National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Exposure to and toxicity of methyl-, ethyl- and propylparaben

A literature review with a focus on endocrine-disrupting properties

RIVM Report 2017-0028

W. Brand et al.

National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Exposure to and toxicity of methyl-, ethyl- and propylparaben

A literature review with a focus on endocrine-disrupting properties

RIVM Report 2017-0028

Colophon

DOI 10.21945/RIVM-2017-0028

W. Brand (author), RIVM
P.E. Boon (author), RIVM
E.V.S. Hessel (author), RIVM
J.A.J. Meesters (author), RIVM
M. Weda (author), RIVM
A.G. Schuur (author), RIVM

Contact:
dr.ir. Walter Brand
Centre for Safety of Substances and Products
walter.brand@rivm.nl

This investigation has been performed by order and for the account of The Netherlands Food and Consumer Product Safety Authority (NVWA), within the framework of research question 9.1.67 ‘Exposure of consumers to substances with possible effects on the endocrine system’.

This is a publication of:
National Institute for Public Health and the Environment
P.O. Box 1 | 3720 BA Bilthoven
The Netherlands
www.rivm.nl/en

Synopsis

Exposure to and toxicity of methyl-, ethyl- and propylparaben
A literature review with a focus on endocrine-disrupting properties

Parabens inhibit the growth of fungi and bacteria and, as such, are substances that can be used as preservatives in a variety of consumer products, such as personal care products, food and medicines. Parabens, however, are suspected of having an endocrine-disrupting effect. Endocrine-disrupting substances can disturb the hormonal system.

On the basis of a literature review, RIVM has investigated whether the three most commonly-used parabens (methyl-, ethyl- and propylparaben) can be considered as endocrine-disrupting substances. However, the available data from animal studies described in the literature do not provide sufficient information to be able to reach this conclusion. This report also examines whether the possible endocrine-disrupting effects are included in the applicable legal assessment frameworks. Because there are insufficient data, this is not the case within the current risk assessment.

Exposure via personal care products has been examined in some detail and generally seems to be the greatest contributor to total exposure. Exposure via food appears to be negligible. Too little information is available for an acceptable estimate of exposure via medicines. The report also shows that the extent to which people are exposed to the individual parabens appears to be lower than the level at which a health effect can be expected. For safety reasons, exposure assessments have been performed with very unfavourable assumptions. However, in practice, people are exposed to a combination of substances. It is still unclear whether and how combined exposure to individual parabens can be included in risk assessment.

In order to fill the knowledge gaps, RIVM recommends that additional research should be undertaken into the possible endocrine-disrupting effect of parabens and refinement of the exposure assessment methods. Recommendations for this are provided.

Keywords: parabens, exposure, toxicity, endocrine disruption, cosmetics, foodstuff, medicinal products, methylparaben, ethylparaben, endocrine disruptors

Publiekssamenvatting

Blootstelling aan en toxiciteit van methyl-, ethyl- en propylparabeen
Een literatuurreview met een focus op hormoonverstorende eigenschappen

Parabenen zijn stoffen die als conserveermiddel in verschillende consumentenproducten kunnen worden gebruikt, zoals persoonlijke verzorgingsproducten, voedsel en medicijnen. Ze gaan de groei van schimmels en bacteriën tegen. Parabenen worden er van verdacht dat ze een hormoonverstorende werking hebben. Hormoonverstorende stoffen kunnen de hormoonhuishouding in de war brengen.

Het RIVM heeft in een literatuurstudie voor de drie meest gebruikte parabenen (methyl-, ethyl- en propylparabeen) onderzocht of deze als een hormoonverstorende stof beschouwd kunnen worden. De beschikbare gegevens uit dierstudies die in de literatuur zijn beschreven, leveren echter onvoldoende informatie om hierover een conclusie te trekken. In de studie is ook bekeken of de mogelijke hormoonverstorende effecten zijn meegenomen in de wettelijke beoordelingskaders die van toepassing zijn. Omdat er onvoldoende gegevens zijn, is dat niet het geval bij de huidige risicobeoordeling.

De blootstelling door persoonlijke verzorgingsproducten is behoorlijk goed onderzocht en lijkt in het algemeen het meest aan de totale blootstelling bij te dragen. De blootstelling vanuit voedsel blijkt verwaarloosbaar. Voor een acceptabele, eerste schatting van de blootstelling vanuit medicijnen is te weinig informatie beschikbaar. Uit de studie blijkt verder dat de mate waarin mensen aan de afzonderlijke parabenen worden blootgesteld naar schatting lager lijkt te zijn dan de hoeveelheid waarbij een gezondheidseffect kan worden verwacht. Voor deze blootstellingsschattingen zijn veiligheidshalve zeer ongunstige aannames gebruikt. In de praktijk worden mensen echter aan een combinatie van verschillende stoffen blootgesteld. Het is nog onduidelijk of en hoe deze gecombineerde blootstelling aan de verschillende parabenen meegenomen kan worden in de risicobeoordeling.

Om hiaten in de kennis te vullen adviseert het RIVM om aanvullend onderzoek te doen naar de mogelijk hormoonverstorende werking van de parabenen en de blootstellingsschatting te verfijnen. Hiervoor worden aanbevelingen aangereikt.

Kernwoorden: parabenen, blootstelling, toxiciteit, hormoonverstoring, cosmetica, levensmiddelen, geneesmiddelen, methylparabeen, ethylparabeen, hormoonverstoorders

Contents

Summary

Chemical substances potentially causing effects on the endocrine system have attracted increasing attention in recent years. For that reason, the Netherlands Food and Consumer Product Safety Authority (NVWA) asked the RIVM to look into chemicals with possible endocrine-disrupting (ED) properties in connection with consumer product safety. Parabens were selected as an example of such chemicals. Parabens are mostly used as a preservative in food as well as in non-food products. The focus of this report is on three parabens: methyl-, ethyl- and propylparaben.

The aim of this report is to provide an overview of the exposure, hazard and risk assessments performed on these parabens, and to assess whether potential ED effects are included in risk assessments and in the derivation of their toxicological reference values. The report describes and summarizes the information on exposure, hazard and risk assessments on these parabens as described in the literature, and formulates recommendations for further research.

Use of and exposure to methyl-, ethyl- and propylparaben
Methyl-, ethyl- and propylparaben can be used as a preservative in various consumer products. Aggregate exposure assessment of these substances includes an assessment for a single substance that takes into account various exposure routes (inhalation, dermal and oral) as well as several exposure sources. In this report, three major sources are considered: personal care products, food (and drinks), and medicinal products. Data on exposure assessments for non-food consumer products other than personal care products are virtually absent. Exposure assessments for the different product sources vary greatly in approach, level of information taken into account, and the quantity and quality of the data available for the assessment.

Exposure via personal care products
Several studies have estimated exposure to parabens via personal care products. According to the studies most relevant to the situation in the Netherlands and/or Europe – both lower-tier aggregate exposure estimations and higher-tier stochastical estimations (97.5th percentile values) – internal exposure to methyl-, ethyl- and propylparaben is estimated for adults to be about 0.8, 0.2 and 0.3 mg/kg bw/day, respectively. For infants and toddlers 1.01, 0.20, and 0.41 mg/kg bw/day (95th percentile values) have been stochastically estimated.

Several factors within the exposure estimation could result in an overestimation, including: the method of aggregation of exposure from different products; assumptions regarding the use frequency of products and the amount of product applied; the assumed concentration of parabens in personal care products; the fraction of available products in which parabens are present; estimation of the fraction of product that remains on the skin after application; and the estimated extent to which parabens are absorbed from the skin into the internal system. Additional relevant data with respect to several of these factors (including recent product use and concentration data) are available that could be used for

a more realistic estimation of current exposure to parabens via personal care products in the Netherlands and/or Europe.

Exposure via food

Exposure to parabens via food may occur as a result of their use as a preservative, of migration from food packaging material, or of their natural occurrence (in some fruits or fruit-derived products). There is no relevant study available into the actual European intake of methyl-, ethyl- and propylparaben via food. Two intake assessments relevant for Europe examined the intake of methyl- and ethylparaben via their use as a food preservative. Propylparaben is not authorized to be used as a food preservative in the EU. The intakes estimated by these studies were, however, very conservative, because of the assumption that all foods in which such preservative(s) are authorized contained the parabens at the maximum permitted level (MPL). These estimates were not further refined, because the intake of both parabens was far below the Acceptable Daily Intake (ADI) for methyl- and ethylparaben of 10,000 µg/kg bw/day. The highest exposure (90th percentile) was calculated for French children aged 13–36 months: 900 µg/kg bw/day.

Only two other studies, one from China and one from the USA, reported realistic concentrations from actual measurements of methyl-, ethyl- and propylparaben in food and used these to assess exposure to these parabens via food. The mean concentrations reported in these studies were far below the EU MPLs. The highest mean concentration reported in the US study was 14.1 ng/g in grain products, and in the Chinese study: 81.1 ng/g in vegetables, both for methylparaben. The highest (95th percentile) exposure was reported for infants in the USA for ethylparaben: 1.74 µg/kg bw/day. It is emphasized that these studies were performed in China and the USA, where regulation of the use of parabens in foods is likely to differ from that in the EU. Consumption patterns may also differ. It is therefore unclear how well these exposure estimates represent the situation in the Netherlands. At best, the estimations may give an impression of the actual level of exposure. In these studies, the sources of parabens in food were not identified (preservative, natural occurrence or migration from food packaging material). The migration of parabens into food via food packaging material was, however, shown not to be an important source of exposure in both the Chinese and the USA studies.

Exposure via medicinal products

Exposure to methyl-, ethyl- and propylparaben via medicinal products may occur concurrently via various administration routes. Few data are available on exposure via medicinal products. An available exposure estimation study from China is considered not representative of the situation in the Netherlands or Europe. According to a reflection paper by the European Medicines Agency (EMA), oral exposure can be calculated from the upper value of the range in which the parabens are formulated in such medicinal products. No estimation was made for exposure via other routes of medication (e.g. topical or parenteral) as no information is available, although it may be assumed that dermal exposure will take place, as parabens are used as a preservative in creams and ointments, as in personal care products. A realistic worst-case oral exposure via medicinal products has been estimated to be maximally 2.3 mg/kg

bw/day for methylparaben and 0.83 mg/kg bw/day for propylparaben. As there are no data on product concentrations for ethylparaben, an analogous calculation for this paraben could not be made and, therefore, exposure to ethylparaben via medicinal products in the Netherlands cannot be estimated. There are few medicinal products containing ethylparaben on the Dutch market (8) compared with products containing methylparaben (260) or propylparaben (180). Most of these eight products are intended for short-term use, i.e. from a few days up to 4 weeks. However, as exposure via medicinal products can be chronic, even over a short duration, but is completely absent in a part of the population, a probabilistic exposure assessment for parabens via medicinal products would be very valuable. The necessary data for such an exposure assessment are, however, not publicly available. The contribution of medicinal products to aggregate exposure could be estimated only very roughly and worst-case for methyl- and propylparaben. For ethylparaben, there were insufficient data available to estimate the contribution by medicinal products to total exposure.

Summary of exposure assessment

Aggregation of exposure to methyl-, ethyl- or propylparaben via personal care products, food and medicinal products, as considered in this report, was difficult (or even impossible) because of varying levels of information quality and uncertainties in the different sources.

If the different exposure estimates were added together, this would result in an aggregate exposure estimate for methylparaben of about 3 mg/kg bw/day for adults and children. The estimate for medicinal products would contribute 70–74% of this value, while the contribution of food would be less than 1%. The high contribution of medicinal products and the uncertainties in its estimation diminish the reliability of the aggregate exposure values. The majority of medicinal products do not contain methylparaben and only a fraction of the population use methylparaben-containing medication regularly or chronically.

Medicinal products as a source were not considered in the aggregate exposure estimate for ethylparaben, because no information on product concentrations was available. Adding together exposure via personal care products and exposure via food would result in an aggregate exposure estimate of 0.2 mg/kg bw/day for ethylparaben for both children and adults and, again, the contribution of food would be less than 1%.

For propylparaben, adding the exposures via personal care products, food and medicinal products would result in an aggregate exposure estimate of 1.2 mg/kg bw/day for both children and adults. As with the aggregate exposure to methylparaben, 64–72% of the exposure would be due to the intake of medicinal products, and less than 1% due to the intake of food.

The worst-case character of these aggregate estimates is supported by several biomonitoring studies (in several specific populations mostly other than European, and using different calculation methods), in which 95th percentile values from urine metabolite concentrations were back-calculated to internal exposure, or daily intake levels. If we consider these values as reliable estimations of actual exposure, there is usually

a difference of 1 or 2 orders of magnitude compared with the estimated internal (compared with back-calculated to internal exposure estimates) or external (compared with daily intake levels) exposure estimates in the present report. It is unclear, however, how well all these exposure estimates represent the current situation in the Netherlands.

Toxicity of methyl-, ethyl- and propylparaben

Dependent of the route of exposure, parabens are absorbed from the gastrointestinal tract or through the skin and metabolized. Interspecies differences in metabolites indicate that parabens are not as effectively metabolized in humans as in rats, at least after dermal exposure. Therefore, rats might not sufficiently represent the biotransformation of methyl-, ethyl- and propylparaben as it occurs in humans, which is important, as the availability of un-metabolized parabens is expected to determine their biological activity and toxicity, including any potential ED activity.

With regard to hazard, all toxicological endpoints are summarized in this report. Methyl-, ethyl- and propylparaben do not irritate the skin in individuals with normal skin, might slightly irritate the eye, are not skin sensitizers, and are not genotoxic or carcinogenic according to the available studies. However, this review focused on studies of developmental and reproductive toxicity, and of ED effects.

With regard to developmental toxicity, for propylparaben an OECD TG 422 study on rats was combined with a reproduction/developmental toxicity screening test up to a dosage of 15,000 ppm (between 1000 and 1500 mg/kg bw/day) in 2012. No adverse effects were identified in the male and female groups and no test item-related findings in pups were noted. For methyl- and ethylparaben OECD TG 414 studies from the 1970s with dosages up to 550 mg/kg bw/day also observed no effects. Thus, in the available studies no developmental effects were identified for any of the parabens.

With regard to reproductive toxicity, for methyl- and ethylparaben, a non-GLP reproductive toxicity study was published in 2004, which performed dosing up to 1000 mg/kg bw/day. This study was not, however, compliant with official OECD Test Guidance (TG) for reproduction toxicology studies, and reproductive toxicity studies according to OECD TGs are currently lacking for methyl- and ethylparaben. A No Observed Adverse Effect Level (NOAEL) of 1000 mg/kg bw/day has been derived by the Scientific Committee on Consumer Safety (SCCS) and the European Food Safety Authority (EFSA) for methyl- and ethylparaben based on the absence of reproductive effects up to that dose in repeated dose toxicity studies. Some *in vivo* studies challenge this NOAEL for methyl- and ethylparaben, as it does not take potential spermatogenic effects into account, nor effects found at lower doses in other studies, such as a delay in the date of vaginal opening in pre-pubertal rats, a decrease in length of the estrous cycle, and increased adrenal gland weight. To draw reliable conclusions on reproductive toxicity, for all three parabens, more data are needed, especially in view of the fact that reproduction toxicology studies for methyl- and ethylparaben that adhere to OECD Guidance are lacking. Propylparaben has been tested in a TG study for reproduction/developmental screening toxicity (OECD TG 422), in which

no effects were detected. Some other studies in young male rats have shown adverse effects on sperm production and testosterone levels from oral exposure to propylparaben, but other, more recently performed studies with the same study design did not confirm these findings. This makes it difficult to draw a final conclusion for propylparaben and its effect on reproduction.

Concerns have been raised about the ED potential of parabens at higher exposure levels. The effects on reproduction in *in vivo* studies on methyl-, ethyl- and propylparaben might be related to ED endpoints, but certain *in vivo* data are missing. Parabens are known to be estrogenic *in vitro*, and estrogenicity appears to increase with side chain length. Additionally, for some parabens, anti-androgenic potential or other ED-related mechanisms have been identified in *in vitro* studies. Therefore, methyl-, ethyl- and propylparaben are on the EU list of potential Category 1 endocrine disruptors with regard to human health. Due to the lack of data, the status of the EU criteria (in areas other than biocides and plant protection products) and the final EFSA-ECHA guidance being not yet being available, it cannot be concluded whether or not methyl-, ethyl- and propylparaben fulfil the criteria of Endocrine Disrupting Chemicals (EDCs) as proposed by the European Commission. The available mechanistic *in vitro* and *in vivo* studies point towards an ED Mode Of Action (MOA) with estrogenic and anti-androgenic properties. However, there is a clear limitation in available *in vivo* studies. Further studies on adverse health effects are needed, especially reproductive toxicity studies, with special attention to hormone-related parameters (e.g. an OECD TG 443 study). On the other hand, the extent of the relevance of these *in vivo* studies to the toxicokinetics of parabens in humans needs to be considered, as metabolic inactivation is possibly more effective in rats than in humans. This could affect the relevance of animal studies in general. Though results from *in vitro* studies on ED effects suggest some similar MOAs, there are too many uncertainties with regard to similar toxicological endpoints *in vivo* to legitimize a cumulative risk assessment.

Risk assessment

From the absence of reproductive effects found in four repeated-dose toxicity studies for methyl- and ethylparaben, a NOAEL of 1000 mg/kg bw/day was established by EFSA and the SCCS. EFSA considered more data necessary to determine a NO(A)EL for propylparaben, and therefore propylparaben is not allowed to be used as a food additive in the EU. The SCCS also considered that more data were necessary on propylparaben to determine a NO(A)EL, but meanwhile proposed to use very conservatively the for butylparaben. This NOEL of 2 mg/kg bw/day is based on a non-TG study in which juvenile rats were subcutaneously exposed for 17 days to butylparaben (only one dose group). Though ED properties were discussed in the SCCS opinions, they were not taken into account in the derivation of the NOAEL for methyl- and ethylparaben. Additionally, the relevance of the animal studies to human risk assessment has been questioned by the SCCS because of the rapid and effective metabolism of parabens in rats – a phenomenon that is not present in humans. In conclusion, although extensive toxicological data on propylparaben in rodents exist, evidence has not been provided for the safe use of propylparaben and no NO(A)EL could be determined.

In order to establish a NO(A)EL, additional data are needed, in particular on the toxicokinetics of propylparaben in humans.

When comparing the aggregate exposure estimate with toxicological reference values for the different parabens, there seems to be a margin of safety (MOS) >100 between the present worst-case aggregate internal exposure estimate of about 3 mg/kg bw/day and the NOAEL of 1000 mg/kg bw/day established for methylparaben. However, there are indications that the current NOAEL does not adequately take ED-related effects (e.g. delay in vaginal opening, decrease in length of the estrous cycle, and increased adrenal gland weight) into account. Assuming a possible lower NOAEL, the MOS could be < 100 for methylparaben. However, based on the worst-case character of the exposure assessment, especially with regard to the contribution from medicines, a refinement of the exposure assessment is expected would sufficiently increase this MOS.
For ethylparaben, the MOS between the aggregate internal exposure of 0.2 mg/kg bw/day for ethylparaben estimated in this report and the NOAEL of 1000 mg/kg bw/day established for ethylparaben is sufficiently large.
For propylparaben, there is no established NO(A)EL with which the present worst-case aggregate internal exposure estimate of 1.2 mg/kg bw/day can be compared. Instead, several effect levels from different studies, ranging from 2 mg/kg bw/day to 1000 mg/kg bw/day, are used to make a risk assessment. Depending on the effect level considered, the MOS could be insufficient, but as with exposure to methylparaben, it is expected that a refinement of the exposure assessment would sufficiently increase this MOS.

In conclusion (with regard to risk assessment):

- Exposure via personal care products has been examined in some detail; estimated exposure via food is very limited. Exposure to methyl- and propylparaben via medicinal products is estimated very roughly in this review, because only a worst-case preventive risk assessment has been performed by the EMA which could be used for this purpose. A refined exposure assessment would contribute to a more realistic aggregate exposure assessment.
- There is currently no concern for methylparaben. Although the currently derived NOAEL may be too high because of missing (specific) data on reproductive and developmental toxicity, the exposure estimation is most likely more than worst-case.
- The MOS between the exposure estimate and the current NOAEL for ethylparaben is sufficient for the conclusion of no concern.
- There is currently no concern for propylparaben, as the use of the NOEL for butylparaben is very conservative and studies have indicated that a NOAEL for propylparaben could be set at a higher level (with regard to reprotoxic and/or developmental effects). In addition, the exposure estimation is most likely more than worst-case.
- The available mechanistic *in vitro* and *in vivo* studies point towards an ED MOA with estrogenic and anti-androgenic properties, but there are too limited *in vivo* data to conclude whether methyl-, ethyl- and propylparaben are endocrine disruptors according to the WHO definition.

- ED properties were not taken into account by EFSA or the SCCS in setting the NOAEL of methyl- and ethylparaben, although the SCCS did discuss them during its risk assessment. The same applies to propylparaben, for which no NO(A)EL could be set; instead, the SCCS very conservatively used the NOEL for butylparaben.
- There are doubts about rat studies representing the human situation with regard to toxicokinetics.

Therefore, the following recommendations for further research can be made:

Recommendations for further research

It is recommended that future studies should be directed to:

- more realistic estimates of exposure via non-food consumer products other than personal care products, and especially medicinal products, in order to refine the exposure assessment;
- better information with regard to (toxico)kinetics, including dermal absorption and metabolic interspecies differences – as metabolic inactivation is likely more effective in rats than in humans, which could affect the relevance of animal studies – in order to set more realistic toxicological reference values;
- recent biomonitoring data representative of the current situation in the Netherlands or Europe in order to derive an actual level of exposure with which to compare the calculated aggregate exposure estimate and – assuming that the toxicokinetics of parabens is further clarified – either confirm the current exposure assessment or produce an alternative;
- when the final EFSA-ECHA guidance becomes available, additional *in vivo* studies on developmental/reproductive toxicity (e.g. according to OECD TG 443), with special attention to hormone-related parameters, in order to facilitate a weight-of-evidence decision on whether methyl-, ethyl- and propylparaben are endocrine disruptors; and
- further studies into the toxicological mechanism of parabens in order to clarify whether a cumulative exposure assessment is justified.

