	Male Crj:Wistar rat	103 and 1030 mg/kg/day for 8 weeks oral administration	No adverse effects noted

With respect to the estrogenic potency of the parabens, it is reported that in the current literature, it was described that as well *in vitro* as *in vivo*, it increased with increasing length and branching

of the alkyl side chains (methyl < ethyl < propyl < butyl < isobutyl), though at all times it remained 1000 to 1,000,000 times below the potency of 17β-estradiol.

3.2 Newly introduced data

Submission I, entered February 2006, consisted, besides literature references and older study protocols, of the following test descriptions:

1. Final report (Protocol 1203-008) on the oral (diet) reproduction toxicity study of Methyl Paraben in male rats [Anonymous 2005a].
2. Final report (Protocol 1203-006) on the oral (diet) reproduction toxicity study of Butyl Paraben in male rats [Anonymous 2005b].
3. *In vitro* dermal penetration and metabolism study - Butyl Paraben [Fasano 2004a].
4. *In vitro* kinetics and metabolism using full thickness human skin - Butyl Paraben [Fasano 2005].
5. *In vitro* dermal penetration and metabolism study - Butyl and Methyl Paraben [Fasano 2004b].

3.2.1 Final reports on the oral (diet) reproduction toxicity studies of Methyl and Butyl Paraben in male rats

According to the COLIPA submission, the newly introduced studies are conducted using the design of the 2001 Oishi study, with the claimed improvement that "*the current studies are run according to GLP, in a more statistically robust manner (more animals were used, i.e. 16 instead of 8 per dosage group), with additional endpoints including detailed histopathological examination of tissues as well as sperm function, adding resolving power"*.

a) Oral reproduction study of Methyl Paraben in the rat

Date of study:	Aug - Oct 2004
Guideline:	no reference stated (Protocol 1203-008)
GLP/QAU statements:	signed statements available
Species/strain:	Crl:(WI)BR rat (strain demonstrated being sensitive to reproductive toxins)
Group size:	16 males / group (64 males in total, offspring of 10 selected dams)
Duration of test:	56 days
Test substance:	Methyl Paraben
Dosage levels:	0, 100, 1000 and 10,000 ppm in food

64 male rats were assigned to four exposure groups, 16 male rats per group. Prepared diets containing Methyl Paraben at constant concentrations of 0, 100, 1000 and 10,000 ppm were available *ad libitum* to the rats for 56 days.

The constant concentrations in the diet corresponded to the following dosage levels:

100 ppm	≈	8.6	-	18.8	mg/kg bw/day
1000 ppm	≈	82.9	-	183.1	mg/kg bw/day

10,000ppm ≈ 874.1 - 1905.2 mg/kg bw/day

All rats were given test diets when weaned on day 21 postpartum. Exposure continued until the day of sacrifice. Viabilities, clinical observations, body weights and feed consumption values were recorded.

Beginning at the start of week 3 of the exposure period, blood samples were collected every other week from each male rat and retained frozen for analysis of LH (luteinizing hormone), FSH (follicle-stimulating hormone) and testosterone.
On the day of sacrifice, all surviving rats were killed and blood samples were collected for future analysis for Methyl Paraben and metabolite levels. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Reproductive organs from all rats, as well as the liver, adrenal glands, thyroid and pituitary gland were weighed. Sperm evaluations were conducted to determine sperm concentration, motility and morphology. The left testis from each rat was collected for evaluation of Daily Sperm Production (DSP) determinations (i.e. testicular spermatid concentration). The liver, adrenal, thyroid and pituitary glands from ten male rats per exposure group were quick-frozen in liquid nitrogen and retained for hormone measurements. Histological examination was performed on the reproductive organs from all rats assigned to the control and high test article concentration groups. Additionally, the liver, adrenals, thyroid and pituitary glands from six rats per group were retained for histopathology. A detailed qualitative examination of the testes was conducted, taking into account the tubular stages of the spermatogenic cycle.

Results
According to the authors of the study, the following findings were made:

No substance-related mortalities occurred.

Retro-orbital bleeding; chromorhinorrhea; chromodacryorrhea; a scab or abrasion on the nose, mouth, right axilla, right forelimb, tip of tail, tail or neck; localized alopecia; excess salivation; microphthalmia; swollen right hindpaw; lacrimation; ulceration on the mouth; missing tip of tail; neck abrasion and soft or liquid faeces occurred in animals of all dosage groups. Since these effects were not dosage-dependent, they were considered not to be related to the administration of the test substance.

