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Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Opinion of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety

Adopted 14 June 2006

Risk assessment of parabens in cosmetics

SUMMARY

The Norwegian Food Safety Authority (Mattilsynet) has asked the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) to conduct a complete risk assessment of the use of parabens in cosmetic products. The case has been assessed by the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics.

Parabens are the alkyl esters of 4-hydroxybenzoic acid and are allowed as antimicrobial preservatives for use in food products, pharmaceutical products and cosmetics. Recent studies have shown that some parabens in varying degree may bind to the oestrogen receptor and exert weak oestrogenic and androgenic activity, butyl paraben being the most potent. *In vivo* studies have also indicated that butyl paraben has the potential to affect postnatal development of the male reproductive system, resulting in decreased sperm production capacity.

In Norway, these scientific findings have lead to a non-governmental organisation, "Grønn hverdag", raising concerns related to the use of parabens in baby care products. To provide an answer to these concerns, a preliminary risk assessment from the Norwegian Public Health Institute (NIPH) was carried out and submitted to the Norwegian Food Safety Authority in February 2004. The conclusions from this preliminary assessment were that the current use of parabens in cosmetics is safe, but it was indicated that more data on toxicity, human skin absorption and metabolism is needed. The NIPH risk assessment was forwarded to the EU Commission.

In January 2005, the EU Scientific Committee on Consumer Products (SCCP) concluded that more information was needed in order to give a decisive response to whether propyl-, butyl- and isobutyl paraben can be safely used up to the maximum authorized concentration in cosmetic products (0.4%). The industry was therefore requested to provide 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 Cosmetic,

Toiletries, and Fragrance Association and the European Cosmetic Toiletry and Perfumery Association (CTFA/COLIPA Task Force) have recently conducted a number of studies in rats to determine the potential for paraben compounds to affect reproduction or development and also to document the dermal absorption of parabens.

Following a review of the request from the Norwegian Food Safety Authority, it was agreed that the risk assessment from VKM should be postponed until the awaited new and necessary documentation from the industry was provided. The present opinion from VKM, has been based on the preliminary evaluation of parabens in cosmetic products performed by the NIPH, taking into account comments from "Grønn hverdag", a report from CANTOX Health Sciences International and a risk assessment of parabens performed by the Danish Institute of Food and Veterinary Research (DFVF).

The VKM Panel is of the opinion that a health risk assessment of parabens should include an evaluation of each paraben separately. As pointed out by SCCP in their opinion from 2005, methyl- and ethyl paraben can be safely used up to the maximum authorized concentration (0.4%) in cosmetics. This conclusion is endorsed by the Panel. The present assessment addresses the new data on butyl paraben. Further assessments regarding propyl-, isopropyl- and isobutyl paraben will be awaited.

The NOAEL for butyl paraben (1000 mg/kg bw/day) presented in the COLIPA dossier was based on oral reproductive and developmental toxicity studies in rats. As information from standard functional fertility studies was not provided, the Panel is of the opinion that the COLIPA dossier does not provide sufficient information to establish such a NOAEL for butyl paraben on reproductive toxicity. Also, the NOAEL for butyl paraben has been determined in a study using oral administration, which may be of limited relevance for dermal exposure, and is therefore, without further toxicokinetic information, not adequate for use in a risk assessment of butyl paraben in cosmetics. The new data from COLIPA indicate that approximately 50% of the dermally applied butyl paraben is systemically available unmetabolised. This information seems sufficient to determine the Systemic Exposure Dose (SED) for a worst case human exposure.

The Panel concludes that the opinion expressed by the SCCP that: "the available data do not enable a decisive response to the question as to whether propyl-, butyl- and isobutyl paraben can be safely used in cosmetic products at individual concentrations up to 0.4%" is still valid.

BACKGROUND

Parabens are alkyl esters of 4-hydroxybenzoic acid (4-HBA) and are allowed as antimicrobial preservatives for use in food products, pharmaceutical products and cosmetics. These compounds, e.g. methyl-, ethyl-, propyl-, (isopropyl-), butyl-, (isobutyl-) and benzyl parabens are widely used in cosmetic products. They are normally used in combinations containing two or more parabens and/or other preservatives. Butyl paraben shows the largest antimicrobial activity, but is less preferred in cosmetics because of low water solubility. Methyl paraben is used at the highest concentrations and is most frequently used in cosmetics as it is the most water-soluble paraben. Nearly all (99%) of the leave-on cosmetics and 77% of rinse-off cosmetics have been found to contain parabens (1). A preferential use of methyl- > ethyl- > propyl- > butyl- > benzyl paraben in various groups of cosmetic products were reported (1).

Because of their substantial antimicrobial capacity, low toxicity, relatively non-irritating and non-sensitising properties, parabens have in various combinations been used as preservatives in cosmetics and toiletries for decades. These products may come into daily contact with the skin, hair, nails, lips, eyes, mouth or other mucous membranes. Parabens also have a long history of use in a variety of pharmaceutical products intended for either injection, inhalation, oral, topical, rectal or vaginal administration (2;3). According to EC Directive 95/2/EC, Annex III, methyl-, ethyl- and propyl parabens and their sodium salts (E214-219) are conditionally permitted for use in a limited number of foods in combination with either sorbates or sorbates and benzoates.

Recent studies, particularly *in vitro*, have shown that some parabens to a varying degree may bind to the oestrogen receptor and exert week oestrogenic and androgenic activity (1000-1 000 000 times less potent than the active hormones in the body), butyl paraben being the most potent (4-7). *In vivo* studies by Oishi (8;9) indicate that butyl paraben has the potential to affect postnatal development of the male reproductive system, resulting in decreased sperm production capacity.