1 Introduction

There is concern about the effects of substances with possible endocrine-disrupting (ED) properties on humans and the environment. However, whether there is a causal relationship between exposure, a mechanism of endocrine disruption and the occurrence of specific diseases is often uncertain. This is partly due to complicating factors, which include the often fluctuating or temporary nature of exposure. In addition, in practice people are exposed to a combination of substances with (suspected) ED properties. Among these suspected ED substances are parabens, some of which are commonly used as a preservative in consumer products. Therefore, people may be exposed to parabens from various sources, including personal care products, food products (including migration from food contact materials) and medicinal products.

Substances with ED properties have recently received attention in relation to products in daily use by consumers. Some such substances have received specific attention, including parabens. The present report aims to investigate exposure (taking into account all possible sources) and toxic effects (with a focus on ED properties) and make risk assessments for the three most used parabens based on information from the scientific literature. This investigation includes:

- an inventory and discussion of estimates of exposure by consumers to parabens via consumer products and food, taking into account actual exposure scenarios at certain life stages (e.g. childhood) based on available information;
- a description of the toxicity of these parabens, with a focus on ED properties, including the current toxicological reference values;
- a statement about the risks related to exposure to parabens, how exposure relates to the current toxicological reference values, and whether the possible ED effects are included in the derivation of these reference values;
- an identification of the uncertainties present in the available data and methodology with regard to exposure, toxicity and risk assessment, and proposals for reducing these uncertainties by additional research, where relevant.

1.1 Parabens

Parabens are a group of substances consisting of several congeners, including methyl-, ethyl-, propyl-, butyl-, isopropyl- and isobutylparaben, all esters of *p*-hydroxybenzoic acid (PHBA). This exploratory report focuses on three of them: methyl-, ethyl- and propylparaben (Figure 1), which are the most used parabens in personal care products, food and medicinal products and are registered under REACH. In this report propylparaben refers to n-propylparaben, not to its isomer isopropylparaben. Often methyl-, ethyl- and propylparaben are used in combination; in particular, methyl- and propylparaben are often used together (combined available as a mixture) in personal care and medicinal products.

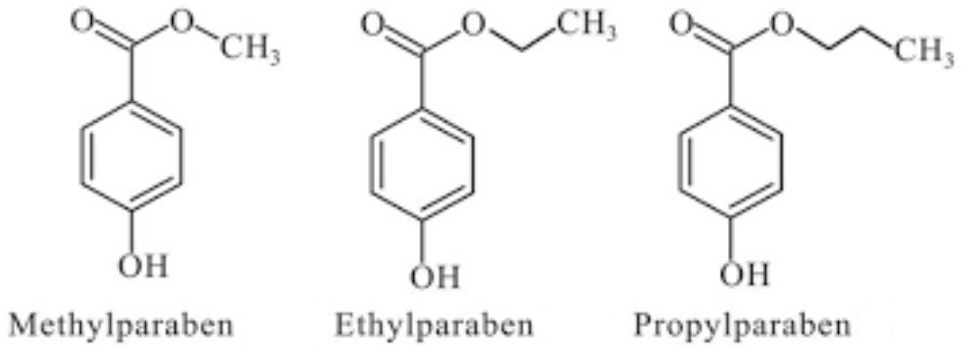

Figure 1. Structural formulae of methyl- (CAS 99-76-3), ethyl- (CAS 120-47-8) and n-propylparaben (CAS 94-13-3)

1.2 Literature study

A literature study was conducted with regard to exposure to and toxicity of methyl-, ethyl- and propylparaben (see Appendix 9.1 for details) and relevant exposure assessments, e.g. by the Scientific Committee on Consumer safety (SCCS) and the European Food Safety Authority (EFSA).

1.3 Report structure

The structure of this report is as follows:

Exposure to methyl-, ethyl- and propylparaben
An overview of the available exposure estimates for the three parabens via different sources (such as personal care products, other consumer products, food, food contact materials and medicinal products) for both adult and child populations is presented in Chapter 2.

Toxicity of methyl-, ethyl- and propylparaben
An overview of the known hazard characteristics of the three parabens is presented in Chapter 3. The hormone-disrupting effects of methyl-, ethyl- and propylparaben and the NOAELs derived by previous studies are described and discussed in relation to the WHO definition of endocrine disruption.

Reviews and risk assessments
An overview of available reviews and risk assessments of the three parabens is presented in Chapter 4.

Legal frameworks
In Chapter 5, the legal framework for cosmetics, food additives and food contact materials and the position within REACH are given.

Conclusions and recommendations for further research
Chapter 6 presents conclusions and suggestions and recommendations for further research.
It should be noted that the various components of exposure assessment (exposure, hazard, ED properties, toxicological reference values, uncertainties) are described as an inventory. Only available literature (scientific publications as well as published opinions) is used, and no exposure assessments are carried out as part of this study. Nor does

this study include an extensive review of available biomonitoring studies.

This report is therefore not exhaustive, *and does not result in a definitive judgement about possible health risks* related to the presence of parabens in consumer products and food. Nor does it address the topic of cumulative exposure and hazard assessment, i.e. the effects of combined exposure to methyl-, ethyl- and propylparaben (as briefly explained in Section 4.5). In sum, this report is a current overview and discussion of exposure, toxicity and risk assessment relating to the three most used parabens, with a specific focus on their potential ED properties.

2 Exposure to methyl-, ethyl- and propylparaben

2.1 Introduction

Methyl-, ethyl- and propylparaben are effective, stable preservatives that are relatively well water soluble [1]. As the chain length of the ester group of the paraben increases, antimicrobial activity increases, but water solubility decreases [1]. Their properties as preservatives make them suitable for use in a variety of products. As a result, exposure to these three parabens can occur via many different sources. The principal sources considered here are: (1) personal care products, (2) food, and (3) medicinal products. These sources of exposure are discussed below in Sections 2.2–2.4. Section 2.5 addresses exposure via other consumer products. In Section 2.6 exposure estimates recalculated from biomonitoring studies are briefly summarized. Section 2.7 summarizes the exposure estimations for the individual parabens via the different sources, and adds up (aggregates) exposure via different sources to produce an aggregate exposure estimate for each paraben. The aggregate exposures are also briefly compared to back-calculated exposure estimates from biomonitoring studies. Summing the aggregate exposure estimates for all three parabens would result in a cumulative exposure estimate, but this is not performed in this report (as briefly explained in Section 4.5). Section 2.8 provides a discussion of the uncertainties in the exposure estimations.

2.2 Exposure via personal care products

Parabens are used as a preservative in a variety of personal care products and cosmetics such as body lotion, soap and shampoo. The application of these products as well as the presence and concentrations of parabens in them vary per product. Estimating aggregate exposure via all personal care products together is therefore a complex exercise. The studies in which an aggregate exposure assessment is performed all follow a similar approach, first estimating exposure per specific product [2–7]. However, the method used to aggregate these exposure estimates into one aggregate exposure estimation for the total of the products differs per study; there are broadly three types of method, in order of increasing complexity: (Tier 1) simple summation, (Tier 2) summation per use pattern, and (Tier 3) probabilistic model simulations.

It should be noted that the exposure estimation for a single product already contains some degree of uncertainty, because of recognized uncertainties as to the dermal and oral absorption of parabens, the concentrations and presence of parabens across different product samples, and the quantities and frequencies in which the products are used [2–7]. The lower tier, simplest aggregate exposure estimations deal with such uncertainties most conservatively, whereas the higher tier, more complex methods are designed to provide more realistic estimates.

Below it is discussed how exposure to parabens is estimated in the available studies, how the recognized uncertainties are dealt with, and how well the outcomes of the available studies apply to exposure to

methyl-, ethyl- and propylparaben in the personal care products and cosmetics available on the Dutch or European market.

2.2.1 *Methodologies and required data/parameters*

The exposure route of parabens for the majority of personal care products and cosmetics is via dermal absorption after a product is applied to the skin. A few personal care products may also lead to oral exposure, such as toothpaste, mouth wash and lipstick [8]. The internal dose (mg/kg bw/day) per product is derived for both oral and dermal exposure as:

$$internal\ dose_{paraben\ per\ product} = \frac{Amount_{product} \times Freq_{product} \times Ret.factor_{product} \times Weight\ frac._{paraben} \times Uptake_{paraben}}{body\ weight}$$

where $Amount_{product}$ refers to the amount of product (g) applied per use event, $Freq_{product}$ refers to the frequency of product use (per day), $Ret.factor_{product}$ refers to the retention factor of the product, i.e. the fraction of the product amount that stays on the skin after application, $Weight\ frac._{paraben}$ refers to weight fraction of paraben in the product (mg paraben per g product), and $Uptake_{paraben}$ refers to the fraction of paraben absorbed through the skin into the internal system of the body (absorption) [5].

Frequencies and amounts of personal care products used

It is not clear whether the product amount/use data considered in the studies of aggregate exposure to methyl-, ethyl- and propylparaben [3–6] adequately represent the current Dutch or European market. The study by Gosens et al. (2011, 2014) [6, 7] was actually performed for the Dutch infant population, and the product use data in their worst-case approach were taken from the Cosmetics Fact Sheet (2006; using European data, as cited in [6,7]). For their probabilistic calculation the dataset of a small Dutch pilot survey was used with a limited number of respondents (n= 28) [7]. On the other hand, the product use data from Cowan-Ellsberry & Robison [5], Guo et al. [4], and Guo & Kannan [3] share the same source: a survey among 360 women aged 19–65 years in the USA performed by Loretz et al. in 2005 [9–12]. It should be noted that these product amounts may no longer be applicable (> 10 years old) and in any case refer to the USA. Hence, the available product use data are regarded as a source of uncertainty in the aggregate exposure estimates. More recent detailed survey data – including data on personal care product use in Switzerland for the year 2015, published by Garcia-Hidalgo et al. (2017) [13] – are available, but these are not used in any current exposure estimation studies, of which this section is an inventory.

Retention factors

The retention factor refers to the fraction of the product amount that stays on the skin after application. The rationale behind retention factors, as explained in safety evaluations of cosmetic products by the SCCS and the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) [14, 15], is rather simple and product-oriented. Personal care products applied dermally are subdivided into hair products, rinse-off products and non-rinse-off products. For products

that are rinsed off directly after application, e.g. shower gel, hand soap and make-up remover, it is assumed that 10% will stay on the skin, whereas for non-rinse-off products a retention factor of 100% is assumed. Hair products are assumed to be applied 90% to the hair and 10% to the scalp, so that their retention factor is set at 10%. Shampoo is both a rinse-off and a hair product, so that its retention factor is considered to be 1% (10% of 10%) [15]. No measurement data are available to validate these retention factors [16], but they are generally accepted in the safety evaluation of cosmetic ingredients [14, 15].

Paraben weight fractions in personal care products

Allowed maximum weight fractions for methyl-, ethyl- and propylparaben are 0.4% (as acid) for a single ester, and 0.8% (as acid) for mixtures of esters, as set by the EU (see also Table A3 in Appendix 9.3) [17]. Data on actual weight fractions of methyl-, ethyl- and propylparaben in personal care products can be collected from analytical studies in which the content of parabens in product samples has been measured [3, 4, 18–20]. Exceedance of maximum weight fractions was found only in a few samples purchased in the USA and China [3, 4]; none was found in samples purchased on the European market [18–21]. Moreover, a survey among manufacturers, importers and distributors of cosmetic products in Denmark (performed in 2013) showed a decrease of paraben use as a preservative compared with 2006, indicating that parabens are currently used at lower concentrations and/or in fewer products [45].

Dermal and oral absorption

Only after skin absorption are substances able to reach internal circulation, meaning that parabens must cross a number of cell layers of the skin. A number of factors play a role in this process, e.g. the lipophilicity of the substance, the thickness and composition of the cell layers, the vehicle (solvent drag), the duration of exposure, and the concentration and amount of product applied [14, 22]. The dermal absorption of substances in personal care products is therefore complex to predict [14]. The studies on aggregate exposure to methyl-, ethyl- and propylparaben deal with this complexity by including a dermal absorption percentage that should be representative for the population [3–6]. In a worst-case approach it is often assumed that 100% of the cosmetic ingredient reaches the internal system of the human body. However, actual dermal exposure is (much) lower, although no accurate value has been determined (see also Section 3.2). Such a calculation is complicated by the fact that during dermal absorption parabens are metabolized into PHBA, but this metabolism seems far more effective in rats, on which studies are normally performed, than in humans (where parabens are additionally metabolized into conjugates). The exposure studies the SCCS refers to report dermal absorption values ranging from 1% to 55%, probably due to differences in matrix effects, species differences and the experimental conditions or artefacts [5, 12, 18, 19, 23–26]. For oral absorption, although there is a lot of uncertainty with respect to the likelihood of first pass metabolism taking place, a figure of 100% is used, as is common in risk assessments when absorption from the gastrointestinal tract is assumed to be higher than 70% [22].

2.2.2 *Simple aggregate exposure estimation by summation (Tier 1)*

Aggregate external exposure can be estimated as the sum of external exposure estimates calculated per product. As an illustration, Table 1 provides the exposure estimates per product, per paraben, as well as the simply summed aggregate exposure per paraben obtained by adding the exposures per products from the study by Cowan-Ellsberry & Robison (2009) [5].

Table 1. External dermal exposure estimates for methyl- (MeP), ethyl- (EtP) and propylparaben (PrP) via several products by adult females (from Cowan-Ellsberry & Robison (2009) [5])

Product	**Calculated external exposure** *(mg/kg bw/day)*		
	MeP	**EtP**	**PrP**
Blush	0.0006	0.0003	0.0002
Body cream	0.5367	0.5367	0.2683
Body lotion	0.5367	0.5367	0.2683
Body wash	0.004	0.0004	0.0042
Conditioner (rinse-off)	0.0042	0	0.0021
Eye-liner	0.0002	0.0003	0.0007
Eye make-up remover	0.004	0	0.002
Eye shadow	0.0014	0.0007	0.0005
Face powder	0.0033	0.0025	0.0017
Face serums	0.0084	0.0166	0.0083
Facial cleanser	0.0167	0	0.01
Facial mask	0.1333	0	0
Facial moisturizer	0.0533	0.0533	0.0267
Foundation (liquid make-up)	0.07	0.4	0.05
Hair styling/sculpting	0.042	0.008	0.04
Hand lotion	0.12	0.1167	0.06
Holding spray	0.0068	0	0
Lip colour	0.0289	0	0.027
Mascara	0.0013	0.0008	0.0005
Night cream	0.0267	0.0267	0.0133
Shampoo	0.0055	0	0.0055
Toner	0.0007	0	0
Under eye lotion/cream	0.0067	0.005	0.0083
Simply summed aggregate exposure	**1.6114**	**1.7047**	**0.7976**

The worst-case estimates of external exposure by (female) adults to methyl-, ethyl- and propylparaben in personal care products derived by Cowan-Ellsberry & Robison (2009) in the USA are 1.61, 1.70 and 0.80 mg/kg bw/day, respectively (rounded values from Table 1) [5]. Internal exposure estimates (including dermal absorption) were calculated for US female and Chinese populations by Guo & Kannan (2013) and Guo et al. (2014) (Table 2) [3, 4]. With regard to children, Gosens et al. (2011,

2014) made first-tier aggregate external exposure estimates for infants/toddlers of 2.32, 0.36 and 1.05 mg/kg bw/day for methyl-, ethyl- and propylparaben [6, 7]. Using dermal absorption factors of, respectively, 36%, 55% and 37%, and an oral absorption factor of 100%, internal exposure estimates of 1.01, 0.20, and 0.41 mg/kg bw/day were calculated. For US infants and toddlers internal exposure values were calculated by Guo & Kannan (2013) in the US (Table 2) [3].

2.2.3 *Including non-use and co-use patterns in summed exposure (Tier 2)*

A first option for refinement is to incorporate into the aggregate exposure assessment co-use and non-use pattern data (Tier 2). The consumer is exposed via a combination of different product uses (it is unrealistic to assume that consumers use all the products listed in the inventory and that all the products used contain parabens). Such combinations of product uses are referred to as co-use patterns, the non-use of certain products as non-use patterns. Cowan-Ellsberry & Robison (2009) analysed data on use patterns from a company survey of 3297 women in the USA [5]. They observed 32 co-use combinations for the five skin care products included in the survey and 233 co-use combinations for all nine personal care products included. These product use combinations were weighted in the total aggregate exposure estimate for the products in the survey [5]. However, the survey included only nine personal care products, whereas the inventory used for the simply summed aggregate exposure assessment (Tier 1) included 23 personal care products [5]. Cowan-Ellsberry & Robison (2009) therefore conservatively assumed that the remaining products were all used, and the sum of the exposures via the products not included in the Tier 2 survey was added in a refined aggregate exposure calculation [5]. This resulted in aggregate external exposure estimates of 0.99, 1.03 and 0.51 mg/kg bw/day for methyl-, ethyl- and propylparaben, respectively, with co-use data for nine products (Table 2) [5]. These exposure values were further refined by using extent-of-use data for the less frequently used ethyl- and propylparaben relative to methylparaben (Table 2). This resulted in aggregate external exposure estimates of 0.99, 0.16 and 0.42 mg/kg bw/day for methyl-, ethyl- and propylparaben, respectively (Table 2) [5].

2.2.4 *Modelling using a probabilistic approach (Tier 3)*

The summed exposure calculations described above mostly express product use and concentration data, with single values estimated from their maximum. Consequently, the aggregate exposure calculated as the sum of the exposures per product is usually unrealistically high. Probabilistic models treat product use and concentration (and sometimes even dermal absorption) as variables that can be represented by a probability distribution. Csiszar et al. (2017) and Gosens et al. (2011, 2014) performed such stochastic model simulations for methyl-, ethyl- and propylparaben [2, 6, 7].

In Csiszar et al. (2017) [2], product use is expressed as a uniform distribution for which the minima and maxima are taken from the survey data of Loretz et al. (2005, 2006, 2008) representing a female US population [9–11]. The concentrations of the parabens in the products are also represented with a uniform distribution, but the minima and maxima are taken from the data sources described in the simple aggregate exposure estimation (Paragraph 2.2.2), i.e. Cowan-Ellsberry & Robison

(2009) and Guo et al. (2014) [3, 5], and in addition from Rastogi et al. (1995), who conducted an analytical study on the presence of parabens in 215 samples of cosmetic products in 1994 [21]. Csiszar et al. (2017) further derived lognormal distributions for dermal absorption of the parabens by reflecting on experimentally derived dermal permeation coefficients across different skin types and media such as creams, alcohols and aqueous solutions [2]. The stochastic simulation itself was performed with the Monte Carlo approach, consisting of 10,000 iterations. Per iteration a value is randomly taken from the given input distributions for which the exposure is calculated in a comparable way as described in Paragraph 2.2.2. However, not all samples of the products listed in the inventory necessarily contain the parabens. Csiszar et al. (2017) therefore adjusted their simulations by adding an appropriate number of zeros representing such non-exposure in the 10,000 iterations performed [2]. From the 10,000 outcomes of all iterations together, a mean and distribution (2.5^{th} to 97.5^{th} percentile values) can be calculated for methyl-, ethyl- and propylparaben (Table 2). The results from the probabilistic modelling approach by Gosens et al. (2011, 2014) were presented in cumulative probability plots, which were used in a risk assessment to determine whether the complete calculated exposed population was below a toxicological reference value for the respective parabens, and the results were presented graphically [6, 7].

2.2.5 Overview of exposure to parabens via personal care products

Aggregate exposure to methyl-, ethyl- and propylparaben has been estimated in several studies using methods that can be represented by three tiers: (1) simply summing the exposure of different products [3–6], (2) refining with co-use and non-use patterns [5], and (3) modelling using a probabilistic approach [2, 6, 7]. Table 2 presents an overview of the aggregate exposure to parabens in personal care products as estimated by the different studies using Tiers 1, 2 and 3. The first tier is the most conservative approach, which yields the highest exposure estimates for methyl-, ethyl- and propylparaben, but they are respectively only a factor 1.3, 1.0 and 1.4 higher than the estimates for highly exposed individuals derived using the more complex third-tier model: compare, for example, the highest exposure estimate for children by Gosens et al. (2011, 2014) with the Tier 3 exposure assessment by Csiszar et al. (2017) (Table 2) [2, 6, 7]. The consumer exposure studies performed by Cowan-Ellsberry & Robison (2009) [5], Guo & Kannan (2013) [3] and Csiszar et al. (2017) [2] agree more or less on the order of magnitude of aggregate internal exposure to methyl- and propylparaben from personal care products for highly exposed adults: 0.15–1.61 and 0.11–0.79 mg/kg bw/day, respectively. The studies agree with each other to a lesser extent on estimated exposure to ethylparaben: Cowan-Ellsberry & Robison (2009) derived an estimate of 0.13–1.70 mg/kg bw/day for US females, which agrees with Csiszar et al. (2017) (0.2 mg/kg bw/day) but not with Guo & Kannan (2013), who estimated aggregate exposure to be 0.044 mg/kg bw/day for the same population (Table 2).