Body weights, body weight gains, absolute and relative feed consumption values were not affected.

Necropsy results showed gross lesions in 4/16 of the 1000 ppm treated animals, including small ventral and dorsal prostate and seminal vesicles, 2 diaphragmatic hernias and a case of small testes and epididymides. Since these effects were not dosage-related, they are considered not to be related to the administration of the test substance.

Terminal body weights, organ weights and ratios of organ weight to body weight were reported not to be affected by oral exposure to Methyl Paraben. A statistically significant increase in the ratio liver weight/body weight occurred at 10,000 ppm, but was considered not treatment-related since the increase of the absolute values was not significant.
Finally sperm motility, sperm count, morphology or daily sperm production were described not being affected during the study. The amount of normal sperms was significantly reduced in the 1000 ppm animals and to a slightly lesser extent in the 10,000 ppm animals. Since this effect was

not considered dosage-dependent, it was not regarded as being related to the oral exposure to Methyl Paraben.
No histopathological changes were observed.

Conclusion
The testing laboratory concluded that on the basis of this study, the NOEL for general toxicity for Methyl Paraben, including histopathology of reproductive organs and sperm analysis, is 10,000 ppm. The authors also concluded that no effects occurred at the highest dosage tested.

Ref.: 5

b) Oral reproduction study of Butyl Paraben in the rat

Date of study:	Jun - Aug 2004
Guideline:	no reference stated (Protocol 1203-006)
GLP/QAU statements:	signed statements available
Species/strain:	Crl:(WI)BR rat (strain demonstrated being sensitive to reproductive toxins)
Group size:	16 males / group (64 males in total, offspring of 10 selected dams)
Duration of test:	56 days
Test substance:	Butyl Paraben
Dosage levels:	0, 100, 1000 and 10,000 ppm in food

64 male rats were assigned to four exposure groups, 16 male rats per group. Prepared diets containing Butyl Paraben at constant concentrations of 0, 100, 1000 and 10,000 ppm were available *ad libitum* to the rats for 56 days.
The constant concentrations in the diet corresponded to the following dosage levels:

100 ppm	≈	8.0	-	17.7	mg/kg bw/day
1000 ppm	≈	81.8	-	188.7	mg/kg bw/day
10,000 ppm	≈	807.2	-	1784.6	mg/kg bw/day

All rats were given diets with test compound when weaned on day 21 postpartum. Exposure continued until the day of sacrifice. Viabilities, clinical observations, body weights and feed consumption values were recorded. Beginning at the start of week 3 of the exposure period, blood samples were collected every other week from each male rat and retained frozen for analysis of LH (luteinizing hormone), FSH (follicle-stimulating hormone) and testosterone.
On the day of sacrifice, all surviving rats were killed and blood samples were collected for future analysis for Butyl Paraben and metabolite levels. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Reproductive organs from all rats, as well as the liver, adrenal glands, thyroid and pituitary gland were weighed. Sperm evaluations were conducted to determine sperm concentration, motility and morphology.

The left testis from each rat was collected for evaluation of Daily Sperm Production (DSP) determinations (i.e. testicular spermatid concentration). The liver, adrenal, thyroid and pituitary glands from ten male rats per exposure group were quick-frozen in liquid nitrogen and retained for hormone measurements. Histological examination was performed on the reproductive organs from all rats assigned to the control and high test article concentration groups. Additionally, the liver, adrenals, thyroid and pituitary glands from six rats per group were retained for

histopathology. A detailed qualitative examination of the testes was conducted, taking into account the tubular stages of the spermatogenic cycle.

Results
According to the authors of the study, the following findings were made:

No substance-related mortalities occurred.

Retro-orbital bleeding; chromorhinorrhea; chromodacryorrhea; a scab or abrasion on the head, neck, mouth, nose and/or left axilla; localized alopecia; dental problems; lacrimation; excess salivation; enlarged eye; corneal opacity; ulceration and bent tail occurred in animals of all dosage groups. Since these effects were not dosage-dependent, they were considered not to be related to the administration of the test substance.

Body weights, body weight gains, absolute and relative feed consumption values were not affected.

No necropsy observations related to Butyl Paraben occurred.

Terminal body weights, organ weights and ratios of organ weight to body weight were not affected by oral exposure to Butyl Paraben.