Regulation

Parabens are regulated by Cosmetic Directive 76/768/EEC, Annex VI, part 1, reference 12 and can accordingly be used as a preservative up to a maximum concentration of 0.4 % in the finished product for one ester and up to 0.8 % (expressed as the acid, 4-hydroxybenzoic acid, 4-HBA) for mixtures of esters (10). The substances are marked with the symbol (+) and therefore may also be added to cosmetic products in concentration other than those laid down in Annex VI for other purposes apparent from the presentation of the product.

If a preservative marked with the symbol (+) is added for non-preservative purpose to a cosmetic product in a concentration higher than that laid down in the Annex VI, data to substantiate its safety should be submitted to the SCCNFP (11).

Overview of the discussion of parabens in Norway

In October 2003, a non-governmental organisation, "Grønn hverdag", raised concern related to the use of parabens in baby care products in Norway. New studies, indicating parabens having an endocrine disrupting potential, were the reason for their concern and in a letter to the Norwegian Minister of Environment and the Norwegian Minister of Health they asked for a ban on parabens in cosmetic products (12). In light of this the Norwegian Food Control Authority (the Norwegian Food Safety Authority (Mattilsynet) from 1 January 2004) requested a human risk assessment of the use of parabens in cosmetic products from the Norwegian Institute of Public Health (NIPH). The preliminary risk assessment from NIPH was submitted on 6 February 2004 to the Norwegian Food Safety Authority (13).

On 17 February 2004, representatives from the Ministry of Environment, the Ministry of Health, the Norwegian Food Safety Authority, the Norwegian Pollution Control Agency, NIPH and "Grønn hverdag" met to discuss if there is a possible environmental and health risk related to parabens. In this meeting, "Grønn hverdag" presented their point of view on the recent preliminary evaluation from NIPH. Their comments were later described in a note where they also suggested which governmental actions should be considered related to the use of parabens in cosmetics in Norway (14).

The conclusions from NIPH´s preliminary assessment in 2004 were that the current use of parabens in cosmetics is safe, but it was indicated that more data on toxicity, human skin absorption and metabolism is needed. The Norwegian Food Safety Authority submitted the NIPH safety assessment to the EU Commission and pointed out that the incomplete data caused some uncertainty in the evaluation of the health risk. The Norwegian Food Safety Authority also urged the importance of asking the industry to carry out an up-to-standards multiple-generation study as concerns reproductive and developmental effects of propyl- and butyl paraben (15;16).

A revised version of the report "Critical evaluation of the endocrine disrupting/oestrogenic potential of parabens" provided by CANTOX Health Sciences International at the request of Johnson and Johnson Consumer Europe was submitted to the Norwegian Food Safety Authority on 14 April 2004 (17). CANTOX informed the Norwegian authorities that the information in one of the *in vitro* metabolism studies used in their initial report was unintentionally misleading. The misleading information was that the maximum skin absorption of parabens probably should be higher than 3.5 % as was reported in the original version of the report (18). Representatives from CANTOX explained this problem and presented the results from their report in a meeting with the authors of the NIPH evaluation in Oslo, in April 2004.

Opinions and evaluations of parabens from national and international bodies

Several evaluations and reviews from different international and national scientific bodies have been published on the toxicity profile and safe use of parabens in food and consumer products. A brief introduction to the conclusions from some of the most important evaluations are summarised below.

Joint FAO/WHO Expert Committee on Food Additives (JECFA) 1974 and 2002
In 1974, JECFA established an ADI of 0-10 mg/kg bw/day for the sum of methyl-, ethyl- and propyl paraben and their sodium salts (19). The ADI was based on chronic toxicity studies made available in the 1950 – 60´s on methyl-, ethyl- and propyl parabens in rats showing a No Observed Effect Level (NOEL) for all three parabens of 2% in the diet, equivalent to 900-1200 mg/kg bw/day. The effect observed at the higher dose level of 8% in the diet was decreased weight gain, accompanied by weight depression and death. JECFA was unable to establish an ADI for butyl paraben.

Butyl paraben, as a flavouring agent, was assessed by JECFA in 2002, and found not to present a safety concern at current low levels of intake (20).

European Commission - Scientific Committee for Food (SCF) 1994
The Scientific Committee for Food (SCF) evaluated parabens in 1994 (21). They reported that acute toxicity of parabens was only seen at high dosages. All the parabens produced similar symptoms with rapid onset of ataxia, paralysis and central nervous system depression, resembling anaesthesia, suggesting their toxicity is related mainly to the free acid. With non-lethal doses recovery is prompt.

Absorption, metabolism and excretion of parabens administrated orally have been studied in rats, rabbits, dogs and humans. The methyl-, ethyl- and propyl esters of 4-hydroxybenzoic acid appeared to be well absorbed, and the ester linkage was readily hydrolysed. Urinary excretion of the unchanged esters was very low, usually less than 1% after oral

administration. Limited *in vivo* data on butyl paraben suggested that it may follow a different metabolic pathway, but studies in dogs had shown no evidence of accumulation of either parent compound or metabolites in the tissues.

Reproduction and teratogenicity studies in rat using ethyl paraben at levels up to 10% in diet showed no adverse effects on reproductive performance. Foetal anomalies, however, were observed, though without a clear dose-response relationship. In view of these equivocal findings, a new oral teratogenicity study in rat was requested by SCF. *In vitro* and *in vivo* mutagenicity studies provided no evidence of genotoxicity for methyl-, propyl- and butyl paraben. A carcinogenicity study with butyl paraben in mice reported no significant difference in tumour rates between treated and control animals, but it was considered inadequate for assessment due to early deaths in treated and control groups and relatively high incidence of some tumours in the control group. A number of special studies indicated that parabens (in particular propyl- and butyl paraben) were able to induce cell proliferation in the forestomach and glandular stomach of rats.