Table 2. Overview of aggregate exposure estimations for methyl- (MeP), ethyl- (EtP), and propylparaben (PrP) via personal care products from several studies for different populations across different tiers. Exposure estimates are for external or internal exposure, as indicated in the Remarks column (dermal absorption values given where applicable)

Tier	Population	**Exposure estimates** *(mg/kg bw/day)*				Reference
		MeP	**EtP**	**PrP**	**Remarks**	
1	Adult females, USA	1.61	1.70	0.80	**External** dermal exposure	Cowan-Ellsberry & Robison (2009) [5]
1	Adult females, USA	0.154	0.044	0.106	**Internal** dermal exposure (40% absorption); exposure to leave-on and rinse-off products with the highest concentration values	Guo & Kannan (2013) [3]
1	Infants (0–1 year), USA	0.766	0.095	0.231	**Internal** dermal exposure (80% absorption); idem	Guo & Kannan (2013) [3]
1	Toddlers (2–3 years), USA	0.474	0.059	0.143	**Internal** dermal exposure (80% absorption); idem	Guo & Kannan (2013) [3]
1	Adults, China	0.49	0.06	0.28	**External** dermal exposure	Guo et al. (2014) [4]
1	0–3-year-olds, the Netherlands	2.32	0.36	1.05	**External** dermal and oral exposure	Gosens et al. (2011, 2014) [6, 7]
1	0–3-year-olds, the Netherlands	1.01	0.20	0.41	**Internal** dermal (36%, 55% and 37% absorption for MeP, EtP and PrP, respectively) and oral exposure (100% absorption)	Gosens et al. (2011, 2014) [6, 7]
2	Adult females, USA	1.29	1.39	0.64	**External** dermal exposure; based on co-use patterns for five PCPs	Cowan-Ellsberry & Robison (2009) [5]
2	Adult females, USA	0.99	1.03	0.51	**External** dermal exposure; based on co-use patterns for nine PCPs	Cowan-Ellsberry & Robison (2009) [5]
2	Adult females, USA	0.99	0.16	0.42	**External** dermal exposure; based on co-use patterns for nine PCPs, refined using extent of use data	Cowan-Ellsberry & Robison (2009) [5]
2	Adult females, USA	0.79	0.13	0.34	**Internal** dermal exposure (80% absorption); based on co-use patterns for nine PCPs, refined using extent of use data	Cowan-Ellsberry & Robison (2009) [5]
3	Adults, USA	0.2 (0.003–0.8)	0.03 (0-0.2)	0.06 (0–0.3)	**Internal** exposure (dermal absorption probabilistically derived from skin permeation coefficients); mean values (2.5^{th}–97.5^{th} percentiles)	Csiszar et al. (2017) [2]

It should be noted that the results of these studies were not obtained entirely independently, since they shared data sources with regard to product use [9–11, 27, 28], retention factors [15, 29, 30] and dermal absorption values [14]. The differences in the Tier 1 aggregate exposure estimates can be attributed to the different data sources used to estimate the weight fractions of methyl-, ethyl- and propylparaben in the products. Guo et al. (2014) and Guo & Kannan (2013) used own measurement data [3, 4], whereas Cowan-Ellsberry & Robison (2009) referred to Steinberg (2002, 2006, 2008) and Elder (1984) (as cited in [5]). The Tier 3 estimate by Csiszar et al. (2017) also used product use data and retention factors from the same literature sources [9–11, 29]. Furthermore, Csiszar et al. (2017) used the weight fraction data presented in Guo et al. (2014) and Guo & Kannan (2013) [2–4]. Csiszar et al. (2017), however, derived dermal absorption as a range by reviewing experimental data on the aqueous dermal permeation coefficients of methyl-, ethyl- and propylparaben across different types of skin [2]. It was found that dermal permeation is about 0.01 cm/h for all three parabens considered [2]. The lower aggregate exposure estimates for methyl-, ethyl- and propylparaben simulated by Csiszar et al. (2017) can thus be attributed to their refined method of aggregation with a probabilistic approach.

The study by Gosens et al. (2011, 2014) was performed for the Dutch infant/toddler population [6, 7]. They used other data sources to characterize product use, paraben weight fractions and dermal absorption fractions. The product use data in their worst-case approach (75th percentile) were taken from the Cosmetics Fact Sheet (2006; using European data, as cited in [6,7]). For their probabilistic approach the dataset of a small pilot Dutch survey was used. The number of respondents was very small: n=28 for the entire population of 0–3-year-olds [6, 7]. Furthermore, Gosens et al. (2011, 2014) applied dermal absorption percentages of 36%, 55% and 37%, for methyl-, ethyl- and propylparaben, respectively [31, 32], derived from the SCCS opinion on parabens from 2010 [33]. Despite the different data sources used by Gosens et al. (2011, 2014), their aggregate exposure estimate for toddlers still agrees in order of magnitude with the results of Guo et al. 2014 for 0–1- and 1–3-year-olds (Table 2).

2.3 Exposure via food

2.3.1 Presence in food

Methyl-, ethyl- and propylparaben can be present in foods for different reasons. They may be intentionally added as a preservative, naturally occurring, or present as a result of migration from materials that come into contact with food. Methylparaben, as E218 and its sodium salt (E219), and ethylparaben, as E214 and its sodium salt (E215), are approved for use as a preservative in food according to Annex II to Regulation (EC) No. 1333/2008 (paragraph 5.2.1). Propylparaben is not allowed in food in the EU. However, all three parabens are allowed in the manufacture of plastic materials and articles intended to come into contact with food (Commission Regulation (EU) No. 10/2011) and may, via migration, thus also enter food (see Paragraph 5.2.2). European data on the migration of the three parabens from food packaging material and consequent concentrations in food are not available. Parabens have also

been reported to occur naturally in food. Methylparaben has been reported to be present in cloudberry, yellow passion fruit juice, white wine, botrytized wine and Bourbon vanilla (Ali et al. (1998) as cited in [1]). Soni et al. (2005) report, however, that the intake of parabens from natural sources is negligible [1].

2.3.2 Four studies on exposure to methyl-, ethyl- and propylparaben via food

The literature search yielded four studies in which the intake of parabens via food was assessed: a study on exposure by young children in France to methyl- and ethylparaben as a food additive [34]; studies on a specific adult population in China [35] and on the general population in the USA [36] into exposure to all three parabens via food; and a study performed as part of the Scientific Cooperation (SCOOP) Task Reports, for which EU Member States provided pooled data from across the EU on issues of concern regarding food safety [37]. One of these tasks considered the dietary intake of food additives in the EU, including methyl- and ethylparaben [37]. Below, these studies are described in detail.

Young children in France

In this study, exposure to methyl- and ethylparaben and their sodium salts, as a group, was estimated in children aged 1–36 months in France. For this, individual food consumption data, collected via a food diary on three consecutive days, were combined with MPLs as set out in Annex II to Regulation (EC) No. 1333/2008 (see Paragraph 5.2.1). The mean exposure per child was subsequently calculated by summing exposure across the foods per day and averaging the exposure over the three days. By dividing this average daily exposure by individual body weight, average exposure per child was estimated (µg/kg bw/day). The result was a distribution of individual mean exposure levels, which was used to derive different exposure statistics. This assessment should be considered to result in a very conservative estimate of the intake of methyl- and ethylparaben, and their sodium salts, via food, as it is assumed that all relevant consumed foods that may contain these additives as a preservative do contain them at the MPL. Table 3 lists the reported exposure estimates.

Table 3. Estimated exposure (µg/kg bw/day) to methyl- and ethylparaben, and their sodium salts, per age group based on MPLs as set in Annex II to Regulation (EC) No. 1333/2008 (see Paragraph 5.2.1 for details)

Age group (N)	**Estimated exposure** *(µg/kg bw/day)*		
	Mean (standard deviation)	**Median** (min–max)	**90th percentile**
1–4 months (124)	0 (0)	0 (0–0)	0
5–6 months (127)	0 (0)	0 (0–0)	0
7–12 months (195)	40 (100)	0 (0–890)	70
13–36 months (259)	350 (790)	175 (0–4500)	900

The combined intake of methyl- and ethylparaben, and their sodium salts, was far below the ADI of 10 mg/kg bw/day. The intake assessment was therefore not refined to obtain a more realistic exposure estimate [34]. This assessment addressed only exposure via food intake.

Adult population in China

In 2013, a study into the presence of six parabens, benzyl-, butyl-, ethyl-, heptyl-, methyl- and propylparaben, in food in China was published [35]. In this study, paraben concentrations were determined in 282 foodstuffs belonging to 13 food groups – cereals and cereal products, meat, fish and seafood, eggs, dairy products, bean products, fruits, vegetables, cookies, beverages, cooking oils, condiments, and others, collected from nine cities in China. The food samples were collected during the summer (July–September) of 2012. The majority of food samples were purchased from large retail stores; a few samples were purchased from local grocery stores. Brands were chosen to represent the foodstuffs commonly consumed by the Chinese, including national, store and specialty brands.

Occurrence

Methyl-, ethyl- and propylparaben were detected in all 13 food groups. The highest concentrations were found in vegetables, condiments, dairy products and cereal products, with the highest concentrations analysed for methylparaben, followed by ethyl- and (lowest) propylparaben. Mean methylparaben concentrations ranged from 0.524 ng/g in beverages (n=4; e.g. juice, liquor, coffee) up to 81.1 ng/g in vegetables (n=60; e.g. mushrooms, peanuts, peppers, seaweed, bamboo shoots, potatoes, edible tree fungus, Chinese cabbage, salted mustard). Corresponding concentrations for ethylparaben were 0.037 ng/g in 'other foods' (n=13; e.g. jelly, black sesame powder, lotus root starch, milk tea powder, coffee powder) and 42.8 ng/g in condiments (n=55; e.g. soy sauce, vinegar, cooking wine, ketchup, bean paste, aniseed, chili powder), and for propylparaben 0.007 ng/g in beverages and 14.7 ng/g in vegetables. The overall mean concentration of methyl-, ethyl- and propylparaben was 22.4, 11.0 and 5.22 ng/g, respectively. Methyl-, ethyl- and propylparaben were the major parabens found, and accounted for 59%, 24%, and 10% of the total paraben concentrations in the analysed food samples, respectively. The source of the presence of parabens (natural occurrence, addition as preservative or migration from food packaging material) was not specified.

Exposure

Based on the analysed concentrations, mean and high exposure levels were calculated for all six parabens. For this, mean and 95th percentile concentration levels per food group were combined with the mean daily intake per food group of adult men and women derived from the literature. The resulting mean and high exposure per sex was divided by a fixed body weight per sex: 62.7 and for men and 54.8 kg for women. Table 4 lists the calculated exposures for methyl-, ethyl- and propylparaben.

Table 4. Estimated mean and high (95th percentile) daily intake (µg/kg bw/day) of methyl- (MeP), ethyl- (EtP) and propylparaben (PrP) via food by adults in China (taken from Liao et al. (2013a))

Population	**Exposure** *(µg/kg bw/day)*					
	MeP		**EtP**		**PrP**	
	Mean	High	Mean	High	Mean	High
Men	0.70	1.49	0.17	0.90	0.12	0.43
Women	0.73	1.56	0.17	0.92	0.13	0.45

Methylparaben contributed most to total exposure to the six parabens in China (69%), followed by ethylparaben (16%) and propylparaben (12%) [35].

General population of the USA

A study similar to that in China was performed in the USA [36]. In this study, 267 foods belonging to 8 food groups – beverages, dairy products, fats and oils, fish and shellfish, grain products, meat, fruits, and vegetables – were analysed for the presence of five parabens: benzyl-, butyl-, ethyl-, methyl- and propylparaben. The foods were collected from the city of Albany (New York) in 2008, 2010 and 2012. Several brands were chosen to represent the variety of available manufacturers, and most of the foods were of US origin.

Occurrence

In total, 91% of the analysed foods contained methylparaben at levels ranging from below the limit of quantification (LOQ) (= 0.01 ng/g) to 409 ng/g, with a mean value of 5.83 ng/g. Ethylparaben was present in 61.8% of the analysed foods at levels ranging from below the LOQ to 258 ng/g, with a mean value of 2.26 ng/g. For propylparaben, 62.9% of the analysed foodstuffs contained this paraben at levels from below the LOQ to 95.4 ng/g, with a mean value of 9.67 ng/g. For the data analysis, samples with a level below the LOQ were assumed to contain the parabens at a level equal to half the LOQ.

All meat (n=52; e.g., beef, pork, chicken, turkey, ham, sausage) and vegetable (n=49; e.g., broccoli, cabbage, carrot, celery, cucumber, mushroom, onion, potato, tomato) samples contained methylparaben at levels above the LOQ. Methylparaben was also detected in more than 85% of the samples of grain products (n=54; e.g. wheat flour, bread, rice, noodles, pie, pasta, pizza, corn products, cookies, cakes), fish and shell fish (n=23; e.g. freshwater and marine fish, shrimp, crabs, clams), dairy products (n=31; e.g. milk, infant formula, yogurt, cheese, ice cream) and fruits (n=20; e.g. apple, pear, pineapple, peach, grape, banana, raisin). The lowest detection frequency was 40% in fat and oil samples (n=5; e.g. salad and cooking oil). Overall, methylparaben accounted for 60% of the total analysed paraben concentrations in this study [36]. Ethyl- and propylparaben were the next major parabens present in food in this study, accounting for 23% and 16% of the total analysed paraben concentrations, respectively. The detection frequencies for both these parabens were lower than for methylparaben: ranging from 39% in samples of beverages (n=33; e.g. bottled water, carbonated drinks, soft drinks, wine, beer, juice) to 74% in vegetable samples for ethylparaben, and from 20% in fat and oil samples to 82% in samples of grain products for propylparaben.
As in the Chinese study, the source of the presence of parabens (natural occurrence, addition as preservative or migration from food packaging material) was not specified.

Exposure

As in the Chinese study, the analysed concentrations were used to assess exposure to the five analysed parabens in the US population. For this, per paraben and food group, the mean analysed concentration was multiplied by the average per capita consumption rate according to the

US Environmental Protection Agency (EPA) Exposure Factors Handbook, and summed across the food groups to obtain an estimate of daily exposure. Calculations were performed for five age groups (Table 5). A high-level exposure was also calculated using the 95th percentile per capita consumption rate per age group obtained from the same Handbook combined with a mean concentration per food group. Body weights to calculate mean and high intakes per kg body weight were also obtained from the Handbook.

The mean and high exposures to methyl-, ethyl- and propylparaben are listed in Table 5. The highest exposures were reported in infants and young children, and the lowest in people aged 11 and above. Note that in infants exposure to ethylparaben was higher than to methylparaben, despite overall higher levels of methylparaben in food (see above). The reason for this is not explained by the authors, but it could be due to a higher mean concentration of ethylparaben than of methylparaben in beverages: 8.53 vs 4.74 ng/g. This food group includes water, which is used in the preparation of infant formula.

Table 5. Estimated mean and high (95th percentile) daily intake (µg/kg bw/day) of methyl- (MeP), ethyl- (EtP) and propylparaben (PrP) via food by five age groups in the USA (taken from Liao et al. (2013b))

Population	**Exposure** *(µg/kg bw/day)*					
	MeP		**EtP**		**PrP**	
	Mean	High	Mean	High	Mean	High
Infants (< 1 year)	0.37	1.38	0.47	1.74	0.10	0.39
Young children (1 to < 6 years)	0.44	1.03	0.24	0.68	0.20	0.45
Children (6 to < 11 years)	0.23	0.58	0.16	0.46	0.08	0.21
Teenagers (11 to < 21 years)	0.13	0.36	0.10	0.32	0.04	0.12
Adults (≥ 21 years)	0.13	0.36	0.14	0.40	0.04	0.10

The authors observed that several food items that may contain parabens were not included in their assessment, for example eggs, condiments, fast food and breast milk [36]. Therefore, the estimated intakes may underestimate actual exposures. Furthermore, the number of analysed samples was low and the paraben concentrations varied widely within food groups.

SCOOP study

In 2001, the intake of food additives, including methyl- and ethylparaben and their sodium salts, was estimated according to a tiered approach (Figure 2) as part of the SCOOP Task Reports [37]. In this tiered approach, Tier 1 resulted in the most conservative and Tier 3 in the most refined exposure estimates [37]. If the calculated intake exceeded the relevant health-based guidance value according to a Tier, the food additive intake was calculated according to the next, more refined, Tier [37]. Based on conservative assumptions regarding food consumption and additive usage (Tier 1), the calculated intake of methyl- and ethylparaben and their sodium salts did not exceed the ADI of 10 mg/kg bw/day in either adults or children. The intake of these food additives was therefore not examined further [37]. Actual calculated intake levels were not reported.

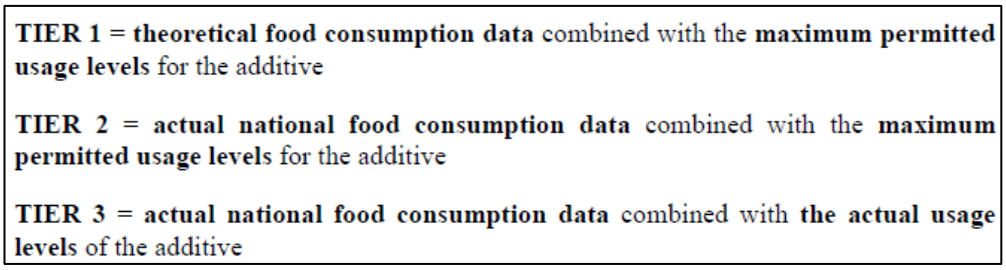

Figure 2. The three Tiers of the approach used in the SCOOP Task Report for the dietary intake of food additives in the EU. Figure is obtained from [37].

2.3.3 Exposure via food packaging material

To establish whether exposure to parabens via food may be the result of migration from food packaging material, the concentrations of the different parabens were compared between four types of packaging materials (can, glass, paper, plastic) in the Chinese study discussed in Paragraph 2.3.2. The results suggested that there was no association between paraben concentrations in foods and the packaging materials used [32]. The same authors repeated this experiment in the USA with the same result (Paragraph 2.3.2) [36]. In both studies, the concentrations of parabens in tinned foods were low.

2.4 Exposure via medicinal products

2.4.1 Presence in medicinal products

Methylparaben

Methylparaben is widely used as a preservative in medicinal products, frequently combined with propylparaben in order to obtain a synergistic antimicrobial effect [38]. A search in the Medicines Information Bank of the Dutch Medicines Evaluation Board (CBG-MEB) revealed about 260 approved medicinal products containing methylparaben.[1] About 50% of these products are oral solutions and suspensions. However, topical formulations (e.g. cutaneous, rectal, vaginal) and parenteral preparations may also contain this preservative. The concentration of methylparaben is usually not stated on the packaging or in the patient information leaflet; the amounts present in the various medicinal products on the market is thus not publicly known. However, handbooks on pharmaceutical excipients indicate concentration ranges of 0.015 to 0.2% [39].

Ethylparaben

There are also a 8 medicinal products on the Dutch market that contain ethylparaben, sometimes combined with propyl- or methylparaben, according to the search in the Medicines Information bank of the CBG-MEB. For ethylparaben, no use concentrations were found in handbooks on pharmaceutical excipients. According to Moreta et al. (2015), the maximum detected value for ethylparaben in several medicinal products in the USA was 0.39 mg/g, in a liquid or cream [40].

Propylparaben

Propylparaben is also widely used in medicinal products and is usually combined with methylparaben. The ratios methylparaben to propylparaben applied are usually 7:1, 7:3 or 3:1 [41]. According to the

[1] Search performed on 24 May 2017; https://www.cbg-meb.nl/geneesmiddeleninformatiebank

search in the Medicines Information bank of the CBG-MEB, there are 180 approved medicinal products containing propylparaben as a preservative on the Dutch market. The handbooks on pharmaceutical excipients mention concentration ranges of 0.01–0.02% in oral liquid preparations, 0.01–0.6% in topical formulations and 0.005–0.2% in parenterals [39].

Moreta et al. (2015) performed measurements on total parabens in several medicinal products in the USA [40]. The maximum value detected for methylparaben was 2 mg/g, in a liquid or cream [40]. For propylparaben the maximum value was also detected in a liquid or cream, namely 0.70 mg/g. They also measured a few samples from India, China, Poland, Italy and Spain, which showed quite different concentrations, indicating a non-uniform application in products across countries [40].

2.4.2 Exposure data

Methyl- and propylparaben

Studies reported in the scientific literature did not reveal data on internal or external exposure to methyl- or propylparaben via medicinal products in the Netherlands or the EU. For methylparaben, according to a reflection paper by the EMA, the maximum concentration of 0.2% results in a maximum oral intake of approximately 140 mg/day [38]. This maximum intake is based on a dose of 70 ml per day of a product containing 0.2% methylparaben. Analogously, for propylparaben, the maximum concentration of 0.06% results in a maximum oral intake of approximately 50 mg/day [38]. This intake is in line with the current posology of medicines and is therefore to be considered as a realistic maximum intake via orally administered medicinal products. Some of the oral medicinal products may be used chronically, while others are applied only short term. Examples of chronic use are oral solutions for the treatment of epilepsy and human immunodeficiency virus. Oral suspensions with antibiotics are usually applied for only one or two weeks. Besides oral medication, a patient may use topical formulations or even parenteral preparations. For dermal products, it is difficult to estimate exposure, since the amount of cream or ointment applied will depend on the surface area to be treated.

If the oral bioavailability of methylparaben is assumed to be 100%, the maximum external exposure of 140 mg/day would lead to an equivalent internal exposure or 2.3 mg/kg bw/day for a 60 kg individual. As no data on specific product concentrations or use patterns are available in order to differentiate between children and adults, this value of 2.3 mg/kg bw/day is also used for the estimation of the aggregate exposure of children. If the oral bioavailability of propylparaben is assumed to be 100%, the maximum external exposure of 50 mg/day would equate to an internal exposure of 0.83 mg/kg bw/day for a 60 kg individual. As no data on specific product concentrations or use patterns are available in order to differentiate between children and adults, this value of 0.83 mg/kg bw/day is also used for the estimation of the aggregate exposure of children.

Ma et al. (2016) performed a survey of parabens in commercial oral medicinal products in China [42]. Using measured concentration and daily ingestion rates (from the ingestion directions) for methylparaben, an estimated daily intake (95th percentile) was calculated for male and

female adults (24.0 and 28.1 ng/kg bw/day, respectively) and for children (28.9 ng/kg bw/day) [42]. For propylparaben an estimated daily intake (95th percentile) was calculated for male and female adults (11.2 and 13.1 ng/kg bw/day, respectively) and for children (17.6 ng/kg bw/day). The body weights used in these calculations were 67.3, 57.5 and 24.1 for men, women and children, respectively [42]. Dodge et al. (2015) determined whether paraben-containing medication contributes to urinary paraben concentrations [43]. Although they used a small sample size, it could be concluded that medication contributes to the urine paraben concentration within hours of use [43].