Sperm motility, sperm count, morphology or daily sperm production were not affected during the study.

No histopathological changes in the reproductive organs or the liver, adrenal glands, thyroid or pituitary, were noted.

With regard to the hormones measured in the blood, statistically significant reductions in testosterone levels were recorded at 1000 and 10,000 ppm after 3 weeks of exposure. A significant increase in testosterone and follicle-stimulating hormone (FSH) was noted at the end of the study in the 10,000 ppm group. The laboratory does not consider these effects substance-related, since the 3 week decrease in testosterone was attributable to abnormal high values in 2 control animals and since a general increase in testosterone and FSH levels occurred in all groups. Finally, significant reductions in luteinizing hormone (LH) after 5 weeks in the 100 and 1000 ppm groups were not considered related to Butyl Paraben, since they only occurred at one time point and were not dosage-dependent.

Conclusion

The testing laboratory concluded that on the basis of this study, the NOEL for general toxicity for Butyl Paraben, including hormone levels for testosterone, LH and FSH, histopathology of reproductive organs, liver, adrenal glands, thyroid and pituitary and sperm analysis, is 10,000 ppm.

Ref.: 4

c) Discussion on reproduction toxicity studies

The above-summarised studies are intended to represent an improvement of the published Oishi studies and result in the proposal of a new NOEL value for Butyl Paraben of 1000 mg/kg/day. For Methyl Paraben, the NOEL had already been determined as 1000 mg/kg/day as described in previous reports.

In order to accept the new NOEL value for Butyl Paraben, it is essential to certify the validity of the presented study. To that respect, some important remarks should be taken into consideration:

- Both introduced so-called "reproduction studies" do not follow any well-established scientific protocol (no OECD number, no Annex V EC B. number).
- The 64 animals are emanating from the restricted number of 10 dams, expected to generate litters large enough to deliver a F1 generation of 64 males. No further details (which pups are from the same dam) are given in the description of the test.
- The body weights of the animals are very divergent. In classical toxicity studies, usually a variation of 20% in body weight is accepted, while the assays under consideration display deviations up to 48% within one dosage group.
- The Methyl Paraben study mentions in its protocol that testosterone, FSH and LH have been measured in the blood, but these values are not discussed in the results section and the raw data cannot be found. Moreover, pages B-45 and B-60 are lacking in the raw data section.
- For Butyl Paraben the hormone levels are available, but they are characterized by large standard deviations, which make their interpretation quite difficult. The protocol states that *the time of each blood collection will be recorded in the raw data*, but these sampling times cannot be found in the information provided. This information is important as measuring hormones at different times of the day is known to generate diverging values.
- Many animals display unexpected clinical symptoms, such as chromorhinorrhea, chromodacryorrhea, etc., which raises questions about their general health at the start of the study.
- Finally, nearly all findings that have statistical significance, have been waived due to the lack of dose-dependency, abnormal high values in control animals, etc.

Taking all the above together, it is extremely difficult to consider the performed tests as valid. A full description of a positive control study in the performing laboratory might have given an indication on the validity and scientific value of the results. But also this parameter in the submitted dossier was of unacceptable quality. Indeed, COLIPA submission I briefly tackled this point, but in the test mentioned to serve as a positive control, not the same parameters as the ones determined in the above-described reproduction studies, have been measured. More specifically, it mentioned the percentage of motile sperms, but also the *% rapid, straight line velocity* and *track velocity*. The positive control substance (α-chlorohydrin) is stated to be added *directly to 10ml sample in M-199 medium*, a procedure rather belonging to an *in vitro* assay than to an animal study. Finally, the positive control is stated to induce a reduction of 7.4% of motile sperms (from 82.6% to 76.2%), while in the performed Butyl Paraben test, the values for these percentages are systematically higher (> 90%) and the mentioned standard deviations easily exceed 7%. Scientifically spoken, a positive control is expected to have an effect largely exceeding the normal standard deviation of a measurement.

Therefore, the presented study for Butyl Paraben cannot be considered as scientifically valid.

d) Additional data

A number of publications, of which the vast majority has already been taken into account in the previous extended opinion, were also included in the data package [Ashby et al. 2003, Daston 1997, Hossaini et al. 2000, Kang et al. 2002, Oishi 2001, 2002 and 2004, Routledge et al. 1998, Daston 2004, FDRL 1972 and 1973, CIR 1994 and 1995, Soni 2001 and 2002]. These publications do not provide an answer to the questions posed by the SCCP and are therefore not further discussed.

e) Final remark

Besides the fact that no additional data on the reproductive effects of Methyl Paraben were asked, the study provided on the methyl ester suffers from the same problems as mentioned under the discussion for Butyl Paraben. As a consequence, it even undermines the decision taken earlier for Methyl Paraben.