An overall No Observed Adverse Effect Level (NOAEL) of 1000 mg/kg bw/day could be derived from several subchronic and chronic oral toxicity tests conducted in rats, dogs and mice. Based on this value, SCF established a temporary ADI of 0-10 mg/kg bw/day, as the sum of methyl-, ethyl- and propyl 4-hydroxybenzoic acid and their sodium salts. The ADI was made temporary, since SCF asked for some additional information with regard to the reproductive effects and more data on the cell proliferation effects of the compounds in the rat forestomach.

The Norwegian Institute of Public Health (NIPH) 2004

The Norwegian Institute of Public Health performed a preliminary evaluation of the use of parabens in cosmetic products in 2003. A slightly revised version was later forwarded to the Norwegian Food Safety Authority in February 2004 (13).

The conclusions of the evaluation were:

- Different parabens have varying endocrine potentials both in cell cultures and in animal studies, but they are 1000 – 1000 000 times less potent than 17β-estradiol or testosterone. Butyl paraben shows the largest oestrogenic effect and testis seems to be the organ in which adverse effects are seen at the lowest intake of parabens.
- The information on parabens does not represent a sufficient basis for a revised and complete risk evaluation. Data on reproduction in long-term animal experiments, data from multiple generation experiments and more detailed knowledge about the pharmacokinetics and the realistic systemic load after dermal exposure of parabens in children and adults are required.
- A preliminary risk assessment based on the maximal permitted concentration of paraben mixture = 0.8% (worst case) implied Margins of Safety (MoS) of 122 and 73 for adults and children respectively. The calculations were performed according to the "Notes of Guidance for Testing of Cosmetic Ingredients and Their Safety Evaluation" from the SCCNFP(22) and by using a temporary NOAEL of 10 mg/kg bw/day (based on the developmental toxicity of propyl- and butyl paraben) and a percutaneous absorption of 3.5 % as reported by Cantox Health Sciences International in 2003 (18). The contribution of dietary parabens (very small) and the biotransformation of parabens into 4-hydroxybenzoic acid in liver and skin were not accounted for.
- Interactions, additive or synergistic effects, between parabens have not been demonstrated in animal experiments and results from cell culture experiments cannot

be used in the risk evaluation. Effects at doses below the ones tested were considered unlikely.

European Food Safety Authority (EFSA) 2004

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food adopted an opinion on the safety of paraben (E 214-219) usage in foods on 13 July 2004 (23).

Recent studies on the developmental toxicity of methyl paraben in rats, mice, hamsters and rabbits, not available to the SCF(21), were evaluated. No evidence of developmental toxicity up to 300 or 550 mg/kg bw/day was observed. Proliferative effects of parabens in forestomach cells in rats were also re-evaluated. It was concluded that the proliferative effect of parabens will only occur above a certain threshold and that the human exposure resulting from the use of parabens as preservatives in food will be much lower than such doses.

The EFSA Panel was of the opinion that no oestrogenic activity could be detected *in vivo* for methyl-, ethyl- and propyl parabens using uterotrophic assays with peroral and subcutaneous administrations of high doses in mice and rats. However, for butyl- and isobutyl paraben (not used in food), a positive oestrogenic effect was seen after subcutaneous injection but not after oral administration. The common metabolite of parabens, 4-hydroxybenzoic acid, was considered to be non-oestrogenic.

Dietary administration of methyl- and ethyl paraben showed no effects on sex hormones and the reproductive organs in juvenile male rats at dosage levels up to 1000 mg/kg bw/day. Therefore 1000 mg/kg bw/day was considered a NOAEL for both methyl- and ethyl paraben. For propyl paraben given in the diet of rats, impaired spermatogenesis, reduced testosterone levels and reduced number of sperm cells were observed, and a LOAEL of 10 mg/kg bw/day was established.

The EFSA Panel set a group ADI of 0-10 mg/kg bw/day for the sum of methyl- and ethyl paraben and their sodium salts on the basis of a NOAEL of 1000 mg/kg bw/day for each compound in long-term toxicity studies and studies on sex hormones and male reproductive organs in juvenile rats. Propyl paraben was not included in this group ADI because of its effects on sex hormones and the male reproductive organs in juvenile rats. An ADI for propyl paraben was not set because of the lack of a clear NOAEL.

Danish Institute of Food and Veterinary Research (DFVF) 2004

The report "Note on Parabens in Food, Cosmetics and Consumer Products" from the Danish Institute of Food and Veterinary Research was published in September 2004 (24). With regard to the oral intake of parabens the document refers to the conclusions of the EFSA report.

In the case of dermal application of parabens, a NOAEL of approximately 750 mg/kg bw/day for the uterothrophic effect of benzyl paraben topically applied to immature mice can be taken as a point of departure for a worst-case evaluation of the oestrogenic potential of dermal paraben application. It was assumed that the human dermal exposure from cosmetic and pharmaceutical products amounts to 60 mg total parabens per day (1 mg total paraben/kg bw/day for a person weighing 60 kg). This exposure, of which a substantial proportion may be due to the considerably less potent methyl-, ethyl- and propyl parabens is several orders of magnitude lower than the NOAEL for benzyl paraben.

The authors stressed that no specific dermal studies have been performed regarding the potential effect of parabens on the male reproductive system. However, the estimated exposure levels to parabens from dermal applications is an order of magnitude lower than the LOAEL obtained from the most sensitive oral studies on propyl parabens in rats. They therefore conclude that this indicates that the effect, if any, of paraben exposure from cosmetic products on reproductive parameters may be of limited biological relevance. However, studies using low levels of paraben exposure are not available.

Scientific Committee on Consumer Products (SCCP) 2005

The EU Scientific Committee on Consumer Products (SCCP) adopted on 28 January 2005 an opinion on the safety evaluation of parabens covering also isopropyl paraben (25).