Ethylparaben

Due to the absence of data, no exposure level could be calculated for products used in the EU. Ma et al. (2016) estimated the daily intake (95th percentile) of ethylparaben via medicinal products in China as 23.2 and 27.2 ng/kg bw/day for men and women, respectively, and 104 ng/kg bw/day for children [42].

2.5 Exposure via other sources

As well as via personal care products, food and medicinal products, exposure – especially to methyl- and propylparaben – can occur via other consumer products such as household pesticides, cleaning products, paints and pet supplies [44, 45]. Furthermore, methyl-, ethyl- and propylparaben have been reported to leach from plastic babies' dummies (pacifiers) [46, 47] and from paper products [48].

Exposure via environmental sources can occur as well, such as via indoor dust [49, 50]. In a study by Wang et al. (2012) 158 indoor dust samples from China, South Korea, the USA and Japan were collected (period 2006–2012) and the concentrations of six parabens and their common metabolite PHBA were determined [49]. Methyl- and propylparaben were the most prevalent substances detected in the samples, with methylparaben accounting for 42–73% of the total paraben concentration (mean concentrations per country ranging from 226 to 1670 ng/g), and propylparaben accounting for 12–46% of the total paraben concentration (mean concentrations per country ranging from 123 to 761 ng/g) [49]. The concentration of ethylparaben was minor, with a mean concentration per country ranging from 8.9 to 91 ng/g). The estimated daily intake of parabens in this study via dust ingestion was 5–10 times higher among children than among adults, and highest in South Korea (3.24, 0.12 and 1.78 ng/kg bw/day for methyl-, ethyl- and propylparaben, respectively) and Japan (3.90, 0.21 and 0.63 ng/kg bw/day for methyl-, ethyl- and propylparaben, respectively) [49].
Altogether, this indicates that exposure to methyl- and propylparaben, and to a lesser extent, to ethylparaben, can occur through a great variety of sources and products other than personal care products, food and medicinal products. Although only limited information on the content and concentration in different products and sources is available, and consequently very limited information is available on exposure via these additional sources, exposure is usually considered to be very minor compared with exposure via personal care products, food and medicinal products.

2.6 Exposure estimates recalculated from biomonitoring data

Methyl-, ethyl- and propylparaben are mainly excreted via the urine. Metabolites and conjugates (mainly glucuronides and sulfates), but also a small fraction of the free parent substance, can therefore be detected in the urine of humans [1, 51]. The major part of the parabens is excreted as metabolites of PHBA, which cannot be directly used to discriminate metabolites of methylparaben from metabolites of ethyl- or propylparaben, or vice versa, or even from metabolites of other parabens (e.g. butylparaben).

There are numerous studies reporting data on the biomonitoring of parabens in urine, often in combination with epidemiological studies. As mentioned in the Introduction, an extensive review of those studies is excluded here. However, biomonitoring data, such as measurements of parabens and metabolites in urine, can be used to estimate aggregate exposure via all routes and sources among individuals in a population [5, 52–55]. For that reason, the topic is addressed here in order to provide insight into the relevance of the estimated modelled exposure via personal care products, food and medicinal products, as a check. Total exposure estimates for methyl-, ethyl- and propylparaben back-calculated from metabolites in urine in different studies are described below [5, 52–55], and summarized in Table 6. Because of the (specific) study populations and periods, their representativeness of the current situation in the general population in the Netherlands and/or Europe is likely very limited. For details of the calculation methods is refered to the respective publications.

Table 6. Back-calculated 95th percentile daily intake values (µg/kg bw/day) for methyl- (MeP), ethyl- (EtP) and propylparaben (PrP) from selected biomonitoring studies

Reference	**Study population and period**	**Exposure, 95th percentile** *(µg/kg bw/day)*		
		MeP	**EtP**	**PrP**
Cowan-Ellsberry & Robison (2009) [5], based on Ye et al. (2006) [51]*	General, USA, 2003–2005	19.9	1.39	8.2
Moos et al. (2016) [55]	Students (age 20–30), Germany, 1995–2012	47.5	7.4	20.6
Kang et al. (2016) [56]	General (age 3–69), South Korea, 2009–2010	241	60	25
Ma et al. (2013) [54]	Female (age ~20), China, 2010	242	79.4	125
Ma et al. (2013) [54]	Male (age ~20), China, 2010	154	84.2	125
Guo et al. (2017) [52]	Agricultural region (age 3), China, 2012–2013	150.8	187.1	118.2

** Instead of daily intake, internal exposure has been calculated here.*

Ye et al. (2006) and Cowan-Ellsberry & Robison (2009)
In a US study performed by Ye et al. (2006), urinary concentrations of methylparaben and its conjugates were examined in a demographically diverse group of 100 adults with no known exposure to parabens [51].

The urine samples were collected from 2003 to 2005. In 99%, 58% and 96% of the urine samples, respectively, methylparaben, ethylparaben or propylparaben, or their metabolites (conjugates of the respective parabens), were detected [51]. In the study by Cowan-Ellsberry & Robison (2009), the 95th percentile values from these measurements were used to estimate internal exposures to methyl-, ethyl- and propylparaben of 19.9, 1.39 and 8.2 µg/kg bw/day, respectively, using a steady-state toxicokinetic model [5].

Kang et al. (2016)
In a South Korean study by Kang et al. (2016) with 2541 urine samples (taken in 2009–2010 from a population aged 3–69 years), in 98%, 97% and 97% of the samples, respectively, methylparaben, ethylparaben or propylparaben were detected (free + deconjugated glucuronide and sulfate metabolites) [53]. On the basis of the measured urinary concentrations, human exposure to the sum of methyl- and ethylparaben was estimated by a simple steady-state toxicokinetic model, resulting in an estimated internal exposure value of 301 µg/kg bw/day [53]. According to Kang et al. (2016), based on the composition profile of parabens in the urine in this study, ~80% of this concentration can be attributed to methylparaben (resulting in an estimated daily intake of about 241 µg/kg bw/day for methylparaben, and 60 µg/kg bw/day for ethylparaben) [53]. The daily intake of propylparaben was estimated to be 25 µg/kg bw/day.

Moos et al. (2016)
In a study by Moos et al. (2016) among German students (20–30 years old) 660 24-hr urine samples were collected from 60 subjects (30 female, 30 male) per year, from 1995 to 2012 [55]. By using calculated urinary excretion factors of 17.4% for methylparaben, 13.7% for ethylparaben and 9.7% for propylparaben, oral equivalent daily intake values were back-calculated from urinary levels [55, 57]. This resulted in median daily intake levels of 5.8, 0.4 and 1.2 µg/kg bw/day for methyl-, ethyl- and propylparaben, respectively [55]. The respective 95th percentile values were 47.5, 7.4 and 20.6 µg/kg bw/day [55]. There was a difference between the male and female sub-populations, with respective median values of 3.0, 0.2 and 0.3 µg/kg bw/day for males, and 9.0, 0.7 and 2.5 µg/kg bw/day for females, with respective 95th percentile values of 31.7, 3.4 and 11.9 µg/kg bw/day for males, and 52.7, 10.1 and 26.9 µg/kg bw/day for females [55]. Interestingly, over the period 1995-2012 a significant increase in intake of methylparaben was estimated, driven by the male sub-population; the estimated intake by the female sub-population did not significantly increase over this period [55]. There were no other significant trends during this period [55].

Guo et al. (2017)
In a recent study by Guo et al. (2017) urinary concentrations were determined in children 3 years of age (n=436) during 2012–2013 in an agricultural region of Jiangsu province, China [52]. In 98%, 95% and 99% of the samples, respectively, methyl-, ethyl- or propylparaben were detected (free + deconjugated glucuronide and sulfate metabolites). Median concentrations of 6.03, 3.17, and 2.40 ng/ml total methylparaben, total ethylparaben and total propylparaben were detected, respectively. The respective 95th percentile values reported

were 82.13, 106.38 and 66.27 ng/ml. From these, median daily intakes were estimated to be 12.10, 5.68 and 4.50 µg/kg bw/day for methyl-, ethyl- and propylparaben, respectively [52]. The respective 95th percentile values were 150.8, 187.1 and 118.2 µg/kg bw/day.

Ma et al. (2013)
Ma et al. (2013) determined urinary concentrations in young Chinese adults (n=109), approximately 20 years of age, in 2010 [54]. In all samples methyl-, ethyl- and propylparaben were detected (free + deconjugated glucuronide and sulfate metabolites). Median concentrations of 4.63, 1.40 and 3.17 ng/ml total methyl-, total ethyl- and total propylparaben were detected, respectively. The respective 95th percentile values were 140, 61.3 and 92.0 ng/ml. From these, male (n=68) median daily intakes were estimated to be 5.20, 1.83 and 2.96 µg/kg bw/day for methyl-, ethyl- and propylparaben, respectively [54]. The respective 95th percentile values were 154, 84.2 and 125 µg/kg bw/day [54]. For females (n=41) median daily intakes were estimated to be 15.5, 2.50 and 10.7 µg/kg bw/day for methyl-, ethyl- and propylparaben, respectively [54]. The respective 95th percentile values were 242, 79.4 and 125 µg/kg bw/day [54].

2.7 Summarizing exposure estimation

For exposure estimation in this report three major sources were considered: personal care products, food, and medicinal products. Table 7 (for adults) and Table 8 (for children) present the most relevant estimates. Exposure to methyl-, ethyl- and propylparaben can also occur via a great variety of consumer products other than personal care products. Unfortunately, very little information on these additional sources is available for inclusion in the aggregate exposure estimation. Aggregation of the exposure via the three major sources considered in this report was difficult because of varying levels of information quality and uncertainties in the different sources.

2.7.1 Exposure via personal care products

Exposure to methyl-, ethyl- and propylparaben from personal care products has been estimated in different studies in different tiers and for different populations (Table 2). Internal exposure to methyl-, ethyl- and propylparaben is conservatively estimated for adults to be 0.79, 0.13 and 0.34 mg/kg bw/day, respectively, based on Cowan-Ellsberry & Robison (2009) [5] (Tables 2 and 7); for infants and toddlers it is estimated at 1.01, 0.20 and 0.41 mg/kg bw/day, respectively, based on Gosens et al. (2011, 2014) [6, 7] (Tables 2 and 8). More realistic but very similar levels for highly exposed adult individuals estimated stochastically are 0.8, 0.2 and 0.3 mg/kg bw/day for methyl-, ethyl- and propylparaben, respectively (97.5th percentile values) by Csiszar et al. (2017) [2] (Tables 2 and 7). Exposure values from other studies are mostly in the same order of magnitude (Table 2) [3]. Nonetheless, these values' representativeness of the present situation in the Netherlands remains uncertain, especially for adults, because the underlying product use data in the available aggregate exposure studies may be outdated (> 10 years old) and/or refer to the USA. With regard to the European situation, more recent product use datasets are available, which could

be used for new estimations of exposure to parabens via personal care products [13, 58–60].

2.7.2 *Exposure via food*

Exposure to methyl-, ethyl- and propylparaben via food was studied using two approaches: use of MPLs according to legislation (the French study and SCOOP study), and actual analysed concentrations (the Chinese and US studies). There is no relevant study into the actual European intake of methyl-, ethyl- and propylparaben via food. The intake assessments most relevant for Europe (the French study and SCOOP study) examined only intake via the use of methyl- and ethylparaben as a preservative in food [34, 37]. The estimated intakes were, however, very conservative, because of the assumption that all foods in which the preservative(s) are authorized contain the parabens at the MPL. On the other hand, natural occurrence of the parabens in foods was not considered in these studies; nor was migration from food packaging material. However, if it is assumed that the concentrations of methyl- and ethylparaben analysed in vegetables, fruits, dairy products and grain products in China [35] and the USA [36] are (mainly) due to natural occurrence, it is not likely that exposure would increase significantly if these sources had also been included in the two assessments. The mean concentrations reported in these studies were far below the EU MPLs. The highest mean concentration reported in the US study was 14.1 ng/g, in grain products; the highest in the Chinese study was 81.1 ng/g, in vegetables – in both cases for methylparaben. The presence of parabens via migration from food packaging material is also likely to be low.

Based on analysed concentrations and mean consumption patterns (US study), 95th percentile estimates of adult exposure to methyl-, ethyl- and propylparaben were 0.36, 0.40 and 0.10 µg/kg bw/day, respectively (Tables 5 and 7). For children (< 1 year), the corresponding 95th percentile estimated exposures were 1.38, 1.74 and 0.39 µg/kg bw/day, respectively (Tables 5 and 8). The reported 95th percentile for exposure to propylparaben by children aged 1 to 6 years was higher: 0.45 µg/kg bw/day (Table 5 and 8). The approach used to assess exposure via food using analysed concentrations best represents potential exposure, although this information was available only for China and the USA (Tables 4 and 5), where the regulation of the use of parabens in foods is likely to be different from that in the EU. Consumption patterns may also differ. It is therefore unclear how well these exposures represent the situation in the Netherlands and/or Europe. At most, the estimations may give an impression of the actual level of exposure. In these studies, the source of parabens in food was not identified (preservative, natural occurrence or migration from food packaging material). The migration of parabens into food via food packaging material was, however, shown not to be an important source of exposure [35, 36].

2.7.3 *Exposure via medicinal products*

Exposure to methyl-, ethyl- and propylparaben from medicinal products may occur concurrently via various administration routes. In the scientific literature, only one study describing an assessment of exposure to methyl-, ethyl- and propylparaben via medicinal products is

available – a study in China. In the EU, according to a reflection paper by the EMA, worst-case oral exposure via medicinal products has been estimated to be 2.3 mg/kg bw/day for methylparaben and 0.83 mg/kg bw/day for propylparaben [38], based on the maximmum value of the range in which the parabens are formulated [39]. No estimation can be made of exposure via other routes of medication (e.g. topical or parenteral), as no information is available, although it is likely that dermal exposure will also take place, as methylparaben is used as a preservative in creams and ointments, as with personal care products. As there are no data on product concentrations for ethylparaben, an analogous calculation for this paraben could not be made, and consequently exposure to ethylparaben via medicinal products in the Netherlands cannot be estimated. There are only a few medicinal products containing ethylparaben on the Dutch market (8), compared with products containing methylparaben (260) or propylparaben (180). Most of these eight products are intended for short-term use, i.e. from few days up to 4 weeks. As exposure via medicinal products can be chronic, even over a short duration, but is completely absent in a part of the population, a probabilistic exposure assessment for methyl-, ethyl- and propylparaben via this source would be very valuable.

2.7.4 Aggregate exposure

Methylparaben

The aggregated methylparaben exposure values for adults (Table 7) and children (Table 8) can be estimated by adding the estimates from three sources, although from different studies with very different levels of detail. The exposure estimation from medicinal products in particular was based on very limited information and is very crude and worst-case. Altogether, the aggregate estimate of internal exposure to methylparaben for adults can be calculated as ~3.1 mg/kg bw/day (Table 7). Medicinal products contribute 74% to this estimate, personal care products 25%, and food < 1%. For children the aggregate internal exposure estimate of methylparaben can be calculated as ~3.3 mg/kg bw/day (Table 8). Medicinal products contribute 70% to this estimate, personal care products 30%, and food < 1%. The high contribution of medicinal products diminishes the reliability of the aggregate exposure values. The majority of medicinal products do not contain methylparaben and only part of the population uses methylparaben-containing medication chronically.

Ethylparaben

To produce an aggregate exposure value for ethylparaben that is comparable to those for methyl- and propylparaben is challenging, as no relevant estimate for ethylparaben in medicinal products is possible. Therefore, this source is omitted from the internal exposure estimate for ethylparaben, which is ~0.2 mg/kg bw/day for both adults and children. Almost 100% of this value consists of the contribution by personal care products, as the contribution by food is < 1%.

Propylparaben

As with methylparaben, the aggregated propylparaben exposure values for adults (Table 7) and children (Table 8) consist of added estimates from three sources, from different studies with very different levels of detail. Here also, the estimation of exposure via medicinal products was based on very limited information and is very crude and worst-case.

Altogether, the aggregate estimate of internal exposure to propylparaben by both adults and children can be calculated as ~1.2 mg/kg bw/day (Tables 7 and 8). Between 64% and 72% of this estimate is contributed by medicinal products, and the remaining 36–28% almost completely by personal care products, as < 1% is contributed by food. The high contribution by medicinal products (in a crude and worst-case estimation) diminishes the reliability of the aggregate exposure values. The majority of medicinal products do not contain propylparaben and only part of the population use methylparaben-containing medication chronically.

Table 7. Estimates of exposure by adults to methyl- (MeP), ethyl- (EtP) and propylparaben (PrP) via three sources from different studies (with very different qualities of estimation); the figures in bold are summed in the total estimated internal exposure

Source	**Estimated external exposure** *(mg/kg bw/day)*			**Estimated internal exposure** *(mg/kg bw/day)*			**Route**	**Quality of estimate**	**Ref.**
	MeP	**EtP**	**PrP**	**MeP**	**EtP**	**PrP**			
Personal care products, USA	1.61	1.70	0.80	0.79	0.13	0.34	Dermal only	External exposure is simple summed exposure; worst-case deterministic exposure estimate of 23 personal care product types. Internal exposure is refined using non-use, co-use and extent of use data, and 80% dermal absorption.	[5]
Personal care products, USA	-	-	-	**0.8**	**0.2**	**0.3**	Dermal only	P97.5 values by stochastic modelling; more realistic. Dermal absorption fraction probabilistically derived from skin permeation coefficients.	[2]
Food, China	1.49 $x10^{-3}$	0.90 $x10^{-3}$	0.43 $x10^{-3}$	1.49 $x10^{-3}$	0.90 $x10^{-3}$	0.43 $x10^{-3}$	Oral	P95 value for male population based on analysed concentrations and mean consumption patterns.	[35]
Food, China	1.56 $x10^{-3}$	0.92 $x10^{-3}$	0.45 $x10^{-3}$	**1.56 $x10^{-3}$**	**0.92 $x10^{-3}$**	0.45 $x10^{-3}$	Oral	P95 value for female population based on analysed concentrations and mean consumption patterns.	[35]
Food, USA	0.36 $x10^{-3}$	0.40 $x10^{-3}$	0.98 $x10^{-3}$	0.36 $x10^{-3}$	0.40 $x10^{-3}$	**0.98 $x10^{-3}$**	Oral	P95 value based on analysed concentrations and mean consumption patterns.	[36]
Medicinal products, EU	2.3	?	0.83	**2.3**	**?**	**0.83**	Oral only	Maximum exposure estimated very roughly and	[38, 39]

Source	**Estimated external exposure** *(mg/kg bw/day)*			**Estimated internal exposure** *(mg/kg bw/day)*			**Route**	**Quality of estimate**	**Ref.**
	MeP	**EtP**	**PrP**	**MeP**	**EtP**	**PrP**			
								worst-case.	
Medicinal products, China	24.0 $x10^{-6}$	23.2 $x10^{-6}$	11.2 $x10^{-6}$	24.0 $x10^{-6}$	23.2 $x10^{-6}$	11.2 $x10^{-6}$	Oral only	P95 value for male population based on measured concentrations an daily ingestion rates.	[42]
Medicinal products, China	28.1 $x10^{-6}$	27.2 $x10^{-6}$	13.1 $x10^{-6}$	28.1 $x10^{-6}$	27.2 $x10^{-6}$	13.1 $x10^{-6}$	Oral only	P95 value for female population based on measured concentrations and daily ingestion rates.	[42]
TOTAL	~3.9	~1.7	~1.6	**~3.1**	**~0.2**	**~1.2**			

Table 8. Estimates of exposure by children to methyl- (MeP), ethyl- (EtP) and propylparaben (PrP) via the three sources from different studies (with very different qualities of estimation); the figures in bold are summed in the total estimated internal exposure

Source	**Estimated external exposure** *(mg/kg bw/day)*			**Estimated internal exposure** *(mg/kg bw/day)*			**Route**	**Quality of estimate**	**Ref**
	MeP	**EtP**	**PrP**	**MeP**	**EtP**	**PrP**			
Personal care products	2.32	0.36	1.05	**1.01**	**0.20**	**0.41**	Dermal/ oral	Simple summed exposure, worst-case deterministic estimate from several personal care products. Internal exposure calculated with dermal absorption fraction of 36%, 55%, and 37% for MeP, EtP and PrP, respectively.	[6]
Food, France	0		-	0		-	Oral	P90 value for children aged 1–6 months estimated on food records and MPLs; therefore very conservative.	[34]
Food, France	0.07×10^{-3}		-	0.07×10^{-3}		-	Oral	P90 value for children aged 7–12 months estimated on food records and MPLs; therefore very conservative.	[34]
Food, France	0.9×10^{-3}		-	0.9×10^{-3}		-	Oral	P90 value for children aged 13–36 months estimated on food records and MPLs; therefore very conservative.	[34]
Food, USA	1.38×10^{-3}	1.74×10^{-3}	0.39×10^{-3}	$\mathbf{1.38 \times 10^{-3}}$	$\mathbf{1.74 \times 10^{-3}}$	0.39×10^{-3}	Oral	P95 value for children aged < 1 year based on analysed concentrations and mean consumption patterns in USA	[36]
Food, USA	1.03×10^{-3}	0.68×10^{-3}	0.45×10^{-3}	1.03×10^{-3}	0.68×10^{-3}	$\mathbf{0.45 \times 10^{-3}}$	Oral	P95 value for children aged 1–6 years based on	[36]

Source	**Estimated external exposure** *(mg/kg bw/day)*			**Estimated internal exposure** *(mg/kg bw/day)*			**Route**	**Quality of estimate**	**Ref**
	MeP	**EtP**	**PrP**	**MeP**	**EtP**	**PrP**			
								analysed concentrations and mean consumption patterns in USA	
Medicinal products	2.3	?	0.83	**2.3**	**?**	**0.83**	Oral only	Maximum exposure estimated very roughly and worst-case.	[38, 39]
Medicinal products	28.9 $x10^{-6}$	104 $x10^{-6}$	17.6 $x10^{-6}$	28.9 $x10^{-6}$	104 $x10^{-6}$	17.6 $x10^{-6}$	Oral only	P95 value based on measured concentrations and daily ingestion rates.	[42]
TOTAL	~4.0	~0.4	~1.9	**~3.3**	**~0.2**	**~1.2**			

2.7.5 Comparison with biomonitoring studies

The biomonitoring studies in which measurements of parabens and their metabolites in urine were used to estimate total exposure (Section 2.6, Table 6) can be compared with the aggregate exposure estimation based on exposure via personal care products, food and medicinal products (Section 2.7). Only the back-calculated *internal* exposure values by Cowan-Ellsberry & Robison (2009) based on Ye et al. (2006) can be compared with the calculated aggregated *internal* exposure estimates.