3.2.2 Dermal absorption of Methyl and Butyl Paraben

The presented studies have been initiated to prove that, although Parabens have the potential to penetrate into human or rat skin, the skin has substantial capability to metabolize the esters, which limits systemic exposure to the parent compounds.

a) *In vitro* dermal penetration and metabolism study (human skin) - Butyl Paraben

Date of study:	July 2004
Guideline:	OECD 428 (2002)
GLP/QAU statement:	Not available
Test system:	Full thickness human skin (1000 µm), 6 samples
Contact time:	24 hours
Test substance:	Butyl Paraben
Test formulation:	oil-in-water emulsion (composition stated) containing 0.4% of radio-labelled Butyl Paraben.
Control substance:	None
Application:	8 - 10 mg/cm² (10 µl/cm²)
Receptor fluid:	Hepes-buffered Hanks' balanced salt solution containing 0.05 mg/ml gentamicin and 3.75% bovine serum albumin

Butyl Paraben was formulated as an oil-in-water emulsion at a target concentration of 0.4%. Samples of fresh full-thickness human skin were mounted *stratum corneum* uppermost, onto a flow-through diffusion cell system with an exposure area of 0.64 cm². The underside of each skin specimen was perfused with sterile filtered Hepes-buffered Hanks' balanced salt solution containing gentamicin (0.05 mg/ml) and bovine serum albumin (3.75%). The formulated emulsion was applied as a finite dose at a rate of 10µl/cm² (n = 6 replicates). Penetration was followed using [^{14}C]-labelled active ingredient, which was uniformly blended into the emulsion. The amount of active applied per area of skin was approximately 31.9 µg/cm². The applied formulation remained in contact with the skin for 24 hours without occlusion. During the 24-hour exposure period, serial receptor fluid samples were collected hourly for the first 6 hours and then every other hour until termination. At the end of the exposure period, the skin surface was washed with a dilute soap solution to remove excess formulation and then tape-stripped to remove the *stratum corneum.*

Distribution of the applied radiolabelled material was determined by liquid scintillation counting. Metabolism was determined by quantitative analysis of serial receptor fluid samples for Butyl Paraben and 4-hydroxybenzoic acid using HPLC-mass spectrometry.

Results

Following application of a 0.4% Butyl Paraben emulsion to viable, full-thickness human skin, total penetration at 24 hours post-exposure was 21.01%:

	Mean	S.D.
Absorbed dose (%)		
Receptor fluid	21.01	6.95
Receptor wash	0.49	0.16
Total absorbed	21.50	7.06
Absorbable dose (%)		
Receptor fluid	21.01	6.95
Receptor wash	0.49	0.16
Skin	36.92	4.97
Total absorbable	58.42	10.39
Unabsorbed dose (%)		
Skin wash	37.85	8.12
Donor chamber	0.82	0.46
Tape strips	1.72	0.40
Total unabsorbed	40.39	8.10
Total recovered	98.81	3.45

The principle metabolite, 4-hydroxybenzoic acid, was detected in the receptor fluid over the course of the exposure phase with barely detectable levels of unmetabolized Butyl Paraben in receptor fluid from only one of the six skin replicates.

Conclusion
According to the authors of the study, these data suggest that the dermal bioavailability of Butyl Paraben from an oil-in-water emulsion was low and that local metabolism resulted in complete hydrolysis to the primary acid metabolite.

Ref.: 13

b) *In vitro* kinetics and metabolism using full thickness human skin - Butyl Paraben

Date of study:	July 2005
Guideline:	OECD 428 (2004)
GLP/QAU statement:	Signed statements available
Test system:	Full thickness human skin (1587-1983 µm), 10 samples (2 donors)
Contact time:	24 hours
Test substance:	Butyl Paraben
Test formulation:	oil-in-water emulsion (composition stated) containing 0.4% of Butyl Paraben
Control substance:	None
Application:	8 - 10 mg/cm² (10 µl/cm²)

Receptor fluid: Hepes-buffered Hanks' balanced salt solution containing 0.05 mg/ml gentamicin and 3.75% bovine serum albumin