SCCP was of the opinion that methyl- and ethyl paraben can be safely used up to the maximum authorized concentration as actually established (0.4%). They further concluded that the available data do not enable a decisive response to the questions from the EU Commission to whether propyl-, butyl- and isobutyl paraben can be safely used in cosmetic products at individual concentrations up to 0.4%. The discussion in this SCCP opinion was based solely upon data in the literature. The industry was therefore requested by SCCP and the EU Commission to provide 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 French Commission of Cosmetology 2005

In a working document provided to a SCCP meeting on parabens on 31 August 2005, the French Commission on cosmetology was of the opinion that more information is needed to confirm the safe use of parabens (especially for propyl- and butyl paraben) in cosmetic products (26). They concluded that there are no safety concerns at the currently allowed maximum levels for methyl- and ethyl parabens. Additional studies concerning reprotoxic effects and pharmacokinetic fate are required in order to evaluate the risk of butyl- and propyl parabens. The aim of these studies should be to confirm or not, the results observed by Oishi with butyl paraben (8;9), and to determine a NOAEL which can be used to calculate a safety margin for butyl- and propyl paraben.

The French experts pointed out that more detailed data on the bioavailability of parabens (both parent form and metabolites) after oral versus topical administration in rats and in humans are needed.

National Toxicology Program (US NTP) 2005

The National Toxicological Program in U.S.A. has recently reviewed the toxicological literature for butyl paraben (27). Human exposure to butyl paraben may occur via inhalation, eye or skin contact, or ingestion. Ingested butyl paraben is rapidly absorbed from the gastrointestinal (GI) tract, metabolised and excreted in urine. Results from one chronic feeding study in mice showed that butyl paraben caused a high incidence of amyloidosis, affecting the spleen, liver, kidney, and/or adrenal gland. Butyl paraben was cytotoxic in isolated rat hepatocytes, mitochondria and in other animal cells *in vitro*. Reproductive studies in mice and rats indicated that maternal exposure to butyl paraben in the diet results in adverse effects on the reproductive system of F1 male offspring. Butyl paraben was not mutagenic in several short-term bioassays, and was reported to be non-carcinogenic in rats and mice.

The NOELs and LOELs for butyl paraben for reproductive, developmental, and short-term and subchronic toxicity studies, are listed in Table 6 in NTP´s report (ANNEX I)(27). The most important reproductive and developmental studies in rodents of butyl paraben are presented in Table 7 in their review (ANNEX II). Dependent on dose and route of administration the different studies showed effects or absences of effects on reproduction and development. Four different studies with oral (diet or gavage) administration of butyl paraben gave disagreeing results as two showed clear effects on reproductive and developmental parameters, while the remaining two did not show statistically significant effects. When butyl paraben was administered subcutaneously, clear effects on reproductive parameters were seen in two studies, while a third study administering a very low dose (2 mg/kg bw/day) was negative.

Enquiry from the Norwegian non-governmental organisation – "Grønn hverdag" 2004

In a note from February 2004, the non-governmental organisation, "Grønn hverdag", gave their comments on the evaluation from the Norwegian Institute of Public Health (14). The most important issues raised by "Grønn hverdag" related to the health risks of parabens are given below:

- Should a health risk assessment of parabens include an evaluation of each paraben separately (especially butyl and propyl) rather than using the mean for a mixture of parabens as done in the evaluation from NIPH?
- The NOAEL used in the NIPH evaluation should be further discussed
- Should worst case calculations be performed by using the maximum authorised concentration of a substance or the concentration which is actually used in a cosmetic product?
- Possible interactions between different parabens should be assessed

New developments relevant for this opinion from the Norwegian Scientific Committee for Food Safety

In January 2005, SCCP concluded that more information was needed in order to give a decisive response to whether propyl-, butyl- and isobutyl paraben can be safely used up to the maximum authorized concentration in cosmetic products. The industry was therefore requested by SCCP and the EU Commission to provide 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 (25).

As a response to this request the Cosmetic, Toiletries, and Fragrance Association (CTFA) and the European Cosmetic Toiletry and Perfumery Association (COLIPA) (CTFA/COLIPA Task Force) have recently conducted a number of studies in rats to determine the potential for paraben compounds to affect reproduction or development, and also to document the dermal absorption of parabens (28).

An industry hearing took place in the Working Group on "Preservatives & Fragrances" of the SCCP on 31 August 2005. Representatives from DG Enterprise of the EU Commission, Agence française de securite sanitaire des produits de sante (AFSSAPS), the French Commission of Cosmetology, the Norwegian Food Safety Authority and the Norwegian Scientific Committee for Food Safety (VKM) attended this meeting. COLIPA presented their

new scientific data on parabens which later was made available and forwarded SCCP, the French Commission of Cosmetology and VKM (28).

After receiving the new information from the industry, the French Commission of cosmetology issued a statement on the use of parabens in cosmetics on 29 September 2005 (29;30). They were of the opinion that both methyl-, ethyl-, propyl- and butyl paraben still can be safely used as preservatives in cosmetic products up to the maximum concentrations allowed in the Cosmetic Directive 76/768/EEC. They had come to such a conclusion by accepting the NOAEL for butyl paraben of 1000 mg/kg bw/day as recently presented by COLIPA. The use of propyl paraben was considered safe based on extrapolation from the new data on butyl paraben. The French Commission of cosmetology made reservations regarding isobutyl paraben, asking the industry to provide more data on skin absorption and metabolism for this paraben.

TERMS OF REFERENCE

The Norwegian Food Safety Authority asked the Norwegian Scientific Committee for Food Safety to conduct a complete risk assessment of the use of parabens in cosmetic products, and to base it on the preliminary evaluation of parabens in cosmetic products performed by the NIPH on 6 February 2004 (13), and taking into account comments from "Grønn hverdag" (14), a report from CANTOX (17), and the risk assessment of parabens performed by the DFVF (24).

Following a review of the request from the Norwegian Food Safety Authority of 8 September 2004, it was agreed that the risk assessment could be postponed until awaited new and necessary documentation from the industry was provided.