For adults, the 95th percentile values for internal exposure to methyl-, ethyl- and propylparaben back-calculated by Cowan-Ellsberry & Robison's (2009) from urine metabolite levels of 19.9, 1.39 and 8.2 µg/kg bw/day for the general US population [5], differ by a factor ~156, ~144, and ~146, respectively, from the estimates of aggregate internal exposure via personal care products, food and medicinal products (exposure to ethylparaben via medicinal products could not be estimated).

On the other hand, if we take the estimated daily intake values from other biomonitoring studies (on specific populations mostly other than European, using different methods – Table 6), based on 95th percentile values of urinary concentrations of parabens, as accurate estimations of actual exposure, the difference (by a factor ~16 to ~82 lower) from the calculated *external* aggregate exposure to methylparaben (Tables 7 and 8) is likely to be explained by the worst-case character of model calculations of the aggregate exposure estimate. However, it is unclear how well these exposures represent the current situation in the Netherlands. At best, the estimations may give an impression of the actual level of exposure. A comparable difference as with methylparaben is found for ethylparaben, with daily intake values from biomonitoring studies a factor ~20 to ~230 lower than the *external* aggregate exposure estimates, although the difference for children falls outside this range, the *external* aggregate exposure estimate of ~0.4 mg/kg bw/day (Table 8) being a factor ~2 higher than the daily intake value for children in a specific Chinese area reported by Guo et al. (2017) [54]. For propylparaben, like methylparaben, the daily intake values from biomonitoring studies are a factor of ~20 to ~230 lower than the external aggregate exposure estimates.

2.8 Discussion on the exposure assessment including uncertainties

The combination of estimates of exposure to the selected parabens from the different sources (Tables 7 and 8) is based on several studies with a wide variety in set-up, type, level of detail and assumptions made. Table 9 presents a summary of the main uncertainties present in the current (worst-case) aggregate exposure estimation, and their implications for exposure values. Overall, uncertainties point in the direction of an overestimation of total exposure. This is also indicated by the comparison with back-calculated exposure levels from biomonitoring studies (Paragraph 2.7.5), which may give an impression of the actual level of exposure.

Table 9. Overview of uncertainty within main factors within the sources of exposure and its possible effect on the exposure assessment (↑ = increases exposure estimate, ↓ = decreases exposure estimate, - = no effect on exposure estimate, ? = effect unknown)

Factor	Description	Effect
Personal care products		
Aggregation method	Method of aggregation of exposure from different products, dependent on tier (Paragraphs 2.2.2, 2.2.3 and 2.2.4)	↑ / -
Product use data	Frequency of products used and amount of product applied (2.2.1)	↑ / ↓
Concentration data	The concentration of parabens in personal care products is possibly less than ~10 years ago, because of the suspicion of ED properties and public awareness of this (2.2.1)	↑ (?)
Presence data	The fraction of products in which parabens are present is possibly less than ~10 years ago, because of the suspicion of ED properties and public awareness of this (2.2.1)	↑ (?)
Retention factor	The fraction that stays on the skin after application is determined theoretically, not empirically (2.2.1)	↑ / ↓
Dermal absorption	Uncertainty about the extent to which parabens are absorbed from the skin into the internal system; in most assessments a worst-case percentage is chosen (2.2.1)	↑
Food		
Concentration data	Use of MPLs (2.3.2.1 and 2.3.2.4) / Use of actual concentrations (2.3.2.2; 2.3.2.3)	↑ / -
Consumption data	• Individual food consumption data (2.3.2.1) • Summary statistics (2.3.2.2; 2.3.2.3) • Theoretical consumption data (2.3.2.4)	- ↑ ↑
Coverage of food sources	Not all food sources of exposure were covered	↓
Mean exposure estimates	• Mean individual exposure over three days (2.3.2.1) • Mean consumption x mean concentration per food group summed over food groups (2.3.2.2; 2.3.2.3)	- -
High exposure estimates	• Mean individual exposure over three days (2.3.2.1) • Mean consumption x 95th percentile concentration per food group summed over food groups (2.3.2.2; 2.3.2.3)	↑ ↑
Populations	Representativeness of the studies for the Netherlands	?
Medicinal products		
Concentration data	• No realistic information; maximum levels for methyl- and propylparaben according to	↑↑

Factor	Description	Effect
	handbook used (2.4.1) • No data for ethylparaben, including maximum levels according to handbook (2.4.1)	↓
Use pattern data	• No use pattern data; realistic maximum as rough, worst-case estimation (2.4.2) • No differentiation between exposure routes; oral exposure equals internal exposure (2.4.2) • Use unevenly distributed in the population (2.4.2) • Daily exposure assumed though use often not chronically, (2.4.2)	↑ ↑ ↑ ↑
Other consumer products		
Exposure data	Virtually no information on use of and exposure to parabens from other sources (Section 2.5); therefore excluded	↓

With regard to personal care products, it seems that the use of parabens as a preservative is decreasing. The uncertainties in the present exposure assessments could be reduced by performing a relevant exposure assessment using more recent product use, concentration and presence data, better representing the current situation in the Netherlands, or Europe. Recent data on product use are available, and by using these a more realistic estimation of exposure to parabens via personal care products would be possible, for example using the Probabilistic Aggregate Consumer Exposure Model (PACEM) [61, 62]. However, as model input, current and relevant (i.e. from the Netherlands) concentration data in non-food consumer products are also needed.

For food, estimates of exposure to parabens using data relevant to Europe overestimate real exposure due to the use of MPLs, and the assumption that all foods within an authorized food category contain the paraben as a preservative. The high exposure levels estimated for populations in China and the USA also overestimate exposure due to the assumption that all analysed food groups contain the parabens at the 95th percentile of the analysed samples. At the mean level, exposure is very likely underestimated, because not all sources of exposure were included in the assessments. However, the aggregate exposure estimates show that the contribution via food is small (< 1%) in comparison with the contribution via personal care products and medicinal products. Therefore, further refinement of the exposure assessment for parabens from food is very likely not relevant.

Medicinal products are the greatest contributor to exposure to methyl- and propylparaben in the summed aggregate exposure estimations for these parabens. However, data on product concentration and use are not available, and therefore exposure via medicinal products has only been estimated roughly and worst-case. For ethylparaben, no estimation was even possible. Data on product content and concentration related to product use would be helpful to better estimate this exposure. As exposure via medicinal products can be chronic, however often over a short period, or absent in a part of the population, a probabilistic

exposure assessment for methyl-, ethyl- and propylparaben via this source would be valuable. This is, however, not possible as long as data on product concentration and use are not available.

Exposure to methyl-, ethyl- and propylparaben can occur through a great variety of sources and products other than personal care products, food and medicinal products. However, quantitative data from which to estimate such exposure are virtually absent from the literature. Nevertheless, exposure via these other sources is usually regarded to be very minor compared with exposure via personal care products, food and medicinal products.

3 Toxicity of methyl-, ethyl- and propylparaben

3.1 Introduction

In this Chapter, an overview is provided of the known toxicokinetics (Section 3.2) and hazard characteristics, including endocrine-disrupting (ED) activity (Sections 3.3–3.8), of methyl-, ethyl- and propylparaben. Since the focus of this evaluation is on the endocrine-related properties of the parabens, studies on reproductive and developmental toxicity relevant to ED-related endpoints will be discussed in detail. A conclusion on hazard characteristics is provided in Section 3.9. The potential hormone-disrupting effects of the parabens described in Section 3.8 are then reviewed with regard to the WHO definition of endocrine disruption (Section 3.10). The overall NOAELs that have been derived by others are summarized and discussed in Section 3.11. Section 3.12 provides a summary discussion of toxicity in the light of the uncertainties that have been identified.

3.2 Toxicokinetics

3.2.1 Kinetics

Depending on the route of exposure, parabens are absorbed from the gastrointestinal tract or the skin. Methyl-, ethyl- and propylparaben absorbed from the gastrointestinal tract are metabolised in rats, rabbits, dogs, cats and humans by esterases in the liver, intestine and kidney [63]. In addition to urinary excretion, there is some excretion via the bile and faeces. The most common metabolite is PHBA (phase I metabolism); minor metabolites are the glycine conjugates, and glucuronide and sulfate metabolites of PHBA acid or the respective paraben (phase II metabolism), as well the intact parent substance. The phase II conjugates of the parent methyl-, ethyl- or propylparaben were detected in humans, not in rats [1]. A study on the UDP-glucuronosyltransferase (UGT) isoenzymes responsible for the glucuronide metabolites was conducted by Abbas et al. (2010) [64].

After application to human skin, it has been argued that methyl-, ethyl- and propylparaben are quickly and nearly completely hydrolysed into PHBA, so the systemic absorption of the parent substance is very low [1]. However, other studies indicate that the biotransformation of the different parabens into PHBA is not as effective as claimed [26, 51]. According to a study by Harville et al. (2007) using subcellular tissue fractions (microsomes and cytosol), the hydrolysis rate in human skin is, depending on the specific paraben, about 300 to 3000 times lower than in rat skin and in liver [23]. The hydrolysis rate in human liver and rat liver is similar, or at least less than 10-fold different [23]. This means that after dermal exposure a greater portion of non-hydrolysed parabens could be available for internal exposure [23]. In humans, in contrast to rats, after the application of parabens to the skin, glucuronyl as well as sulphate conjugates are found in serum and urine, which indicates that at least part of the parabens pass the skin un-hydrolysed to PHBA [51, 65]. In addition, human spot urine samples indicate the presence of methyl-, ethyl- and propylparaben as parent substances [7]. Altogether, this indicates that in humans parabens, after dermal and/or

oral exposure, are not completely hydrolysed to PHBA, and therefore rats might not sufficiently represent the biotransformation of methyl-, ethyl- and propylparaben as it occurs in humans. This is important, as the availability of un-metabolised parabens is expected to determine potential ED activity [33]. Therefore, better information with regard to (toxico)kinetics, including dermal absorption and metabolic interspecies differences, especially during dermal uptake, is required.

3.2.2 Dermal and oral absorption values

Dermal

The available *in vitro* dermal absorption studies are considered of poor scientific quality by the SCCS (2010, 2013) [33, 66], and the results of biomonitoring studies show the presence of un-metabolised parabens in the plasma of human volunteers [26, 67]. According to the SCCS (2010, 2013), data for the conversion from rat to human dermal absorption are still lacking, especially for the absorption and metabolism of the parent substance in the skin. As discussed above, the dermal absorption and, especially, dermal metabolism of parabens are very different in rat skin than in human skin. Determining a dermal absorption factor for humans is therefore challenging. A wide range of dermal absorption values, from 1 to 55%, are reported in the SCCS opinion [33]. The SCCS chose a dermal absorption value of 3.7% by using a pragmatic weight-of-evidence approach: taking the highest dermal absorption in an *in vitro* split-thickness dermal human skin study of 37% with butylparaben and using a correction factor of 10 to account for skin metabolism which metabolizes parabens as seen in full thickness skin experiments [33]. In the risk assessment by Gosens et al. (2011, 2014), however, a much higher dermal absorption factors of 36%, 55% and 37% for methyl-, ethyl- and propylparaben were used, respectively [6, 7], these being the highest values for methyl-, ethyl- and propylparaben from two peer-reviewed, non-TG *in vitro* dermal absorption studies with human skin [31, 32]. Some other risk assessment studies on parabens also use high dermal absorption values. For example, Guo & Kannan (2013) used a dermal absorption value of 40% for all parabens for adults, and 80% for children (twice the value for adults). In the risk assessment by Cowan-Ellsberry & Robison (2009) a conservative value of 80% is chosen for all parabens because of the high variability of dermal absorption values reported [5].

Oral

Although there is a lot of uncertainty with respect to oral bioavailability, and first pass metabolism is likely taking place, a default oral absorption factor of 100% is used, which is commonly done in risk assessments when absorption from the gastrointestinal tract is assumed to be higher than 70% [22]. An oral absorption of 100% is also assumed by the SCCS, EFSA and others [33, 66, 68].

3.3 Acute toxicity

Low acute toxicity has been identified in the OECD TG 401 studies on the acute oral toxicity of methyl-, ethyl- and propylparaben in different rodent species (mice, rats, dogs and guinea pigs) after oral administration. LD50 values lie between 2000 and 8000 mg/kg bw [69]. For other routes such as subcutaneous and intraperitoneal administration, the LD50 values are around 10 times lower [7]. No to

moderate irritation was reported after dermal exposure and inhalation of methyl-, ethyl- and propylparaben [7, 69, 70].

3.4 Irritation / corrosion / sensitisation

Methyl-, ethyl- and propylparaben did not irritate the skin in the OECD TG 404 studies on acute dermal irritation/corrosion. Data regarding human exposure are available and parabens are not irritating in people with normal, undamaged skin [1, 69]. Methyl-, ethyl- and propylparaben did not irritate the eyes, either. Methyl-, ethyl- and propylparaben were not considered to be a skin sensitizer when tested in skin sensitization OECD TG 406 and/or OECD TG 429 studies.

3.5 Repeated dose toxicity

Based on the available repeated-dose toxicity studies, repeated oral exposure to methyl-, ethyl- or propylparaben is not considered to cause serious effects to health. No data were available on toxicity relating to repeated dermal exposure and inhalation [69].

3.6 Genotoxicity / carcinogenicity

Based on the weight of evidence from the available *in vitro* and *in vivo* genotoxicity studies, methylparaben is not considered to be genotoxic. Some *in vitro* genotoxicity tests indicated weakly positive results, but all *in vivo* tests were negative [69]. Ethyl- and propylparaben did not induce statistically significant increases in the *in vitro* or *in vivo* assays for genotoxicity. From the available *in vivo* carcinogenicity studies on methyl-, ethyl- and propylparaben it can be concluded that they are not considered to be carcinogenic [1, 7, 45].

3.7 Developmental and reproductive toxicity

Since the focus of this report is on the endocrine-related properties of the parabens, studies on reproductive and developmental toxicity relevant to ED-related endpoints will be extensively discussed in the hazard assessment.

3.7.1 Developmental toxicity

In the OECD TG 414 studies on prenatal development no effects were observed in rodents and rabbits after exposure to methylparaben up to a level of 550 mg/kg bw/day. For ethylparaben no OECD TG study was performed with regard to developmental toxicity. For propylparaben an OECD TG 422 combined repeated-dose toxicity study and reproduction/ developmental toxicity screening test was performed up to a dosage of 15,000 ppm (between 1000 and 1500 mg/kg bw/day) in 2012. These studies were reported by the applicant in the REACH dossier, available from the European Chemicals Agency (ECHA) database. No adverse effects were identified in adult males or females and no test item-related findings in pups were noted.

In the OECD TG studies no developmental effects were identified for any of the parabens. Preferably, usual dosages up to 1000 mg/kg bw/day could have been given, but that has not been done for methylparaben. No explanation was given for the choice of the maximum dosage level of 550 mg/kg bw/day. However, these studies were performed around

1970–1975 (except the OECD TG 422 study on propylparaben, performed in 2012), which might be the explanation for this [33].

3.7.2 *Reproductive toxicity*

No OECD TG studies have been performed on the reproductive toxicity of methyl- and ethylparaben (such as an OECD TG 443 extended one-generation reproductive toxicity study or an OECD TG 416 two-generation reproduction toxicity study). For propylparaben, an OECD TG 422 combined repeated-dose toxicity study and reproduction/ developmental toxicity screening test was performed up to a dosage of 15,000 ppm (between 1000 and 1500 mg/kg bw/day). The parameters measured and recorded for developmental and reproductive toxicity, as reported by the applicant in the REACH dossier available from the ECHA database, were: estrous cyclicity, fertility indices mean precoital time, post-implantation losses, mean litter size, pup sex ratios and viability indices, litter size, live births, still births, gross anomalies, pup weight, sperm motility, count and morphology, histology and weight of the reproductive organs. No effects were identified for these parameters in any of the dose groups. The lowest dose tested was around 100 mg/kg bw/day.

Reproduction effects were investigated in non-TG studies in female and male animals [71–75]. The study design and main findings of these studies were also taken into account in the opinions by the SCCS. The studies are described below.

Oishi (2004)

Rats 25–27 days old were exposed to methyl- or ethylparaben via their diet at doses of 0.1% and 1.0% (8 animals per group). Dosages were calculated to be 100 and 1000 mg/kg bw/day, respectively. Rats were sacrificed after 8 weeks and the weights of the testes, epididymides, prostates, seminal vesicles and preputial glands were determined. There were no treatment-related effects of either substance on the organ weights in any of the study groups. Neither substance exhibited anti-spermatogenic effects or changes in levels of testosterone, LH or FSH at any dose up to 1000 mg/kg of bw/day. A NOAEL of 1000 mg/kg bw/day was therefore determined for methyl- and ethylparaben in this study [71].

Oishi (2002)

Propylparaben was administered in the diet to 3-week-old rats, which were divided into 4 groups (n=8 per group), at doses of 0 (control group), 0.01, 0.10 and 1.00%. The average propylparaben intakes calculated from the amount of food consumed were 12.4, 125 and 1290 mg/kg bw/day for the 0.01, 0.10, and 1.00% groups, respectively. At the end of 4 weeks, the rats were sacrificed and the weights of testes, epididymides, prostates, seminal vesicles and preputial glands were determined. There were no treatment-related effects of propylparaben on the organ weights in any of the study groups. The cauda epididymal sperm reserves and concentrations decreased in a dose-dependent manner in the 0.10 and 1.00% groups. Daily sperm production and its efficiency in the testis of all groups receiving propylparaben were significantly decreased. The serum testosterone concentration decreased in the 1.00% group [76]. A Lowest Observed Adverse Effect Level (LOAEL) of 12.4 mg/kg bw/day was determined in this study.

Hoberman et al. (2008)
Rats 22 days old were fed for 8 weeks with a diet containing 100, 1000, or 10,000 ppm methylparaben (comparable to 11.2, 110.0 or 1141 mg/kg bw/day, respectively) compared with a control group. Parameters evaluated included organ weights, histopathology of reproductive tissues, sperm production, motility and morphology of the organs. Methylparaben up to a dose of 10,000 ppm (1141 ± 58.9 mg/kg bw/day) did not affect any male reproductive organs or parameters, but methylparaben did affect the percentage of abnormal sperm at 1000 ppm (110 mg/kg bw/day) and 10,000 ppm (1141 mg/kg bw/day). A NOAEL of 11.2 mg/kg bw/day was determined on the basis of the percentage of abnormal sperm [72].

Vo et al. (2010)
In this study, a female pubertal assay, the effects of methyl-, ethyl- and propylparaben in Sprague Dawley rats were investigated. Rats were orally exposed to the parabens from postnatal day 21–40 at 62.5, 250 or 1000 mg/kg bw/day. Rats were sacrificed at postnatal day 41. The dose of 1000 mg/kg bw/day resulted in a significant delay in the date of vaginal opening and a decrease in the length of the estrous cycle in the methylparaben-exposed rats, but not in the ethyl- and propylparaben-exposed rats. Organ weight in the methylparaben-exposed rats (thyroid, liver, adrenal gland and ovary), in the ethylparaben-exposed rats (kidney and adrenal gland) and in the propylparaben-exposed rats (adrenal gland) was affected at 1000 mg/kg bw/day [74]. A NOAEL for all parabens of 250 mg/kg bw/day was determined in this study.

Gazin et al. (2013)
Propylparaben was administered orally to juvenile male Wistar rats (n=20 per group) from postnatal day 21 up to 11 weeks of age (8-week treatment period) at a dosage of 3, 10, 100 or 1000 mg/kg bw/day. The selected treatment period covers the juvenile (postnatal day 21–35), peri-pubertal (postnatal day 35–55), pubertal (postnatal day 55–70) and early adult stages in the male rat. The onset of puberty, reproductive organ weights, sperm count, motility and plasma hormone levels were measured at the end of the treatment. Blood samples were taken from additional satellite animals after dosing on postnatal day 21 and postnatal day 77 for toxicokinetic analysis. There was no evidence of an effect of propylparaben on the weight of the male reproductive organs, epididymal sperm parameters, hormone levels or histopathology. A NOAEL of 1000 mg/kg bw/day for propylparaben was determined from this study.