Butyl Paraben was formulated as an oil-in-water emulsion at a target concentration of 0.4%. Samples of fresh full-thickness human skin were mounted *stratum corneum* uppermost, onto a flow-through diffusion cell system with an exposure area of 0.64 cm². The underside of each skin specimen was perfused with sterile filtered Hepes-buffered Hanks' balanced salt solution containing gentamicin (0.05 mg/ml) and bovine serum albumin (3.75%).
The formulated emulsion was applied as a finite dose at a rate of 10µl/cm² (n = 10 replicates representing 2 donors). Penetration was followed using [^{14}C]-labelled active ingredient, which was uniformly blended into the emulsion. The amount of active applied per area of skin was approximately 37.6 µg/cm². The applied formulation remained in contact with the skin for 24 hours without occlusion. During the 24-hour exposure period, serial receptor fluid samples were collected hourly for the first 6 hours and then every other hour until termination. At the end of the exposure period, the skin surface was washed with a dilute soap solution to remove excess formulation and then tape-stripped to remove the *stratum corneum.* Distribution of the applied radiolabelled material was determined by liquid scintillation counting. Local metabolism was determined by quantitative analysis of serial receptor fluid samples for Butyl Paraben and 4-hydroxybenzoic acid using HPLC-mass spectrometry.

Results
Following application of a 0.4% Butyl Paraben emulsion to viable, full-thickness human skin, total penetration at 24 hours post-exposure was 14.9%:

	Mean	S.D.
Absorbed dose (%)		
Receptor fluid	14.90	3.73
Receptor wash	0.32	0.14
Total absorbed	15.20	3.78
Absorbable dose (%)		
Receptor fluid	14.90	3.73
Receptor wash	0.32	0.14
Skin	14.80	4.67
Total absorbable	30.10	7.08
Unabsorbed dose (%)		
Skin wash	47.00	9.23
Donor chamber	1.50	1.49
Tape strips	5.16	1.75
Total unabsorbed	53.60	8.83
Total recovered	83.70	10.90

The principle metabolite, 4-hydroxybenzoic acid, was detected in the receptor fluid over the course of the exposure phase with barely detectable levels of unmetabolized Butyl Paraben in receptor fluid from five of the ten skin replicates.

Conclusion

According to the authors of the study, these data suggest that the dermal bioavailability of Butyl Paraben from an oil-in-water emulsion was low and that local metabolism resulted in complete hydrolysis to the primary acid metabolite.

Ref.: 14

c) *In vitro* dermal penetration and metabolism study (rat and human skin)-

Butyl and Methyl Paraben

Date of study:	Mar - Jun 2004
Guideline:	OECD 428 (2002)
GLP/QAU statement:	Signed statements available
Test system:	rat and human skin (dermatomed to 450 µm, per species at least 10 samples from ≥ 3 donors)
Contact time:	24 hours
Test substance:	Methyl Paraben or Butyl Paraben
Test formulation:	oil-in-water emulsion (composition stated) containing 0.8% of Methyl Paraben or 0.4% of Butyl Paraben
Control substance:	None
Application:	8 - 10 mg/cm² (10 µl/cm²)
Receptor fluid:	Hepes-buffered Hanks' balanced salt solution containing 0.05 mg/ml gentamicin and 3.75% bovine serum albumin

Methyl Paraben and Butyl Paraben were formulated as oil-in-water emulsions at target concentrations of 0.8% and 0.4% respectively. Samples of fresh rat and human skin were dermatomed to approximately 450µm and mounted *stratum corneum* uppermost, onto a flow-through diffusion cell system with an exposure area of 0.64 cm². The underside of each skin specimen was perfused with sterile filtered Hepes-buffered Hanks' balanced salt solution containing gentamicin (0.05 mg/ml) and bovine serum albumin (3.75%). Each formulated emulsion was applied as a finite dose at a rate of 10µl/cm² to rat (n = 10 replicates) or human (n = 13 replicates) skin. Penetration was followed using [^{14}C]-labelled active ingredient, which was uniformly blended into the emulsion. The amount of active applied per area of skin was approximately 65 µg/cm² and 36 µg/cm² for the Methyl Paraben and Butyl Paraben emulsions, respectively. The applied formulation remained in contact with the skin for 24 hours without occlusion. During the 24-hour exposure period, serial receptor fluid samples were collected hourly for the first 6 hours and then every other hour until termination. At the end of the exposure period, the skin surface was washed with a dilute soap solution to remove excess formulation and then tape-stripped to remove the *stratum corneum.* Distribution of the applied radio-labelled material was determined by liquid scintillation counting. Local metabolism was determined by quantitative analysis of serial receptor fluid samples for Methyl Paraben or Butyl Paraben, and the principal metabolite 4-hydroxybenzoic acid using HPLC-mass spectrometry. Receptor fluid samples representing each formulation, from both rat and human experiments, were also analyzed by radiochromatography and LC-MS.