In reply to the request by the Norwegian Food Safety Authority, the following issues have been addressed:

- Should a health risk assessment of parabens include an evaluation of each paraben separately?
- Review of updated information on reproductive toxicity
- Review of updated information on systemic exposure after dermal application
- Are the new data available sufficient to perform a complete safety assessment?

ASSESSMENT

The assessement was performed by the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety.

Should a health risk assessment of parabens include an evaluation of each paraben separately?

The safety of each paraben should, because of different toxicological properties, be evaluated separately assuming a worst case situation where it is used in a maximum authorised concentration (22).

SCCP has pointed out that methyl- and ethyl paraben can be safely used up to the maximum authorized concentration as actually established (0.4%)(25). This conclusion is endorsed by the VKM Panel on Food additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics.

Regarding propyl-, isopropyl-, butyl- and isobutyl paraben, the SCCP is of the opinion that each of these additives should be further assessed with respect to their safe use in cosmetic products at individual concentrations up to 0.4% (25).

The new data presented in the dossier from COLIPA is limited to methyl paraben and butyl paraben (28). As the use of methyl paraben in cosmetics is considered safe, the present assessment addresses the new data on butyl paraben. Further assessments regarding propyl-, isopropyl- and isobutyl paraben will be awaited.

In theory, different parabens may act additively or synergistically to cause adverse effects even at individual dose levels lower than the respective NOAELs. However, no *in vivo* data are available in relation to possible interactions between different parabens. To explore this, additional studies are needed, but this should be done after the effects of each paraben have been characterised separately.

Review of updated information on reproductive toxicity

New data from CTFA/COLIPA Task Force 2005
A brief summary of the results from the new CTFA/COLIPA studies (28) is given below:

Butyl paraben was evaluated for developmental toxicity in rats in a good laboratory practice (GLP) compliant study at oral doses of 0, 10, 100 or 1000 mg/kg bw/day on gestation days 6-19. At day 20, mothers were killed and foetuses examined. Maternal toxicity was observed at the highest dose as reduced food consumption and reduced weight gain. No developmental effects were found and the results indicate that butyl paraben given orally is not a developmental toxicant. These results are also published (31).

The toxicity to male fertility of butyl- and methyl paraben was investigated by Charles River Laboratories using the same non-guideline experimental design as in the studies conducted by Oishi (9). The reported findings by Oishi (9), that butyl paraben had a potential to affect the development of the male reproductive system and to decrease sperm production at an oral dose as low as 10 mg/kg bw/day were not confirmed in the CTFA/COLIPA study. The study was run according to GLP, in a statistically more robust manner and with additional endpoints (histopathological examination of tissues and sperm evaluations to determine sperm concentration, motility and morphology). There were no effects up to 1000 mg/kg bw/day, which was the highest dose level tested.

Comments by VKM
The COLIPA dossier is, as requested by the EU Commission and SCCP, repeating and complementing the data presented in the research papers by Oishi (8;9;32;33). There are several new studies referenced in the COLIPA dossier. Two new male fertility studies conducted by Charles River and absorption studies by Beiersdorf AG and E.I. du Pont (34-39) were reported. The male fertility study in rats conducted by Charles River (1203-008) on methyl paraben (35), is of minor interest as SCCP has pointed out that methyl paraben can be

safely used up to the maximum authorized concentration as actually established in cosmetics (0.4%).

The male fertility study on butyl paraben (34) is designed similar to that of Oishi (9), and was not found to affect male reproductive organs. The conduct and the report from this study are of high quality, the study complies with GLP, and the data are reliable. Additional animals and parameters are included compared to the study design of Oishi. The main conclusion of the study, that the NOEL is 10 000 mg/kg of diet (corresponding to 1090 mg/kg bw/day) for general toxicity, including hormone levels for testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), histopathology of reproductive organs, liver, adrenal glands, thyroid, pituitary, and sperm analysis, is agreed upon. The Panel has no explanation for the discrepancy in results from the previous Oishi study (9) and the more extensive study by Charles River (CTFA/COLIPA). The Panel regards the findings by Oishi (9) as uncertain as they were not reproduced.

However, in the design of the CTFA/COLIPA study on male reproduction, there are also certain shortcomings. The study is not a functional fertility study, but includes only the toxicity parameters and sperm analysis. It is preferred to have a "standard functional fertility study" also including females and the mating phase. Even if the female fertility has not been questioned in the request from SCCP, this would have been valuable data in order to make a complete assessment. It is also noted that the differences between the negative and the positive control (alpha-chlorohydrin) are small. Clear positive controls are important to verify these non-standard assays. Another aspect of the study design is the relevance of the administration route (for parabens with an intended dermal use). This has not been justified and is further questioned below.

Previous data
The following is a brief summary of published studies of the effect of parabens on reproductive parameters.

Parenteral administration
In male neonatal rats, daily subcutaneous injections of butyl paraben (2 mg/kg bw/day) for up to 18 days showed no detectable effects on any reproductive parameter (40).

When doses of 100 or 200 mg/kg bw/day butyl paraben were administered by subcutaneous injections to pregnant rats from gestation day 6 to postnatal day 20, the offspring was affected. Both tested doses showed clear effects, amongst which a decrease in sperm count and sperm motile activity in the epididymis. Also testicular expression of oestrogen receptor was increased at the high dose (41).

When administered subcutaneously to immature rats for three successive days, butyl paraben produced a significantly increased uterus weight at a dose of 400 mg/kg bw/day and above. Dosing to ovariectomised rats significantly increased uterus weight at 1200 mg/kg bw/day and vaginal cornification at 1000 mg/kg bw/day (4).

Oral administration
In the study by Oishi (9) mentioned previously, 10 mg/kg bw/day butyl paraben administered to post-weaning male rats for eight weeks through their diet caused decreases of cauda epididymal sperm reserve, in sperm count, in daily sperm production and in serum testosterone (9).