SCCS conclusions on methyl- and ethylparaben (2010)
The SCCS considered all the studies on reproduction toxicity described above and concluded that the Oishi (2004) study was to be used to derive the current NOAEL of 1000 mg/kg bw/day for methyl- and ethylparaben [33]. The SCCS considered the shortcomings and limitations of the Hoberman study (2008) as follows: (1) the study was not performed in accordance an official or well established scientific protocol such as OECD TG, (2) the raw data provided were considered to be insufficient (e.g. the study report mentioned that the 64 animals of the repeat assay were from 10 dams, but failed to provide further details), (3) the body weights of the animals varied considerably, (4) in the methylparaben study protocol it was mentioned that testosterone, FSH and LH were measured

in the blood, but these values were not present in the raw data provided, (5) 26% of the animals displayed unexpected clinical signs such as chromorhinorrhea and chromodacryorrhea, which raised general questions about the husbandry of the animals, and (6) too many adverse effects with statistical significance were dismissed due to the lack of dose-dependency and/or abnormally high values in control animals. Because of these shortcomings, according to the SCCS, the Hoberman et al. (2008) study could not be used to refute the findings by Oishi (2004) study. On the other hand, the Oishi (2004) study could not be properly assessed by the SCCS due to the unavailability of raw data [33]. In 2010, the SCCS concluded that *in vivo* studies on parabens published between 2008 and 2010 only showed effects with relatively high dosage levels (mainly about 1000 mg/kg bw/day) for methyl and ethylparaben. However, these findings do not explain the diverging results between the Oishi (2004) and Hoberman et al. (2008) studies in male rats.

SCCS conclusions on propylparaben (2010, 2013)
The potential of propylparaben (and butylparaben) to modify the endocrine system is a concern related to the use of parabens in personal care products. Therefore, the availability of a sound *in vivo* reproductive toxicity study is essential to the hazard assessment of propylparaben (and butylparaben). However, at this moment no conclusion can be drawn from the available male reproductive toxicity studies [71, 75]. Contradictory results are available and none are considered to be scientifically acceptable. Therefore, the SCCS was not able to determine an adequate NO(A)EL value for propyl- and butylparaben in 2010.

In 2013 the SCCS examined the Gazin et al. (2013) study on the reproductive toxicity of propylparaben. This study was performed on the same strain of juvenile male rat as the Oishi (2002) study (Wistar Crj: WI (Han)) and treatment started on postnatal day 21. However, the duration of exposure was extended from 4 to 8 weeks (postnatal day 77) and Gazin et al. (2013) used gavage (once daily) instead of a dietary mixture. Furthermore, an additional lower dose level group (3 mg/kg bw/day) was included in this study in order to determine a NOAEL, and an additional group of animals was included to evaluate the reversibility of any toxic signs during a 26-week treatment-free period. Additional endpoints were included in order to determine the mechanisms of the expected testicular and epididymal effects (this included histopathology and serum LH and FSH levels) [66]. This study showed no effects on the reproductive parameters in rats.

However, the SCCS concluded that this study neither reduced nor increased the concerns previously expressed by the SCCS with respect to the lack of scientifically sound data on the link between dermal exposure by rats and humans, since all these studies assess effects in rodents after oral exposure [33, 66]. In the SCCS 2013 opinion the relevance of the animal studies to human risk assessment is questioned because the rapid and effective metabolism of parabens in rats is not present in humans [66]. In conclusion, although much toxicological data on propylparaben in rodents exists, evidence has not been provided for the safe use of propylparaben in personal care products. For these reasons, the SCCS requests an improvement of the data, in particular on the exposure of humans, including children, to propylparaben in cosmetic products and on

the toxicokinetics of propylparaben in humans. For methyl- and ethylparaben, the SCCS (2013) stated that earlier conclusions were made conservatively and therefore there was no argument based on these findings to change the conclusions on these parabens.

RIVM conclusions on developmental and reproductive toxicity
For methyl- and ethylparaben no OECD TG studies on reproductive toxicity were performed, but relevant peer-reviewed studies were performed and summarized in this paragraph. All these studies investigated the effects of methyl-, ethyl- and propylparaben exposure on the reproduction of male and female animals. As previously stated, for methyl- and ethylparaben, a NOAEL of 1000 mg/kg bw/day was derived from the study by Oishi (2004) [33, 71]. The NOAEL of 1000 mg/kg bw/day from this study also supported the establishment of the ADI for methyl- and ethylparaben by EFSA (see Section 3.11) [68]. Oishi (2004) did not find any reproductive effects in rats after methyl- and ethylparaben exposure up to a level of 1000 mg/kg bw/day [71]. This NOAEL does not take possible spermatogenic effects identified by Hoberman et al. (2008) into account [72], nor the delay in the date of vaginal opening in pre-pubertal rats and decrease in length of the estrous cycle with a NOAEL of 250 mg/kg bw/day identified by Vo et al. (2010) [7, 74]. The Vo et al. (2010) study was also taken into account by the SCCS. Vo et al. (2010) identified a NOAEL of 250 mg/kg bw/day and a LOAEL of 1000 mg/kg bw/day (effects on the date of vaginal opening, the length of the estrous cycle and affected organ weight (thyroid, liver, adrenal gland and ovary)). The SCCS concluded that this study could not be used to determine the NOAEL since it was not an OECD TG study and the effects were not dose-response-related.

The RIVM does not completely agree with the SCCS opinion, since effects on the estrous cycle and organ weights occurred only at the highest dose level tested (1000 mg/kg bw/day). The study by Vo et al. (2010) was well designed and the measured effects on vaginal opening, estrous cycle and organ weights are relevant. Nevertheless, the RIVM recommends that further study for these or comparable effects is needed at the same dose levels. No effects for ethyl- and propylparaben on the date of vaginal opening or length of the estrous cycle were identified by Vo et al. (2010).

3.8 Endocrine-disrupting activity

3.8.1 Update on the hormonal (estrogenic/anti-androgenic) properties of methyl-, ethyl- and propylparaben following the SCCS opinion of 2010/2013

The SCCS opinions of 2010 and 2013 focused on the ED properties of parabens. The SCCS evaluated all the studies to date that had investigated possible ED effects of these substances: *in vitro* studies as well as *in vivo* developmental toxicity studies and reproductive toxicity studies. For this report, a literature search was performed to select all recent *in vitro* and *in vivo* studies on the ED properties of methyl-, ethyl- and propylparaben published after the most recent SCCS 2013 opinion (see Appendix 9.1 for details of the literature search) to investigate whether the SCCS conclusions still hold. The selected *in vivo* and *in vitro* studies are summarized in Tables A1 and A2, respectively (Appendix 9.2).

3.8.2 *In vitro studies on ED properties*

The general results or conclusions from the *in vitro* studies for the different endocrine endpoints are described below.

Estrogenic activity

Parabens increased cell growth and proliferation in human breast cancer cell lines (such as MCF-7 and MCF-10A) in different *in vitro* studies. In a study by Khanna and Darbre (2013), methylparaben ($1x10^{-4}$ M) or propylparaben ($1x10^{-5}$ M) resulted in a greater number of colonies per dish and an increased average colony size in the MCF-10A cells (17β-oestradiol at 17 nM) [77]. Another study, by Khanna et al. (2014), showed that long-term exposure of MCF-7 cells to methyl- or propylparaben increased migration. Increased migratory activity was also demonstrated in long-term paraben-exposed T-47-D and ZR-75-1 cells using a scratch assay and time-lapse microscopy (concentrations: 5×10^{-4} M methylparaben (MCF-7/T-47-D cells) or $1x10^{-4}$ M methylparaben (ZR-75-1 cells), 1x10–5 M propylparaben, $1x10^{-8}$ M 17β-oestradiol). 17β-oestradiol showed similar results. Ethylparaben was not measured in this study [78].

Gene or protein expression of ED-related pathways was investigated in the human breast cancer cell lines mentioned above, as well as in the GH3 rat pituitary cancer cell line (Table A1). Estrogen-related pathways were activated *in vitro* after exposure to the parabens at different concentrations, identifying an estrogenic MOA (Table A1) [79–83], but not for all parabens in all studies [84]. Another study showed that cell cycle and apoptosis pathways are affected by the different parabens [85]. Estrogenic activity increases as a function of chain length *in vitro*, i.e. is higher for propylparaben than ethylparaben, and for the latter higher than methylparaben [82, 83]. Parabens are not metabolized in MCF7 *in vitro* cell lines [86], but that says nothing about their metabolism *in vivo*. An *in vitro* nuclear receptor coactivator recruiting assay was used to evaluate the binding activities of parabens via antagonist competitive binding on the human estrogen-related receptor γ (ERRγ). Antagonist activity on ERRy was determined by the 50% relative effective concentrations (REC50). The REC50 values of the different parabens were 4.79×10^{-7} M for methylparaben, 3.73×10^{-7} M for ethylparaben, and 3.45×10^{-7} M propylparaben. Results were not compared with a positive control [87].

Androgenic activity

After exposure to five selected parabens (methyl-, ethyl-, propyl-, butyl- and iso-butylparaben), only isobutylparaben antagonized the androgen receptor (AR) in the AR reporter gene assay in the AR-transfected Chinese Hamster Ovary (CHO) cells [88]. Anti-androgenic activities were also studied in a yeast-based human androgen receptor assay (YAS). Concentration-dependent anti-androgenic properties were found for methyl- and propylparaben. The anti-androgenic potencies of propylparaben were higher than those of methylparaben (IC50 methylparaben: $2.3x10^{-4}$ M, IC50 propylparaben: $3.9x10^{-4}$ M). No positive control was tested. [89]. Ethylparaben was not measured in this study.

Other ED-related mechanisms

A study by Hu et al (2013) found that parabens promote adipogenesis (or adipocyte differentiation) in murine 3T3-L1 cells, as revealed by adipocyte morphology, lipid accumulation and mRNA expression of adipocyte-specific markers at concentrations of 1000 μM [90]. The adipogenic potency of parabens is increased with increasing length of the linear alkyl chain in the following potency ranking order: methyl- < ethyl- < propyl- < butylparaben [90]. In another study using the same model, ethylparaben induced differentiation of the 3T3-L1 cells at 200 μM and propylparaben at 100 and 200 μM; methylparaben did not induce differentiation up to a concentration of 200 μM [91].

Summary

In conclusion, new *in vitro* studies have been performed since the SCCS opinions (summarized in Table A1 in Appendix 9.2). Overall, an MOA has been identified in these studies showing estrogenic and anti-androgenic properties of methyl-, ethyl- and propylparaben *in vitro*. Estrogenic and anti-androgenic activity and effects on adipogenesis seem to increase as a function of chain length [33, 89, 90]. The relevance of this *in vitro* data to the measurement of possible adverse effects *in vivo* is still under debate.

3.8.3 In vivo studies on ED properties

The new *in vivo* studies on ED properties are summarized in Table A2 (Appendix 9.2) and are discussed below.

Sun et al. (2016)

The uterotrophic activities of methyl- and ethylparaben were investigated in immature Sprague Dawley rats. The expression of the following genes was affected in the methyl- and ethylparaben-exposed group (0.8, 4 and 20 mg/kg bw/day): Icabp, Itmap1, CaBP-9k, Pgr. Relative uterine weight was increased in the ethylparaben-exposed group (4 or 20 mg/kg bw/day) and in the methylparaben-exposed group (20 mg/kg bw/day) [92]. The RIVM concluded that the study was performed properly; however, it focused on a limited set of effects. The measured effects (gene expression, uterine weight) suggesting an ED MOA should be confirmed by other studies. By themselves the results are not sufficient to derive a NOAEL.

Manservisi et al. (2015)

This study determined whether low doses of methylparaben affect the development and proliferative activity of the mammary glands. Female animals treated with methylparaben (0.1050 mg/kg bw/day) showed evident histological differences from controls: the alveoli of the mammary gland were not always milk-filled and an increase in adipose tissue was noted. The collapsed alveolar and duct structures showed residual secretory content. Gene expression was affected [93]. The RIVM noted that part of this study was performed in a low number of animals (n=3 dams per group). Additionally, it was not described how the statistical significance of mortality and pup numbers was identified. Furthermore, the quantification of the histopathological findings was not explained; therefore, the quality of the study was poor.

Ahn et al. (2012)
The effects of methyl- and propylparaben exposure on the early phase of folliculogenesis and steroidogenesis in the ovaries of neonatal rats were studied [94]. Propyl- and butylparaben interrupted ovarian follicle development by increasing the number of primordial follicles and decreasing early primary follicle numbers. Methylparaben did not. The dosages used were 62.5, 250 or 1000 mg/kg bw/day. These results suggest that estrogenic parabens suppress the transformation of primordial follicles into early primary follicles in the rat ovaries. The RIVM noted that not all data appear consistent. Effects were observed after subcutaneous application and no adverse effects were observed, but only intermediate effects were measured. This is in line with the conclusions of the SCCS (2013) about this study.

Lee et al. (2017)
The influence of methyl- and propylparaben on ovarian folliculogenesis and steroidogenesis in Sprague Dawley rats was investigated. Diestrus phases in the propylparaben group were longer than those in the vehicle and methylparaben groups. Methylparaben-treated rats showed a regular estrous cycle, whereas propylparaben-treated animals showed a shortened interval of the estrous cycle. There was no effect on the number of primary follicles, and secondary follicles showed a decrease in total number in all treated groups [95]. The RIVM noted that only one dosage was used and questions whether the control group was representative, since this group seemed to deviate from the other groups.

Gopalakrishnan et al. (2017)
In this study the effects of methylparaben on the histology and transcriptome profiles of normal (noncancerous) mammary glands of Sprague Dawley rats were studied. Dosages were chosen that mimicked human exposure (0.105 mg/kg bw/day, orally). Animals were exposed across several key developmental stages: perinatal (GD1–GD20, n=10 or PND1–PND21, n=10), prepubertal (PND21–PND42, n=5) and pubertal (PND42–PND63, n=5). There were also long-term exposures from birth to lactation (PND1–PND146, n=3). Perinatal methylparaben exposure decreased amounts of adipose tissue and increased expansion of the ductal tree within the fat pad. Prepubertal methylparaben treatment was associated with a significant reduction in adipose tissue and more abundant glandular tissue. Pubertal methylparaben exposure elevated the amounts of glandular tissue compared with controls. This was visible as a higher degree of branching relative to the total gland area. Long-term methylparaben treatment from birth to lactation did not result in significant histological changes. In the pubertal window gene expression changes were observed [96]. The RIVM opinion is that all these effects were intermediate effects, suggesting an ED MOA.

Costa et al. (2017)
The ED effects of methylparaben on the adult gerbil prostate were studied. Methylparaben caused morphological changes in gerbil prostates in all experimental groups. These animals displayed similar alterations, such as prostate epithelial hyperplasia, increased cell proliferation, and a higher frequency of AR-positive cells [97]. The RIVM noted that no adverse effects were measured, but it is unclear how the morphological effects were quantified. Only one dosage was measured.

Gazin et al. (2013)

This study has already been described in Paragraph 3.7.2.

Summary

Findings in the *in vivo* studies performed after the SCCS opinions did not contradict the current NOAEL of 1000 mg/kg bw/day for methyl- and ethylparaben. The seven available *in vivo* studies all have weaknesses in study design (e.g. with regard to statistics, small number of animals, no dose–response relationship measured) and in some, no adverse ED effects were found. The (intermediate) endpoints measured in the studies described above suggest an endocrine MOA for all the parabens evaluated [92–95, 97], but more data with regard to *in vivo* effects are needed.

3.9 Conclusions on hazard characteristics

Table 10 summarizes the regular toxicological endpoints for methyl-, ethyl- and propylparaben. Results from the reproductive toxicity studies by Vo et al. (2010) and Hoberman et al. (2008) might suggest that effects occur below the level of the established NOAEL of 1000 mg/kg bw/day for methyl- and ethylparaben. For propylparaben no NO(A)EL has yet been established. Pragmatically, several effect levels from different studies are used within risk assessment, ranging from 2 to 1000 mg/kg bw/day [98]. The value of 2 mg/kg bw/day is proposed on on the basis of a non-TG study by Fisher et al. (1999) in which juvenile rats were subcutaneously exposed for 17 days (only one dose group) to butylparaben [99]. This NOEL for butylparaben, which has a higher toxicity than propylparaben, can very conservatively be used for propylparaben.

Table 10. Summary of hazard characteristics for the main toxicological endpoints for methyl- (MeP), ethyl- (EtP) and propylparaben (PrP)

Toxicological Endpoint	MeP	EtP	PrP
Acute toxicity	Low in OECD TG 401	Low in OECD TG 401	Low in OECD TG 401
Irritation / corrosion / sensitisation	None in OECD TG 406 or TG 429	None in OECD TG 406 or TG 429	None in OECD TG 406 or TG 429
Repeated dose toxicity	Negative	Negative	Negative
Genotoxicity	Some positive *in vitro*, negative *in vivo*	Negative *in vitro* and *in vivo*	Negative *in vitro* and *in vivo*
Carcinogenicity	Negative	Negative	Negative
Developmental toxicity	Negative in OECD TG 414 up to 550 mg/kg bw /day (highest dose) during early seventies	Read across from MeP	(no effects on reproduction in screening study (OECD TG 422))
Reproductive Toxicity	Not determined (in OECD TG) Oishi (2004): Negative (NOAEL 1000 mg/kg bw/day) Hoberman et al. (2008): effects on sperm (NOAEL 11.2 mg/kg bw/day) Vo et al. (2010): delay vaginal opening, decrease length estrous cycle and organ (thyroid, liver, adrenal gland, ovary) weight effects (NOAEL 250 mg/kg bw/day)	Not determined (in OECD TG) Oishi (2004): Negative (NOAEL 1000 mg/kg bw/day) Vo et al. (2010): organ (adrenal gland, kidney) weight effects (NOAEL 250 mg/kg bw/day)	Negative in OECD screening TG 422 up to ~1000–1500 mg/kg bw/day (highest dose) in 2012 Oishi (2002): effects on sperm (LOAEL 12.4 mg/kg bw/day) Vo et al. (2010): organ (adrenal gland) weight effect (NOAEL 250 mg/kg bw/day) Gazin et al. (2013): Negative (NOAEL 1000 mg/kg bw/day)

With regard to ED properties, current available information is insufficient, and more data are needed to conclude whether methyl-, ethyl- and propylparaben have ED properties *in vivo* and can be identified as endocrine disruptors. *In vitro* and *in vivo* studies have identified a mechanism of action with estrogenic, anti-androgenic or other ED properties for these parabens, but data on adverse effects in an intact organism are still lacking. There is a clear limitation in available ED

endpoints of *in vivo* studies that warrants further studies on adverse health effects, especially reproductive toxicity studies with special attention to hormone-related parameters (e.g. an OECD TG 443 study). However, the relevance of rat studies is under debate because of differences in metabolism compared with humans. In conclusion, parabens have an endocrine MOA and there are different indications that they affect ED-related *in vivo* endpoints, but more *in vivo* studies are needed (though the toxicokinetic relevance of such studies needs to be taken into account).

3.10 WHO definition and EU criteria for ED substances

The World Health Organization (WHO) defined an Endocrine Disrupting Chemical (EDC) in 2002 as 'an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations' [100]. In recent decades the issue of EDCs and their potential adverse effects on human health has been a subject of debate in the scientific literature and among regulatory agencies. Based on the WHO definition, the European Commission has developed scientific criteria to identify EDCs in the Plant Protection Products Regulation (PPPR) and for Biocidal Products Regulations (BPR). Though there is an intention to use these criteria in other legal frameworks, it remains to be seen whether (or when) this will happen and whether the criteria are applicable to those legal frameworks, including those covering personal care products, food, and medicinal products, which are relevant with regard to exposure to parabens. An EFSA-ECHA guidance document is currently being developed that describes how to implement the ED criteria with the available data from the different *in vivo* and *in vitro* OECD TG studies.

The WHO definition includes three key elements: (1) adverse health effects (adversity) in an intact organism, (2) endocrine mode (or mechanism) of action (MOA) and (3) the underlying causal relationship (biological plausibility) between these two. These elements will be used in order to evaluate the effects of the parabens. The EU criteria for substances include the following (which are in fact refinements of the corresponding elements of the WHO definition): (1) that a substance shows an adverse effect in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences; (2) that it has an endocrine MOA, i.e. it alters the function(s) of the endocrine system; and (3) that the adverse effect is a consequence of the endocrine MOA [101].

Most, possibly all, of these key elements need to be supported by experimental data in intact animals, or adequately validated alternative test systems predictive of adverse effects in humans or animals (in case these are available), of which only few are requested by EU legal frameworks [102]. Due to a ban on animal testing if a substance is used only in cosmetics (i.e. personal care products), it will probably not be possible to identify a substance as an endocrine disruptor in the legal framework for cosmetics, for which *in vivo* animal testing seems to be

needed based on the WHO definition above [102]; currently no validated and accepted alternatives are available.

Recently, the RIVM published a report entitled 'Endocrine-disrupting substances in the EU legal frameworks: human health perspectives' [102]. The aim of this report was to evaluate the ED substance-related aspects of the EU legal frameworks and to analyse the challenges for the identification and regulation of ED substances by the current ED criteria with the available required information. The report gave recommendations for future research on ED substances with in benefit of the legal frameworks [102]. One of its chapters described the OECD mammalian screening and testing methods, which could detect substances with ED properties. This information is relevant to this report. Currently, as no validated alternatives are available, *in vivo* information is required, as described above. The OECD TG 407, 408, 415, 414, 426, 443 and 416 provide some, but not exhaustive, *in vivo* data on adverse effects relevant to endocrine endpoints. The inclusion of both *in vitro* and *in vivo* screening studies that detect relevant key events at the basis of ED effects, the inclusion of the extended one-generation study as a more general requirement and an update of repeated-dose studies would facilitate the identification and risk assessment of ED substances.

3.10.1 *Methyl-, ethyl- and propylparaben and the WHO definition*

Based on the available information described in this report and described by the SCCS, *in vitro* and *in vivo* mechanistic studies indicate that methyl-, ethyl- and propylparaben have a potential endocrine MOA (modulating properties of estrogenic and anti-androgenic activity). However, no clearly related adverse effects (e.g. impacted anogenital distance) have been identified in the available *in vivo* studies, or the available data showed contradicting results. No TG studies for reproductive toxicity have been performed for these parabens, either. In addition, only limited parameters for endocrine disruption have been examined in the available studies, but in general the study quality was poor and therefore these cannot be used for methyl- and ethylparaben.