Results

A. Methyl Paraben, 0.8% in an oil-in-water emulsion

Following application of a 0.8% Methyl Paraben emulsion to viable rat and human skin, a greater amount of total radioactivity penetrated human skin (79.36%) compared to rat skin (54.94%). A major portion of the total radioactivity that had penetrated rat skin was metabolized to 4-hydroxybenzoic acid (53.9%), with a smaller portion (23.8%) accounted for as unmetabolized Methyl Paraben. By comparison, a lesser portion of the total radioactivity that had penetrated viable human skin had been metabolized to 4-hydroxybenzoic acid (35.1%), with the majority (60.3%) accounted for as unmetabolized Methyl Paraben. Exclusive of species, analysis of receptor fluid pools by radiochromatography and LC-MS revealed the presence of Methyl Paraben, 4-hydroxybenzoic acid, and Ethyl Paraben.

B. Butyl Paraben, 0.4% in an oil-in-water emulsion

Following application of a 0.4% Butyl Paraben emulsion to viable rat and human skin, a greater amount of total radioactivity penetrated human skin (73.51%) compared to rat skin (54.23%). A majority of the total radioactivity that had penetrated rat skin was metabolized to the primary acid metabolite 4-hydroxybenzoic acid (52.3%), with a smaller portion (5.51%) accounted for as unmetabolized Butyl Paraben. For human skin, 32.8% of the total radioactivity in the receptor fluid had been metabolized to 4-hydroxybenzoic acid, with a greater portion (49.7%) accounted for as unmetabolized Butyl Paraben. As was observed for the Methyl Paraben formulation, analysis of receptor fluid pools by radiochromatography and LC-MS revealed the presence of Butyl Paraben, 4-hydroxybenzoic acid, and Ethyl Paraben.

Results for both esters in <u>human skin samples</u>:

	Methyl Paraben		Butyl Paraben	
	Mean	**S.D.**	**Mean**	**S.D.**
Absorbed dose (%)				
Receptor fluid	79.36	15.62	73.51	10.34
Receptor wash	0.46	0.11	0.72	0.21
Total absorbed	79.82	15.60	74.23	10.32
Absorbable dose (%)				
Receptor fluid	79.36	15.62	73.51	10.34
Receptor wash	0.46	0.11	0.72	0.21
Skin	4.88	2.01	6.92	1.77
Total absorbable	84.69	15.46	81.15	10.65
Unabsorbed dose (%)				
Skin wash	14.65	8.76	15.65	8.29
Donor chamber	0.42	0.94	0.60	0.60
Tape strips	6.13	12.01	1.41	1.11
Total unabsorbed	21.21	20.48	17.66	9.38
Total recovered	105.91	15.10	98.81	8.65

Results for both esters in <u>rat skin samples</u>:

	<u>Methyl Paraben</u>		<u>Butyl Paraben</u>	
	Mean	**S.D.**	**Mean**	**S.D.**

Absorbed dose (%)				
Receptor fluid	54.94	5.92	54.23	9.89
Receptor wash	0.43	0.20	0.44	0.10
Total absorbed	55.37	5.92	54.67	9.88
Absorbable dose (%)				
Receptor fluid	54.94	5.92	54.23	9.89
Receptor wash	0.43	0.20	0.44	0.10
Skin	12.23	5.57	13.01	9.44
Total absorbable	67.61	6.06	67.69	9.06
Unabsorbed dose (%)				
Skin wash	17.81	2.82	18.29	6.33
Donor chamber	0.03	0.01	0.44	0.95
Tape strips	5.65	1.12	12.27	5.45
Total unabsorbed	23.49	2.40	30.99	7.94
Total recovered	91.09	5.66	98.68	5.64

Conclusion
According to the authors of the study, these data show, based on a dermatomed viable rat and human skin model and analysis of receptor fluid samples, that the dermal bioavailability of Methyl Paraben and Butyl Paraben from the oil-in-water emulsions was greater for human skin than rat skin, and that a small portion of the total radioactivity that had penetrated the skin, exclusive of species and formulation, was Ethyl Paraben.