When Oishi in another study administered to male mice through the diet, 0.01% (14.4 mg/kg bw/day), 0.10% (146 mg/kg bw/day) or 1.00% (1504 mg/kg bw/day) butyl paraben for 10 weeks, there were several adverse effects on the male reproductive system and the later steps of spermatogenesis were affected. At 1%, absolute and relative weights of the epididymis were increased and there was a dose-dependent decrease of spermatid counts. Also the serum testosterone concentration decreased (8).

Oral gavage feeding to immature rats for three successive days produced a small but insignificant increase in uterus weight at a dose level of 800-1200 mg/kg bw/day (4).

Review of updated information on systemic exposure after dermal application

New data from CTFA/COLIPA Task Force 2005
A brief summary of the results from the new CTFA/COLIPA studies (28) is given below:

Studies on dermal penetration/metabolism by Beiersdorf AG showed that both butyl- and methyl paraben have a potential to penetrate pig skin. The dermal absorption of butyl paraben through pig skin was determined to be 33%.

In vitro percutaneous penetration studies with butyl paraben were conducted by E.I. du Pont Laboratories. Both full thickness and split thickness rat and human skin were tested using emulsions containing ^{14}C-labelled butyl paraben. It turned out that 73.51% (49.7%) and 54.23% (5.51 %) of the radioactivity penetrated split thickness human and rat skin respectively (figures in parentheses are unmetabolised absorbed butyl paraben). Considerably less butyl paraben penetrated full thickness skin.

Comments by VKM
The dermal absorption study performed by Beiersdorf AG is important since it concluded that the dermal absorption of butyl paraben through pig skin was as high as 33% (36). The E.I. du Pont study showed even higher absorption through human skin and a major fraction being unmetabolised (37-39).

Previous data
After oral or intravenous administration of butyl paraben to dogs, about 40-50% of the administered dose is recovered in urine as the 4-HBA glucoronate conjugate after 24 to 30 hours. However, after intravenous injection to dogs, unchanged butyl paraben was also recovered from brain, spleen and pancreas, and high concentrations of metabolites were detected in the liver and kidneys (publications cited by JECFA 2001)(42).

Numerous *in vitro* studies have been conducted reporting the permeability of butyl paraben through skin (27). Skin penetration seems to vary with pre-treatment of skin, presence of penetration enhancers and between animal species. *In vivo* skin penetration was considerably greater than that observed *in vitro*.

Are the new data available sufficient to perform a complete safety assessment?

Safety assessment by CTFA/COLIPA
COLIPA used the NOEL of 1000 mg/kg bw/day from the oral reproductive and developmental toxicity studies (above-mentioned) as the starting point for their risk

assessment. By assuming absorption of 50% unmetabolised paraben across skin, based on exposure from general cosmetic use (17.76 g/day) and taking into account a permitted exposure maximum of 0.4% for a single paraben, an exposure of 0.59 mg/kg bw/day was calculated for a 60 kg person. This would lead to a MoS (margin of safety) of 1690. If these data for butyl paraben are extrapolated to parabens used in combination with a permitted exposure maximum of 0.8%, an exposure of 1.2 mg/kg bw/day was calculated for a 60 kg person. The MoS for a the total mixture of parabens would therefore be 840 (28).

Comments by VKM

The most relevant route of administration to test most cosmetic ingredients is the dermal one. This route is, however, difficult and inconvenient in long term animal studies on potential adverse effects on reproduction and development. Therefore, alternative routes of administration such as oral, subcutaneous or intraperitoneal, have traditionally been used to establish a NOAEL for the cosmetic ingredient. MoS is defined as the ratio NOAEL/Systemic Exposure Dose (SED). SED is defined as the systemically available dose of the cosmetic ingredient after a worst case human dermal application. SED is therefore dependent on the dermal absorption of the ingredient. Traditionally, the oral NOAEL obtained is used for the derivation of the MoS for cosmetic ingredients. However, this presumes that 100% of the orally applied test dose is absorbed and made systemically available.

The Panel regards the new data on skin penetration of butyl paraben sufficient for the determination of the SED for a worst case human exposure. This information indicates that a considerable fraction (50%) of the unmetabolised butyl paraben, following dermal use, is systemically available.

The new NOAEL for butyl paraben was determined in a study with oral administration. However, there is no information on how much of this oral dose is available for the systemic circulation. Although the pharmacokinetics is not fully understood, it seems clear from the literature (43) that a significant amount of ingested parabens are hydrolysed in the intestine, and hydrolysed and further metabolised in the liver, before they enter the systemic circulation and the various potential target organs, including the foetus. This first-pass degradation in the gut and liver is, however, circumvented by transdermal parabens. It is thus conceivable that the systemic exposure of unchanged parabens by dermal administration is higher than by oral administration. Moreover, the route of administration is also of particular concern since the few studies in the literature with parenteral systemic administration indicate higher toxicity, in particular to the reproductive function (4;41). The site of action of a reproductive toxicant will be the reproductive organs or the developing foetus, which will be exposed only after systemic absorption and distribution. In order to eliminate any uncertainty of bioavailibility, the test compound can be administered systemically i.e. intravenous or subcutaneous. Studies by the intravenous route are recommended. Hence, the new NOAEL for butyl paraben as presented by COLIPA may be of limited relevance for dermal exposure, and is therefore, without further toxicokinetic information, not acceptable for use in a risk assessment of butyl paraben in cosmetics.

Furthermore, the existing data on reproductive toxicity of parabens are still scarce and most reports are from explorative research using non-standard protocols. Also, the available reports have conflicting findings, which might be due to methodology and study design.

All together, the new available data are not sufficient to establish a NOAEL and perform a risk assessment of butyl paraben, and th6e suggested MoS of 1690 for butyl paraben, as presented in the dossier by COLIPA cannot be accepted.