For methyl- and ethylparaben, non-TG studies such as those by Oishi (2004), Vo et al. (2010) and Hoberman et al. (2008) did include some ED-related endpoints such as sperm quality, thyroid, time of vaginal opening, organ weights (thyroid, adrenal gland and ovary) and length of the estrous cycle [71, 72, 74]. These studies were described in detail in Section 3.7. The Oishi (2004) study did not show any effects on the examined ED-related endpoints up to a level of 1000 mg/kg bw/day. The effects on sperm quality and the design of the Hoberman et al. (2008) study were too limited to include these results in the hazard assessment. Although the Vo et al. (2010) study also had some limitations, the RIVM is of the opinion that the effects identified in this study must be taken seriously. Exposure to methylparaben at 1000 mg/kg bw/day resulted in a significant delay in the date of vaginal opening, a decrease in length of the estrous cycle and affected organ weight (thyroid, liver, adrenal gland and ovary) [74]. For ethylparaben at 1000 mg/kg bw/day only kidney and adrenal gland weight were affected. The effects on adrenal gland weight could also suggest an ED-mediated endpoint. This indicates that ED-related effects

might occur after exposure to methyl- and ethylparaben at the dosage level corresponding to the current NOAEL.

Additionally, *in vitro* data (described in Paragraph 3.8.2) show that an MOA has been identified in these studies showing that methyl-, ethyl- and propylparaben have estrogenic and anti-androgenic properties. Estrogenic and anti-androgenic activity and effects on adipogenesis seem to increase as a function of side chain length *in vitro*.

For propylparaben, several *in vivo* studies with relevant ED endpoints have recently been performed, but the relevance of animal studies to humans was questioned by the SCCS due to the fact that the metabolic inactivation of parabens in rats is rapid and effective compared to humans. This results in low systemic exposures to free parabens after oral exposure and may protect the rats from potential adverse effects of paraben exposure. In conclusion, the oral rat model is possibly of limited relevance to human risk assessment. More data are needed on toxicokinetics to clarify interspecies differences and provide insight into the human relevance of animal studies on propylparaben (but also on ethyl- and methylparaben). Therefore, on the one hand, more *in vivo* data are needed to identify the ED properties of parabens, while on the other hand, the relevance of the *in vivo* studies to humans is under debate.

In conclusion, parabens have an endocrine MOA and there are indications that they affect ED-related *in vivo* endpoints, but more *in vivo* studies are needed. If more information is available, a final weight-of-evidence decision can be made on whether methyl-, ethyl-, and propylparaben are endocrine disruptors.

3.11 Conclusions of previous hazard assessments

This section summarizes the conclusions of the hazard assessments performed by EFSA and the SCCS.

EFSA (2004)
In 2004, the former EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food established a full group ADI of 0–10 mg/kg bw for the sum of methyl- and ethylparaben and their sodium salts on the basis of the NOAELs of 1000 mg/kg bw/day for each substance in long-term toxicity studies and studies on sex hormones and the male reproductive organs in juvenile rats [68]. This was based on the ADI of 10 mg/kg bw/day derived from the NOAEL of 1000 mg/kg bw/day by the EU Scientific Committee for Food (SCF) in 1994 [104], and supported by Oishi (2004) [71]. ED properties were not taken into account in the derivation of this ADI. The Panel considered that propylparaben should not be included in this group ADI because propylparaben, unlike methyl- and ethylparaben, had effects on sex hormones and the male reproductive organs in juvenile rats. The Panel was unable to recommend an ADI for propylparaben because of the lack of a clear NOAEL [68]. In the EU, propylparaben can therefore not be added to food.

SCCS (2010)
The potential of butylparaben and propylparaben to modify the endocrine system is the major concern related to the use of parabens in personal care products, according to the SCCS (2010). 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, 2002b, 2004) with butylparaben and/or propylparaben [71, 72, 76, 103, 104]. They deliver contradictory results and none of them is considered to be scientifically acceptable. Therefore the SCCS cannot determine an adequate NO(A)EL for the paraben esters under consideration from these studies [33].

SCCS (2013)
A new study was performed by Gazin et al., reported in 2012 [75]. This study did not allay the concerns previously expressed by the SCCS with respect to the lack of scientifically sound data. In the SCCS (2013) opinion the relevance of the animal studies (oral exposure) to human risk assessment was questioned because of the rapid and effective metabolism of parabens in rats, which does not place in humans [66]. For these reasons, the SCCS stated that more data were needed, in particular on exposure by humans, including children, to propylparaben in cosmetic products and the toxicokinetics of propylparaben in humans. For methyl- and ethylparaben conclusions were made conservatively, and there is no argument to change those conclusions on the basis of these findings [66].

3.12 Discussion and uncertainties

There are uncertainties with regard to the hazard and toxicological reference values for methyl-, ethyl- and propylparaben (Table 11). The established NOAELs for methyl- and ethylparaben of 1000 mg/kg bw/day are derived from repeated-dose toxicity studies based on the absence of reproductive effects, though these studies might not have sufficiently taken reproductive toxicity into account. For methyl- and ethylparaben no OECD TG studies on reproductive toxicity have been performed. Therefore, especially for methylparaben, the NOAEL of 1000 mg/kg bw/day is challenged by the effects found by other studies, including possible spermatogenic effects found by Hoberman et al. (2008) [70], and the delay in the date of vaginal opening in pre-pubertal rats and the decrease in length of the estrous cycle, with a NOAEL of 250 mg/kg bw/day, identified by Vo et al. (2010) [74]. The latter study especially should be taken into account, as it is well designed and the effects on vaginal opening, estrous cycle and organ weights as found for methylparaben are relevant. Further studies for these or similar effects are needed at the same dose levels. Interestingly, no effects on the date of vaginal opening or length of the estrous cycle were identified for ethyl- and propylparaben in the Vo et al. (2010) study up to 1000 mg/kg bw/day [74]. On the other hand, a NOAEL could not be established for propylparaben, although many recent studies indicate that this paraben has a less reproductive toxic effect than methylparaben [98]. Some bodies (e.g. the SCCS) use a NOEL for butylparaben of 2 mg/kg bw/day, based on a non-TG study where juvenile rats were subcutaneously exposed for 17 days (only one dose group) [99]. The use of this NOEL for propylparaben is likely to be over-conservative in this respect. There is a

call to re-evaluate the status of propylparaben and to derive a NOAEL from all the available studies [98].

Table 11. Overview of the main discussion points and uncertainties in the hazard assessment (↑ = increases toxicological effect level, ↓ = decreases toxicological effect level)

Factor	Description	Effect on toxicological effect level
Hazard assessment		
Toxicokinetics	Metabolism of parabens into less potent metabolites is more effective in rats than in humans (especially during dermal uptake). The relevance of animal studies is therefore under debate.	↓
NOAEL methyl- and ethylparaben	Methyl- and ethylparaben share a NOAEL of 1000 mg/kg bw/day, derived from a non-TG reproductive toxicity study. Some other studies show reproductive effects at lower dose levels.	↓
NOAEL propylparaben	For propylparaben no NOAEL has been derived. An OECD TG study, however, shows no effects on reproductive toxicity up to a level of 1500 mg/kg bw/day.	↑
Developmental toxicity	For methyl- and ethylparaben developmental toxicity studies from the 1970s were evaluated. Dose levels were up to 550 mg/kg BW day. This raises the question whether these studies are reliable enough to exclude developmental effects by methyl- and ethylparaben exposure.	↓
In vitro ED properties	*In vitro* studies identify that methyl-, ethyl- and propylparaben have an endocrine mode of action (modulating properties), suggesting estrogenic and anti-androgenic activity.	?
In vivo ED properties	*In vivo* studies provide indications that ED endpoints are affected, but more studies with *in vivo* endpoints are needed.	↓
WHO definition	If more information becomes available, a weight-of evidence decision can be made on whether methyl-, ethyl-, and propylparaben are endocrine disruptors.	?

In its 2013 opinion, the SCCS did take the ED properties of parabens into account [66], and subsequent *in vitro* studies (as discussed in the present report) identify an endocrine MOA pointing to estrogenic and anti-androgenic properties of methyl-, ethyl- and propylparaben. The estrogenic and androgenic activity and effects on adipogenesis seem to increase as a function of chain length *in vitro*. However, data on adverse effects in an intact organism are still lacking. Though the findings in the *in vivo* studies performed after the SCCS opinion sometimes question the current NOAEL of 1000 mg/kg bw/day for methyl- and ethylparaben, they all have weaknesses in study design or find no ED effects. Some of the (intermediate) endpoints measured in the studies suggest an endocrine MOA for methyl-, ethyl- and propylparaben. As discussed above, no OECD TG studies on reproductive toxicity have been performed for methyl- and ethylparaben, either. As there is a clear

limitation in the available ED endpoints of *in vivo* studies, further studies on adverse health effects – especially reproductive toxicity studies, with special attention to hormone-related parameters (e.g. an OECD TG 443 study) – are warranted.

It is clear that parabens have an endocrine MOA and there are indications that they affect ED-related *in vivo* endpoints. Nevertheless, currently available information is insufficient to conclude whether methyl-, ethyl- and propylparaben have ED properties *in vivo* and can be identified as endocrine-disruptive chemicals, as conclusive *in vivo* studies are lacking.

4 Reviews and risk assessments

Reviews and risk assessments of methyl-, ethyl- and propylparaben have been conducted by several organizations within different frameworks. An overview is provided in the following sections.

4.1 EFSA opinion

The EFSA review panel used the 1000 mg/kg bw/day level for methyl- and ethylparaben, but considered more data to be necessary to determine a NO(A)EL value for propylparaben (EFSA, 2004) [68]. Propylparaben is therefore not allowed to be used as a food additive in the EU. With regard to exposure, the EFSA Panel noted that human exposure resulting from the use of parabens in food in Europe has not been adequately assessed. Some references are mentioned, such as Soni et al. (2002), who assessed exposure to parabens from all sources in the USA [105]. Total paraben exposure was estimated to be 77.5 mg/day (or 1.29 mg/kg bw/day for a 60 kg individual) [105].

4.2 SCCS opinions

History

The EU Scientific Committee on Consumer Products (SCCP) and its successor, the Scientific Committee on Consumer Safety (SCCS), have published several opinions on parabens. In 2005, the 'Extended opinion on the safety evaluation of parabens' [106] and the 'Extended opinion on parabens, underarm cosmetics and breast cancer' [107] were published. In 2006, the SCCP published a reaction to newly introduced data [108], and in 2008 a description of the outcome of an industry hearing and some additional reports [109]. The SCCS updated the opinion in 2010 after a pharmacokinetic study and a survey by the Danish authorities [33]. In 2013, another update followed, this time on propyl- and butylparaben, based on new toxicity studies [66]. To aid understanding of the SCCP/SCCS opinions over time, a chronological summary is given below.

2005

In 2005, the SCCP concluded that there was no concern for methyl- and ethylparaben [106]. A preliminary exposure assessment using a total global exposure to all cosmetic products of 17.7 g, a percutaneous absorption percentage (based on human *in vitro* studies) of 3.5%, a mean human body weight of 60 kg, a maximum permitted concentration of paraben mixture of 0.8%, and a larger body surface per body mass of children versus that of adults by a factor 1.7 resulted in an exposure estimate of 0.08 mg/kg bw/day for adults and 0.14 mg/kg bw/day for children. It should be noted that extensive biotransformation of parabens into PHBA (liver, skin) was not accounted for, and the contribution of dietary parabens (very small) was not considered. The SCCP confirmed an ADI of 10 mg/kg bw/day based on the NOAEL of 1000 mg/kg bw/day established by the SCF in 1994 [110]. ED properties were not taken into account in the derivation of the ADI. According to the SCF, based on acute, subacute and chronic toxicity studies in rats, dogs and mice, parabens had proven to be practically

non-toxic, non-carcinogenic, non-genotoxic, non-co-carcinogenic and non-teratogenic [110]. Parabens were not expected to accumulate in tissues and the ester linkage of the parabens was expected to be readily hydrolyzed [110]. Therefore, the SCCP concluded that 'methyl- and ethylparaben can be safely used up to the maximum authorized concentration as actually established (0.4%)' [106].

The SCCS failed to derive a NOAEL for propylparaben, but it suggested that the potency of propylparaben is clearly lower than the potency of butylparaben [104], and the proposed NOEL value of 2 mg/kg bw/day for butylparaben [99] can be conservatively used for propylparaben [106]. More data with regard to the reproductive and developmental toxicity of propylparaben, with special attention to the male reproductive system, was requested [106].

2006

The SCCP drafted an updated opinion because of a new reproduction toxicity study of male rats relating to the oral, dietary intake of methylparaben and butylparaben, *in vitro* metabolism studies on the dermal penetration of butylparaben and methylparaben, and an *in vitro* kinetics and metabolism study on the dermal penetration of butylparaben using full thickness human skin [108]. With regard to the new studies, it was concluded that they had too many shortcomings to be considered scientifically valid. Therefore, the conclusions of the earlier (2005) opinion [106] remained unchanged.

2010

In November 2009, the Danish authorities submitted a report entitled 'Survey and health assessment of the exposure of 2-year-olds to chemical substances in consumer products' [111], published by the Danish EPA, for evaluation by the SCCS. In addition, in December 2009 The European Cosmetic Toiletry and Perfumery Association (COLIPA) submitted a pharmacokinetic study on methyl-, propyl- and butylparaben, together with a justification for its decision not to conduct a study on human volunteers. No data for other PHBAs, salts or esters (parabens), such as iso-alkyl- or benzylparaben, were submitted. In February 2010 the Danish authorities submitted a further report by the Danish National Food Institute (DTU) called 'Update on uptake, distribution, metabolism and excretion (ADME) and endocrine-disrupting activity of parabens 2009', subsequently published as a scientific article by Boberg et al. (2010) [63].

The SCCS agreed that, on the basis of currently available *in vitro* data and *in vivo* rodent test results, the estrogenic properties displayed by parabens appeared to increase with increasing chain length. Nevertheless, the SCCS stressed that the displayed potency levels remained about 3 to 6 orders of magnitude lower than the potency of the positive controls. The SCCS considered 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 did not exceed 0.19% (of esters) [33]. This conclusion was 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 substance in the skin. The latter can be addressed only through additional human data.

With regard to methylparaben and ethylparaben, the previous opinion, stating that use at the maximum authorized concentrations can be considered safe, remained unchanged [33].

2013

On 21 March 2011, Denmark notified the EC that it had banned propyl- and butylparaben, and their isoforms and salts, in cosmetics (i.e. personal care products) for children up to 3 years of age. On 10 October 2011, the SCCS adopted a clarification of its previous opinion (2005) in the light of the Danish safeguard clause. The Committee (SCCS/1446/11) concluded that:

- For general cosmetic products containing parabens, excluding specific products for the nappy area, there was no safety concern in children.
- For leave-on cosmetic products designed for application on the nappy area and in the case of children below the age of 6 months, a risk could not be excluded in the light of both the immature metabolism and the possibly damaged skin in this area.

In March 2012, a Member State presented the results of a study on the reproductive toxicity of propylparaben to the Working Group on Cosmetic Products of the SCCS. The study showed no effects on the reproductive parameters and therefore did not confirm the conclusions of the previous studies that pointed towards negative effects on reproduction. This new study, to be published as Gazin et al. (2013) [75], did not remove the concerns previously expressed by the SCCS with respect to the lack of scientifically sound data. In the SCCS 2013 opinion the relevance of the animal studies (oral exposure) to human risk assessment was questioned because of the rapid and effective metabolism of parabens in rats, which is not the case in humans [66]. For these reasons, the SCCS stated that more data were needed, in particular on the exposure of humans, including children, to propylparaben in cosmetic products and the toxicokinetics of propylparaben in humans. For methyl- and ethylparaben, conclusions were made conservatively and there was no case for changing them on the basis of these findings [66].

The SCCS also reported that the studies suffered from data gaps and questionable data on:

- dermal uptake/absorption of parabens by human skin *in vivo* and *in vitro;*
- the dermal and systemic metabolism of parabens in humans, in particular in neonates and early infants;
- systemic exposure to free parabens as seen in biomonitoring studies, in particular the contribution of carboxylesterases to the inactivation of parabens;
- human exposure to parabens in cosmetic products.

4.3 Other reviews

4.3.1 Soni et al. (2001, 2002, 2005)

M.G. Soni and coworkers published several safety assessments on parabens: Soni et al. (2001) on propylparaben, Soni et al. (2002) on methylparaben, and Soni et al. (2005) on parabens in general [1, 105,

112]. According to these reviews, acute, subchronic and chronic studies in rodents indicated that parabens were practically non-toxic and were rapidly absorbed, metabolized and excreted. In individuals with normal skin, parabens were, for the most part, non-irritating and non-sensitizing. However, the application of compounds containing parabens to damaged or broken skin had resulted in sensitization. Genotoxicity testing of parabens in a variety of *in vitro* and *in vivo* studies primarily gave negative results. The paraben structure was not indicative of carcinogenic potential, and experimental studies supported these observations. Some animal studies had reported adverse reproductive effects of parabens. In an uterotrophic assay, methylparaben (and butylparaben) administered orally to immature rats was inactive (while subcutaneous administration of butylparaben produced a weak positive response). The ability of parabens to transactivate the estrogen receptor *in vitro* increased with alkyl group size. The detection of parabens in a small number of breast tumour tissue samples and adverse reproductive effects of parabens in animals had provoked controversy over the continued use of these substances. However, the possibility of parabens constituting an estrogenic hazard on the basis of the available studies was equivocal, since these studies failed to consider the metabolism and elimination rates of parabens, which are dose-, route- and species-dependent. In the light of the recent controversy over the estrogenic potential of parabens, Soni et al. (2005) recommended the commissioning of a reproductive toxicity study [1].

4.3.2 Cosmetic Ingredient Review (CIR)

2008

In 2008, the US Cosmetic Ingredient Review (CIR) reported on the safety of parabens as used in cosmetic products, in a scientific publication referred to as Andersen (2008) [70]. Herein, an exposure estimate was performed based on the assumption that 0.4% of a single paraben was used in a cosmetic product (0.8% for multiple parabens), although industry indicated lower use concentrations. An average daily personal care products use amount of 17.76 g for adults and 378 mg for infants was assumed. This resulted in an adult human systemic dose of 0.59 mg/kg bw/day of a single paraben (based on 50% absorption through skin) and an infant systemic dose of 0.166 mg/kg bw/day (also based on 50% absorption through skin).

The CIR Expert Panel compared estimates of exposure to parabens resulting from use of cosmetic products with a NOAEL of 1000 mg/kg bw/day based on the most statistically powerful and well conducted study of the effects of butylparaben on the male reproductive system. The margin of safety for adults ranged from 1690 for single paraben products to 840 for multiple paraben products. The margin of safety for infants ranged from approximately 6000 for single paraben products to approximately 3000 for multiple paraben products. The Expert Panel considered these margin of safety determinations to be conservative and likely to represent an overestimate of the possibility of an adverse effect (e.g. use concentrations may be lower, penetration may be less) and therefore endorsed the safety of cosmetic products in which parabens were used as a preservative [70].

The Expert Panel did consider data relating to endocrine disruption, including the results of various male reproductive toxicity and estrogenic activity studies. Reiterating the absence of human data that could identify adverse effects associated with endocrine-active chemicals, it was stated that animal studies were necessary. It was crucial that such studies be designed to maximize the likelihood that adverse effects would be detected.

2012
In 2012, the CIR panel reconsidered parabens, carefully reviewing the SCCS opinions, and concluded that there was little new information concerning parabens. It therefore reaffirmed that the use of parabens in cosmetics (i.e. personal care products) was safe.

4.3.3 Danish EPA (2013)

According to the Danish Environmental Protection Agency (EPA), concerns had been raised about the ED potential of parabens at high exposure levels [45]. Some studies in young male rats had shown adverse effects on sperm production and testosterone levels following oral exposure to parabens (propyl- and butylparaben), but other, more recent, studies with the same design did not confirm these finding even at very high doses [45]. Both the studies with positive and negative findings on reproductive toxicity had shortcomings, which made it difficult to assess and weigh the results [45]. According to the Danish EPA, parabens were known to be estrogenic *in vitro* and in uterotrophic assays *in vivo*, and estrogenicity appeared to increase with side chain length. Therefore, methyl-, ethyl-, propyl- and butylparaben were on the EU list of potential endocrine disruptors in category 1 (for human exposure). Isopropyl- and isobutylparaben were not on the EU list. Category 1 substances are substances for which ED activity has been documented in at least one study of a living organism and are given the highest priority for further study. The Danish EPA concluded that the best method of evaluating parabens for their ED potential and their kinetics was still not agreed upon [45]. In addition, discussions on the most relevant NOEL/NOAEL and the dermal absorption values had not yet come to a conclusion. Thus, considering the ED effects, a final risk assessment still awaits which NOEL/NOAEL and which dermal absorption fraction to be used, and further identification of the overall exposure [45]. Few studies were available on the combined exposure to several parabens from several products [45]. A new study on reproductive toxicity was currently being assessed by the SCCS.

4.3.4 NICNAS (2015)

The Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) performed a human health Tier 2 assessment for parabens in 2015 [69]. According to this, current risk management measures were considered adequate to protect public and workers' health and safety, provided that all requirements were met under workplace health and safety and poisons legislation, as adopted by the relevant state or territory [69]. The available data did not indicate any risks associated with exposure to the chemicals in this group [69]. The chemicals had been shown to have weak estrogenic activity, but there were no established adverse outcome pathways for this effect. The assessment concluded that, should further information on adverse

outcome pathways in mammals associated with weak estrogenic activity become available, further assessment of these chemicals at Tier 3 could be required [69].