Ref.: 12

d) Discussion of the performed *in vitro* absorption studies

The same shortcomings can be noted for the three studies:

- The stability of the test substance(s) was checked in the vehicle, but not in the receptor fluid.
- Only one concentration of the concerned Paraben(s) was tested.
- The dosage levels appear to be too high for a finite dose experiment.
- In study b), the total recovery was rather low, namely 83% (normally accepted 85-115%).
- For the calculation of the absorbed %, the amount in the skin has not been taken into account.
- Reference compound data are lacking.
- The number of samples is 10 coming from at least 3 donors but is not as asked by the SCCNFP basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients [SCCP/0750/03], namely 6 per donor of 3 different donors.
- Ethyl Paraben was found in the receptor fluid pools, although not intentionally added to the experiment.

Taking the results of the three studies together, it becomes clear that there is a significant dermal absorption of Butyl Paraben in the human skin and in one study, a large portion of the substance is even detected in its unmetabolized form. Butyl Paraben also appears to penetrate more easily through human skin compared to rat skin. This is unexpected, comparing the skin barrier functions of both species and the available literature stating in fact the opposite.

It must, however, be emphasized that, without data on the solubility of the different esters in the receptor fluid, any interpretation of the obtained results remains questionable.

e) Additional data

The submission contains a brief and incomplete description (study report) of an examination of the dermal absorption and penetration of Methyl Paraben, Ethyl Paraben, Propyl Paraben, Butyl Paraben and 4-Hydroxybenzoic Acid after application on freshly excised pig skin for 24 hours (according to OECD 428).
The results indicate that only a minor part of the unchanged esters is found in horny layer or epidermis. According to the authors, this indicated the penetration of the Parabens in the deeper layers of the skin, where they are metabolized into 4-hydroxybenzoic acid, the only compound that was detectable in the receptor fluid [Diembeck and Duesing 2005].
An unexpected finding, however, was that the dermal absorption is higher for the methyl, ethyl and propyl ester (75%) than for the butyl ester (33%). In general, dermal absorption of a more lipophilic substance is higher than for a more hydrophilic substance. Since the study report was very incomplete (number of samples, contact time, dosage level, vehicle composition, stability of test substances, reference data, protocol description were lacking) and since the mass balance for the methyl, ethyl and propyl esters was low (around 60%) whereas that of the butyl ester was around 100%, it is impossible to evaluate the results obtained.

A second additional report describes the kinetics of Butyl Paraben metabolism in male Sprague Dawley rat skin S9. The conclusions of the test are that there is a high level of esterase activity in rat skin, that Butyl Paraben is a good substrate for these enzymes and that their capacity is high (V_{max} = 8.8 nmol/min./mg; K_m = 28.6 μM). The kinetic characteristics of the rat skin esterases suggest that even high concentrations of Butyl Paraben applied to the skin are unlikely to saturate metabolism [Laezer 2004].
Some incomplete dermal penetration data in Franz cell experiments under non-standardized conditions were also provided. They showed vehicle effects on the dermal absorption of Butyl Paraben, but seen the nature of these data, they do not provide useful results for the problem under discussion.

Finally, the submission includes a number of publications with regard to dermal absorption of Parabens [Bando et al. 1997, Hotchkiss 1998, Cross and Roberts 2000, Dal Pozzo and Pastori 1995, Lobemeier et al.1996], which all mention the metabolization of the esters in the skin.

4. CONCLUSION

As explained in detail under section 3 of the present opinion, the tests provided in Submission I of February 2006 contain too many shortcomings in order to be considered as scientifically valid.

Therefore, the conclusion of opinion SCCP/0873/05 remains unchanged.

5. MINORITY OPINION

Not applicable

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Dr. C. Chambers	Prof. J.-P. Marty	
Prof. R. Dubakiene	Dr. S.C. Rastogi	
Dr. R. Grimalt	Prof. J. Revuz	
Dr. B. Jazwiec-Kanyion	Prof. V. Rogiers	(Rapporteur)
Prof. V. Kapoulas	Prof. T. Sanner	
Prof. J. Krutmann	Dr. I.R. White	(Chairman)
Prof. C. Lidén		