CONCLUSIONS

- The health risk assessment of parabens should include an evaluation of each paraben separately. As pointed out by the EU Scientific Committee on Consumer Products (SCCP), methyl- and ethyl paraben can be safely used up to the maximum authorized concentration as actually established (0.4%). The Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety supports this conclusion.
- The new data presented in the dossier from COLIPA is limited to methyl paraben and butyl paraben. The dossier from COLIPA does not provide sufficient information to establish a NOAEL for butyl paraben on reproductive toxicity. Information from standard functional fertility studies is not provided.
- The NOAEL for butyl paraben presented in the COLIPA dossier was based on oral reproductive and developmental toxicity studies in rats. The derived NOAEL is not relevant for dermal exposure, since a 100% systemic availability is assumed following oral administration. This is not likely. Consequently, the systemic exposure after oral administration of butyl paraben must be determined.
- The new data from COLIPA indicate that approximately 50% of the dermally applied butyl paraben is systemically available unmetabolised. This information seems sufficient to determine the Systemic Exposure Dose (SED) for a worst case human exposure.
- The Panel concludes that the opinion expressed by the SCCP that: “the available data do not enable a decisive response to the question of whether propyl-, butyl- and isobutyl paraben can be safely used in cosmetic products at individual concentrations up to 0.4%” is still valid.

Recommendations

It is thus recommended that the following additional information and documentation are made available in order to make a complete safety assessment:

- Additional information was requested for 4 different parabens; propyl-, isopropyl-, butyl- and isobutyl paraben. It is proposed that focus, in the first instance, is limited to butyl/isobutyl paraben.

- With reference to the SCCNFP´s Notes of Guidance for the testing of cosmetic ingredients (22), the relevant tests on reproductive toxicity are: Two generation reproduction toxicity test (OECD 416) and Teratogenicity test in rodent/non rodent (OECD 414), alternatively a Combined Reproduction/Developmental toxicity screening test (OECD 421). All testing should comply with GLP regulations.

- For all reproductive toxicity studies the route of administration should be justified. This implies that the systemic exposure dose of the unchanged test compound should be similar to or above that resulting from dermal application. This can be achieved by characterising the toxicokinetics of the substance (according to the OECD protocol

417) or by administering the substance systemically, i.e. by intravenous or subcutaneous injection. The intravenous route is preferred.

ASSESSED BY

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ANNEX I Table 6. Selected NOELs and LOELs for Butylparaben

(From Butylparaben [CAS No. 94-26-8] Review of Toxicological Literature - NTP 2005 (27))

Species, Strain, Age, and Sex	Exposure Route/Duration	NOEL or LOEL (mg/kg bw/day)	Endpoint	Reference
Mice, ICR/Jcl, 8-wk-old, M and F	Oral; diet 6 wk	NOEL = 900	subchronic toxicity: significant atrophy of lymphoid tissue in organs and multifocal degeneration and necrosis in liver parenchyma	Inai et al. (1985)
Mice, Crj:CD-1, 4-wk-old, M	Oral; diet 10 wk	NOEL = 100	reproductive toxicity: significant increase in epididymides weights and decrease in spermatid counts and serum testosterone level	Oishi (2002a)
Rats, Fischer 344, (weanling), M	Oral; diet 9-27 days	LOEL = 1600[1]	subchronic toxicity: damage to the forestomach epithelium	Rodrigues et al. (1986)
Rats (strain, age, and sex n.p)	Oral; intubation 13-15 wk	NOEL = 50	subchronic toxicity: no significant effects on body weight, no sporadic deaths, and no histological differences	Ikeda and Yokoi (1950; cited by JECFA, 2001)
Rats, Wistar, age n.p., M and F	Oral; diet 12 wk	NOEL = 2000	subchronic toxicity: reduced growth rate, decreased body weight and motor activity, and myocardial depression (in females)	Matthews et al. (1956)
Rats, Alpk:AP, 21- to 22-day-old, F	Oral; gavage 3 days	NOEL = 1200[2]	reproductive toxicity: statistically insignificant increases in uterus wet and dry weights	Routledge et al. (1998)
Rats, Sprague-Dawley, age n.p., F	Oral; gavage GD 6-19	NOEL = 100	reproductive toxicity: decreases in maternal weight gain; statistically significant decreases in weight gain on GD 18-20; decrease in food consumption (GD 6-20)	Daston (2004)
Rats, Sprague-Dawley, age n.p., F	Oral; gavage GD 6-19	NOEL = 1000	developmental toxicity: no changes in embryo/fetal viability, fetal weight, and external, visceral, and skeletal abnormalities	Daston (2004)
Rats, Wistar, 3-wk-old, M	Oral; diet 8 wk	LOEL = 40[3]	reproductive toxicity: significant decrease in epididymides weights and serum testosterone levels	Oishi (2001)
Rats, Alpk:AP, 21- to 22-day-old, F	s.c; 3 days	LOEL = 400[3]	reproductive toxicity: increase in uterus wet weights	Routledge et al. (1998)
Rats, Wistar, 2- to 12-day-old, M	s.c.; 2-18 days	NOEL = 2[1]	developmental toxicity: testis weight, aquaporin-1 immuno-expression, and effects on rete testis morphology or efferent duct epithelial cell height	Fisher et al. (1999)
Rats, Sprague-Dawley, 9-wk-old, F	s.c.; GD 6 to PND 20	LOEL = 100[4]	reproductive toxicity: significant decrease in proportion of pups born alive	Kang et al. (2002b)

[1] based on a single dose level used in the study; [2] based on the highest dose reported; [3] based on the lowest dose reported to cause a significant effect; [4] based on doses given to pregnant and lactating dams for reproductive toxicity studies
Abbreviations: bw = body weight; F= female(s); GD = gestation day; LOEL = lowest observable effect level; M = male(s); NOEL = no observable effect level; n.p. = not provided; PND = postnatal day; s.c. = subcutaneous; wk = week(s)

ANNEX II Table 7. Reproductive Toxicity and Teratology of Butylparaben

(From Butylparaben [CAS No. 94-26-8] Review of Toxicological Literature - NTP 2005 (27))

Species, Strain, and Age, Number, and Sex of Animals	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Mice, Crj:CD-1, 4-wk-old, 8M/dose group	Butylparaben, ≥99% pure	oral; 0.01, 0.10, or 1.00% (average intake of ~14.4, 146, and 1504 mg/kg [0.0741, 0.752, or 7.743 mmol/kg] per day) in diet for 10 wk; sacrificed at 10 wk	There were no treatment-related effects on the liver, ventral prostates, seminal vesicles, and preputial glands. At 1.00%, absolute and relative weights of the epididymides were significantly increased compared with controls. Round and elongated spermatid counts were dose-dependently decreased in stages VII-VIII seminiferous tubules; the latter was significantly lower in all groups. Serum testosterone level also dose-dependently decreased; significance was seen at 1.00%.	Oishi (2002a)
Rats, Wistar, 2- to 12-days-old (neonatal), number n.p., M	Butylparaben, purity n.p.	s.c.; ~2 mg/kg (0.01 mmol/kg) in corn oil given on days 2-18 inclusive; animals sacrificed (4 h after daily injection) and observed on day 18	No alteration in testis weights compared to controls was seen. Additionally, no gross changes in aquaporin-1 (AQP-1) immuno-expression were observed. There was no detectable effect on rete testis morphology or in efferent duct epithelial cell height.	Fisher et al. (1999)
Rats, Sprague-Dawley, 9-wk-old, 22F	Butylparaben, purity n.p.	s.c.; 100 or 200 mg/kg (0.515 or 1.03 mmol/kg) in DMSO given from GD 6 to PND 20, with a 2-day interruption at parturition; animals sacrificed at PND 21; observed up to PND 90	No clinical signs of toxicity or effects on body weight or food consumption were observed. At both doses, the proportion of pups born alive and proportion of pups that survived up to the weaning period were decreased. At 100 mg/kg, vaginal opening occurred several days earlier in treated rats compared to controls. In male F1 offspring: At 100 mg/kg, body weight was significantly decreased on PND 49. Testicular weight was significantly increased at PND 21 but significantly decreased at PND 49. Additionally, prostate gland weight was significantly decreased at PND 49 and PND 90, while the weight of the seminal vesicles was significantly decreased at PND 49. At 200 mg/kg, testicular weight was significantly increased at PND 90. At both doses, the number and motility of sperm in caudal epididymis were significantly decreased. The total cell numbers of round and elongated spermatid in the seminiferous tubules at stage VII were significantly decreased. In female F1 offspring: At both doses, body weights were significantly decreased at PND 49 to 90. There were no effects on the weights of female reproductive organs.	Kang et al. (2002b)
Rats, Alpk:AP, 21- to 22-days-old, 5F per dose group	Butylparaben, >99% pure	s.c.; 40, 200, 400, 600, 800, 1000, or 1200 mg/kg (0.21, 1.03, 2.06, 4.12, 5.148, 6.178 mmol/kg) daily for 3 successive days	Immature rats: At 400-800 mg/kg, significantly increased uterus wet weights were reported; at 1200 mg/kg; weights were ~170% that of controls. Ovariectomized rats: At 1200 mg/kg, significantly increased uterus wet and dry weights were up to 150% of controls. At 1000 mg/kg, vaginal cornification was significantly increased. (An increase was seen at 800 mg/kg,	Routledge et al. (1998)

			but the response was not statistically significant.)	
Rats, Alpk:AP, 21- to 22-days-old, 5F per dose group	Butylparaben, >99% pure	oral (gavage); 4, 40, 400, 800, or 1200 mg/kg (0.02, 0.21, 2.06, 4.12, 6.178 mmol/kg) daily for 3 successive days	At 800-1200 mg/kg, small but statistically insignificant increases in uterus wet and dry weights were reported in immature rats.	Routledge et al. (1998)
Rats, Wistar, 3-wk-old, number n.p., M	Butylparaben, purity n.p.	oral; 0.01, 0.10, or 1.00% (average intake of ~10.4, 103, or 1026 mg/kg [0.054, 0.53, or 5.28 mmol/kg] in diet for 8 wk; animals sacrificed at 8 wk	Absolute and relative weights of the epididymides and serum testosterone levels were dose-dependently decreased; statistical significance was seen at ≥0.1%. At all dose levels, the cauda epididymal sperm reserve and daily sperm production in the testis were also significantly lowered compared to controls.	Oishi (2001)
Rats, Sprague-Dawley, age n.p., 25F per dose group	Butylparaben, purity n.p.	oral; 10, 100, or 1000 mg/kg (0.051, 0.515, or 5.148 mmol/kg) daily on GD 6-19; caesarean performed on GD20	At the high dose, decreases in maternal weight gain were observed during some measurement intervals; statistical significance was seen during GD 18-20. Maternal food consumption was significantly decreased over the dosing period (GD 6-20). The maternal NOAEL was 100 mg/kg/day. No differences in developmental parameters (including embryo/fetal viability, fetal weight, malformations, and variations) were seen between treated rats and controls. The NOAEL for developmental toxicity was 1000 mg/kg/day.	Daston (2004)

Abbreviations: DMSO = dimethylsulfoxide; F= female(s); GD = gestation day(s); h = hour(s); M = male(s); n.p. = not provided; NOAEL = no observable adverse effect level; PND = postnatal day; s.c. = subcutaneous(ly); TCDD = 2,3,7,8-tetrachlorodibenzo-p-dioxin; wk = week(s)