4.4 Summarizing the reviews and risk assessments

When the SCF considered the group of parabens together in 1994, an overall NOAEL for methyl- and ethylparaben of 1000 mg/kg bw/day was established, resulting in an ADI of 0–10 mg/kg bw/day. CIR established an overall NOEAL for all parabens of 1000 mg/kg bw/day in 2008. The SCCP (2005) also concluded on a NOAEL of 1000 mg/kg bw/day for methyl- and ethylparaben, but failed to indicate a clear NOAEL for propylparaben. Pragmatically, a NOEL of 2 mg/kg bw/day was proposed based on a non-guideline study by Fisher et al. (1999) in which juvenile rats were subcutaneously exposed for 17 days (only one dose group) to butylparaben, which has a higher toxicity than propylparaben [99]; very conservatively this value is also used for propylparaben.

It was concluded by most reviews that there was no risk to human health from the use of methyl- and ethylparaben in food, and no risk from the use of methyl-, ethyl- and propylparaben in personal care products. However, uncertainty existed and more information was said to be needed on the (dermal) absorption and metabolism of parabens, as well as on the reproductive effects of parabens.

Comparing the exposure estimates, and taking into account that these had mostly been performed in a rough or worst-case manner, it was concluded that there was no risk from the use of methyl- and ethylparaben in personal care products (SCCS, CIR) or food (EFSA) or both (NICNAS, Soni et al.). Because of the lack of a clear NOAEL for propylparaben, the substance was not allowed to be used in food (EFSA). The SCCS regards propylparaben as safe for general cosmetic use, excluding specific products for the nappy area for young children.

With respect to ED properties, though discussed by the SCCS, these were not taken into account in the setting of NOAELs or the ADI for methyl- and ethylparaben. Most risk assessments mention endocrine disruption as a point of discussion. In several risk assessments, it was noted that more studies were needed and that further assessments would be required when more information on these effects became available.

4.5 Discussion and uncertainties

Existing risk assessments do not prompt any concern that the use of parabens poses health risks. However, with respect to dermal exposure, a final risk assessment still awaits a proper definition of the dermal absorption fraction to use and clarification of the uncertainty about interspecies difference (rat–human) with regard to toxicokinetics. It is generally agreed that an update will be needed when the required studies on reproduction effects become available.

There is uncertainty with regard to the toxicological reference values for methyl-, ethyl- and propylparaben. The NOAEL values for methyl- and ethylparaben of 1000 mg/kg bw/day could be too high. For propylparaben no official NOAEL has been established. Very conservatively, a NOEL of

2 mg/kg bw/day from butylparaben is sometimes used, though the results from many studies indicate that a NOEAL for propylparaben should be much higher. As a result, looking at these numbers for methylparaben and propylparaben, the MOS between the present worst-case aggregate internal exposure estimate of ~3 and ~1.2 mg/kg bw/day, respectively, might not be protective enough. However, it is expected that a refinement of the exposure assessment could sufficiently increase this MOS.

Studies have reported there is a difference in toxicokinetics between rats and humans in terms of metabolism. There are indications that rats metabolize parabens much more effectively than humans, at least after dermal exposure (because of differences in metabolism in the skin). This could be relevant, especially with regard to the effects of dermal exposure to specific personal care products, or medicinal products applied to the skin. Where first pass metabolism after oral uptake in rats (from which the NOAELs are derived) is very effective, but after dermal exposure in humans is not, this could lead to a relatively higher internal exposure in humans, and could influence the MOS considered sufficient between an aggregate exposure estimate (including dermal exposure) and the current NOAELs.

In the present report, the exposure estimates for the individual parabens via different sources were added up (aggregated). For a cumulative exposure estimate, these exposure estimates for all three parabens would be summed as well, but this is not being performed. Though results from *in vitro* studies on ED effects suggest some similar MOAs, there are too many uncertainties with regard to similar toxicological endpoints *in vivo* for cumulative exposure estimation and risk assessment to be legitimized.

5 Legal frameworks

For methyl-, ethyl- and propylparaben, and other parabens, some use restrictions are laid down in different regulations, such as the Cosmetics Regulation and the Food Additives Regulation. More information can be found in the following sections, as well as in Appendix 9.3.

5.1 Cosmetics Regulation

Based on SCCS opinions (see Section 4.3), the regulation of the use of the different parabens in personal care products is regulated by the Cosmetics Regulation 2009 [17], and revised in 2014 [113]. The SCCS confirms that methyl- and ethylparaben are safe at the maximum authorized concentrations, which are 0.4% for single esters and 0.8% for a mixture of esters. Since 16 April 2015, parabens have been 'regulated as preservatives in entry 12 of Annex V to Regulation (EC) No. 1223/2009 on cosmetic products under the denomination p-hydroxybenzoic acid (PHBA) and its salts and esters, with a maximum concentration of 0.4% for single ester and 0.8% for mixtures of esters.' (see Table A3 in Appendix 9.3).

With regard to propylparaben (and butylparaben), the SCCS concludes that their use as preservatives in finished cosmetic products is safe to the consumer, as long as the sum of their individual concentrations does not exceed 0.19% (as esters). This figure was taken up into entry 12a of Annex V to Regulation (EC) No. 1223/2009: 'In the absence of any indication to the contrary from the SCCS, the maximum concentration of 0.8% for the sum of *all* parabens contained in a cosmetic product already foreseen by entry 12 of Annex V to Regulation (EC) No. 1223/2009 should be maintained'. However, the SCCS maintained that concerning propyl- and butylparaben present in leave-on cosmetic products designed for application on the nappy area of children below the age of 6 months, a risk could not be excluded in the light of both the immature metabolism of such children and the possibility of damaged skin in the nappy area. Based on a worst-case assumption of exposure, safety concerns might be raised (see Appendix 9.3). And therefore the Cosmetics Regulation states:

In light of the concerns raised by the SCCS regarding the use of parabens in leave-on cosmetic products designed for application on the nappy area of children under the age of six months, and for practical reasons linked to the fact that products for infants are usually marketed for children under three years, butylparaben and propylparaben should be prohibited in leave-on cosmetic products designed for application on the nappy area of children below three years.

Within the EU the use of the following parabens in cosmetic products is prohibited due to the lack of data necessary for reassessment: isopropyl-, isobutyl-, phenyl-, benzyl- and pentylparaben (Annex II of the Cosmetics Regulation, see Table A4 in Appendix 9.3).

5.2 Food

5.2.1 Food Additive Directive Regulation EC No. 1333/2008

Methyl- and ethylparaben, as E218 and E214, respectively, and their sodium salts (E219 and E215, respectively) are approved for use as preservatives in food according to Annex II to Regulation (EC) No. 1333/2008. For this group of four preservatives, maximum permitted levels (MPLs) have been set in eight food categories (Table 12). The additives may be added individually or in combination. For four food categories, the MPL is applicable to a group of food additives that also includes sorbic acid – sorbates (E200, E202 and E203) and/or benzoic acid – and benzoates (E210, E211, E212 and E213) (Table 10). MPLs range from 300 to 1,000 mg/kg. For surface treatment of dried meat products, the four parabens may be added up to *quantum satis*[2]. No other parabens are approved for use in food according to Annex II to Regulation (EC) No. 1333/2008. Propylparaben is not allowed to be used in food as a preservative in the EU.

Table 12. Maximum permitted levels (MPLs) of methylparaben (E216), ethylparaben (E218) and their respective sodium salts (E217 and E219) in foods according to Annex II to Regulation (EC) No. 1333/2008

Food category number	Food categories	E number/ group	Restrictions/ exceptions	MPL (mg/kg or mg/L as appropriate)[1]
05.2	Other confectionery including breath- freshening microsweets	E214-219[2]	Except candied, crystallized or glacé fruit and vegetables	300
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	E214-219	-	300
08.2.1	Non-heat-treated processed meat	E200-203, E210-213, E214-219[3]	Only surface treatment of dried meat products	*Quantum satis*[4]
08.2.2	Heat-treated processed meat	E200-203, E214-219[5]	Only pâté	1000[4]
08.2.3	Casings and coatings and decorations for meat	E200-203, E214-219[4]	Only jelly coatings of meat products (cooked, cured or dried)	1000[4]
11.4.1	Table-top sweeteners in liquid form	E200-219[2]	Only if the water content > 75%	500[4]
15.1	Potato-, cereal-, flour- or starch-based snacks	E214-219	-	300
15.2	Processed nuts	E214-219	Only processed nuts	300

[1] The additives may be added individually or in combination.
[2] Including methyl- (E218) and ethylparaben (E214), and their sodium salts (E219 and E215, respectively).
[3] Including sorbic acid – sorbates (E200, E202 and E203) and benzoic acid – and benzoates (E210, E211, E212 and E213).
[4] The maximum level is applicable to the sum and the levels are expressed as the free acid.
[5] Including sorbic acid – sorbates (E200, E202 and E203).

[2] Quantum satis shall mean that no maximum numerical level is specified and substances shall be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided the consumer is not misled (Regulation (EC) No. 1333/2008, article 3).

5.2.2 Food contact material (Regulation (EC) No. 10/2011)

Methyl-, ethyl- and propylparaben (as PHBA, methyl-, ethyl- or propyl-ester) are allowed for use in the manufacture of plastic materials and articles intended to come into contact with food (Food Contact Materials Commission Regulation (EU) No. 10/2011). The amount added should not result in a migration of these parabens to foods in quantities that exceed the generic Specific Migration Limit (SML) of 60 mg/kg food. With this SML, the exposure remains below the temporary group ADI of 10 mg/kg bw/day for the sum of methyl-, ethyl- and propylparaben, as set by the SCF in 1994 [110].

As stated in Paragraph 5.2.1, methyl- and ethylparaben can also be directly added to food as a preservative according to Regulation (EC) No. 1333/2008. As stated in Commission Regulation (EU) No. 10/2011, the amount migrating from plastic food contact material (FCM) into food should 'not exceed the restrictions provided for in Regulation (EC) No. 1333/2008 […] for foods [whose] use is authorised as [a] food additive'. Apart from methyl- and ethylparaben, propylparaben (as PHBA or (iso)propyl ester) is also allowed in plastic FCM with an SML of 60 mg/kg food. However, for FCM an overall migration limit (sum of all substances migrating from the FCM) applies, which also equals 60 mg/kg food.

5.3 REACH

5.3.1 Registration dossier

Several parabens have been preregistered or registered within the REACH legislation. The ECHA website offers information on manufacture and use.

Methylparaben

Methylparaben (CAS 99-76-3) is manufactured and/or imported in the European Economic Area (1000–10,000 tonnes per year). According to registration data, it is used in the following products: cosmetics and personal care products, adhesives and sealants, biocides (e.g. disinfectants, pest control products) and perfumes and fragrances. This substance has an industrial use resulting in manufacture of another substance (use of intermediates). However, the ECHA has no registered data on the types of manufacturing in which this substance is used, or indicating whether or into which articles the substance might have been processed. The overall Derived No Effect Level (DNEL) by the registrant for chronic oral effects was established at 1.04 mg/kg bw/day. The point of departure is a NOAEL of 250 mg/kg bw/day based on a 28-day rat study. At 1000 mg/kg bw/day one male and one female were sacrificed on day 14 and 24, respectively, after showing several clinical signs of ill health. Microscopic examination revealed minimal/slight erosions in the stomach, correlating with the irregular surface recorded at necropsy, slight red pulp atrophy of the spleen and slight/moderate lymphoid atrophy of the thymus, correlating with the reduced size recorded at necropsy. Since these deaths occurred in the highest dose group, and there were corresponding clinical signs in the surviving animals at 1000 mg/kg bw/day, a relation to treatment with the test substance cannot be excluded. The DNEL derived by the registrant was derived using an extrapolation factor of 240 (not further specified) [114].

Ethylparaben
Ethylparaben (CAS 120-47-8) is manufactured and/or imported in the European Economic Area (100–1000 tonnes per year). According to registration data, it is used in the following products: cosmetics and personal care products, and perfumes and fragrances. The overall DNEL by the registrant for oral chronic effects was established at 15 mg/kg bw/day. The point of departure is a NOAEL based on a repeated dose toxicity study in rats of 1200 mg/kg bw/day. The DNEL was derived using an extrapolation factor of 80 (not further specified by the registrant) [115].

Propylparaben
Propylparaben (CAS 94-13-3) is manufactured and/or imported in the European Economic Area (100–1000 tonnes per year). According to registration data, it is used in the following products: cosmetics and personal care products, and perfumes and fragrances. The overall DNEL by the registrant for oral chronic effects was established at 4.08 mg/kg bw/day. The point of departure is a NOAEL based on a repeated dose toxicity study in rats of 980 mg/kg bw/day. The DNEL was derived using an extrapolation factor of 240 (not further specified by the registrant) [116].

5.3.2 Community Rolling Action Plan (CoRAP)

Methyl-, ethyl- and propylparaben have been placed on the Community Rolling Action Plan (CoRAP), and therefore are being evaluated or will be evaluated over the coming years by ECHA.

Methylparaben
In 2013, methylparaben was placed by Member State France on the CoRAP for 2014. The initial justification was concerns for consumer use, carcinogenic, mutagenic or reprotoxic (CMR) properties, high exposure by sensitive populations, high (aggregated) tonnage, potential ED properties and wide dispersive use. Further information was requested on toxicological properties, exposure and use to clarify the risk [117]. The current status of the substance is: further information requested, including on environmental ED properties [118].

Ethylparaben
In 2017, ethylparaben was placed by member State Germany on the CoRAP for 2019. The initial cause for concern was that it is a potential endocrine disruptor. The evaluation has not yet started, but the preliminary indication is that information may be requested on ED potential [119]. Based on a preliminary evaluation of the data related to the ED properties of ethylparaben, chronic studies using aquatic vertebrates could be requested to clarify estrogenic effects in the environment. A detailed evaluation of the available data may lead to further information requirements [119].

Propylparaben
In 2015, propylparaben was placed by member State Belgium on the CoRAP for the same year. The initial grounds were suspected reproduction toxicity, being a potential endocrine disruptor, consumer use, environmental exposure, exposure of sensitive populations and wide dispersive use. The current status of the substance is: further information requested, namely an extended one-generation reproductive

toxicity study (OECD TG 443), a Daphnia magna reproduction test (OECD TG 211) and a fish sexual development test (OECD TG 234) [120].

5.4 Specific legislation

On 15 March 2011, Denmark introduced a national partial ban on certain parabens in cosmetics (i.e. personal care products) intended for children. This includes a ban on propyl- and butylparaben and their isoforms and salts in personal care products for children less than 3 years (http://eng.mst.dk/media/mst/Attachments/Engelskparabenbekendtgrelse.pdf).

6 Conclusions and recommendations for further research

6.1 Conclusions

With regard to consumer exposure, we performed an inventory and discussion of estimates of exposure by consumers to parabens via personal care products, food and medicinal products, taking into account actual exposure scenarios at certain life stages (i.e. childhood) based on available information. The following can be concluded:

- Methyl-, ethyl- and propylparaben are present in personal care products, in other consumer products, in food and in medicinal products. In food, the presence can originate from the addition of methyl- and ethylparaben (or their sodium salts) as a preservative (food additive E218/E219 and E214/E215; propylparaben is not allowed as a food additive in the EU), from food packaging material, and from natural sources.
- Exposure estimations from literature are provided, and show a wide variety with regard to types of study, methodology, level of detail and assumptions, as well as in the resulting estimates themselves (see Tables 7 and 8). An overview of the main sources of uncertainty in the risk assessment of parabens is given in Section 2.8.
- A worst-case aggregate internal exposure assessment for methylparaben, summed from the results of different studies, taking into account personal care products, food and medicinal products, resulted in internal exposure estimates of ~3.1 mg/kg bw/day for adults (Table 7) and ~3.3 mg/kg bw/day for children (Table 8). For propylparaben a similar summation resulted in an internal exposure estimate of 1.2 mg/kg bw/day for both adults and children. The internal exposure estimate for ethylparaben, which took into account cosmetic and personal care products and food, was 0.2 mg/kg bw/day for both adults and children (Tables 7 and 8). For ethylparaben, no estimation of exposure via medicinal products could be made because of a lack of information.
- Medicinal products contributed most to the aggregated estimates of exposure for methyl- and propylparaben, although exposure via medicinal products is estimated very roughly and is likely more worst-case than the estimated exposure via personal care products and food. This is because there are no relevant studies that combine product concentrations of these parabens in medicinal products with use patterns. This consequently diminishes the accuracy of these aggregate exposure values. The contribution of medicinal products to the exposure value for ethylparaben could not be estimated, as there was very little information available.
- Estimated exposure via food is very small (< 1%) compared with personal care products (ethylparaben) and medicinal products (methyl- and propylparaben).
- The worst-case character of the aggregate estimates is supported by several biomonitoring studies, where 95th percentile values were back-calculated from urine metabolite concentrations to internal exposure or daily intake levels (see Paragraph 2.7.5).

- In addition to the lack of data with regard to medicinal products, information on actual levels of parabens in non-food consumer products in the Netherlands, or Europe, is currently missing.

With regard to hazard, we aimed to describe paraben toxicity including their possible endocrine-disrupting (ED) effects, with reference to the current toxicological reference values. The following can be concluded:

- Studies demonstrate that metabolism of parabens is more effective in rats than in humans, especially during dermal uptake. Therefore, the relevance of rat studies to the assessment of human hazard is under debate. More data are needed on toxicokinetics to clarify interspecies differences and provide insight into the human relevance of animal studies for propylparaben and to a lesser extent for ethyl- and methylparaben.
- Due to differences in dermal absorption of parabens between rats and humans, including in metabolism during absorption, good human dermal absorption data for calculating precise internal exposure via the skin are missing. Therefore, potentially over-conservative values are used in the different calculations of exposure via the dermal route (which only concerns exposure from personal care products in the present report).
- Methyl-, ethyl- and propylparaben have low acute toxicity and low repeated dose toxicity, do not irritate the skin in individuals with normal skin, are not skin sensitizers, might slightly irritate the eye, and are not genotoxic or carcinogenic, according to the available studies.
- No effects were observed for methyl- and ethylparaben in OECD TG 414 developmental toxicity studies performed in the 1970s with dosages up to 550 mg/kg bw/day. Propylparaben does not demonstrate developmental toxicity, either, in a modern official guideline study for reproduction/developmental screening toxicity (OECD TG 422) with high doses.
- No OECD TG studies have been performed on the reproductive toxicity of methyl- and ethylparaben. Propylparaben has been tested in a recent OECD TG study of reproduction/developmental screening toxicity (OECD TG 422), in which no effects were detected. Several non-guideline studies have been performed. A NOAEL of 1000 mg/kg bw/day has been derived for methyl- and ethylparaben (Oishi, 2004). Some studies indicate that adverse effects (decrease in length of the estrous cycle, delay in vaginal opening and increased adrenal gland weight) occur at the same dose level at which the current NOAEL is set, but more studies are needed to confirm this.
- *In vitro* and *in vivo* studies indicate that methyl-, ethyl- and propylparaben have an endocrine MOA (modulating properties), suggesting estrogenic and anti-androgenic activity.
- *In vivo* endpoints suggest ED-related properties, but there are contradictory results from different *in vivo* studies and limitations in available ED endpoints in these studies. This warrants further studies on adverse health effects, especially reproductive toxicity studies with special attention to hormone-related parameters (preferably in official guideline studies, e.g. an OECD TG 443 study).

- In conclusion, parabens have an endocrine MOA and there are many indications from *in vivo* studies that these parabens affect ED-related *in vivo* endpoints, but more *in vivo* studies on adversity and potency are needed to be able to make a final weight-of-evidence decision on whether methyl-, ethyl-, and propylparaben are endocrine disruptors based on the information available.
- ED properties were not taken into account by EFSA and the SCCS in setting the NOAEL of methyl- and ethylparaben, although the SCCS did discuss them during its risk assessment. The SCCS also takes into account propylparaben, for which no NO(A)EL could be set, but very conservatively uses the NOEL for butylparaben. Some toxicological studies have indicated a potential NOAEL for propylparaben at a higher level, though there are doubts about rat studies representing the human situation with regard to toxicokinetics. Some studies indicate that the NOAELs of methyl- and ethylparaben might be set too high because of possible reprotoxic and/or developmental toxic effects.

With regard to risk assessment the following can be concluded (including a statement about the risk related to the exposure estimates in the present study:

- From four repeated-dose toxicity studies for methyl- and ethylparaben a NOAEL of 1000 mg/kg bw/day was established by EFSA and the SCCS on the basis of the absence of reproductive effects. For propylparaben more data were considered necessary to determine a NO(A)EL. In the interim, the SCCS proposes that the NOEL for butylparaben of 2 mg/kg bw/day can be very conservatively used for propylparaben.
- **Methylparaben**: For methylparaben there seems to be a MOS > 100 between the present worst-case aggregate internal exposure estimate of ~3.1 mg/kg bw/day for adults and ~3.3 mg/kg bw/day for children and the established NOAEL of 1000 mg/kg bw/day. However, there are indications that the current NOAEL does not adequately take ED-related effects into account. In case of a lower NOAEL (e.g. 250 mg/kg bw/day), the MOS would be < 100. However, based on the character of the exposure assessment, it is expected that a refinement would sufficiently increase this MOS .
- **Ethylparaben**: For ethylparaben there is a sufficient MOS between the estimated aggregate internal exposure of 0.2 mg/kg bw/day for both adults and children (which, however, excludes a potential contribution by medicinal products) and the NOAEL of 1000 mg/kg bw/day.
- **Propylparaben**: There is no established NOAEL with which the estimated worst-case aggregate internal exposure estimate of 1.2 mg/kg bw/day for both adults and children given in this report can be compared. Pragmatically, several effect levels from different studies are used for risk assessment purposes. These range from 2 to 1000 mg/kg bw/day. Depending on the effect level considered, the MOS could be insufficient, but as with exposure to methylparaben, it is expected that a refinement of the exposure assessment would sufficiently increase this MOS.

- The MOS between the actual exposure and the (possibly lower) NOAELs for methyl and ethylparaben, and the potential NOAEL for propylparaben show no cause for concern. Better information (on reproduction and developmental toxicity, as well as toxicokinetics) with regard to the hazard of methyl-, ethyl- and propylparaben, as well as a refined exposure assessment, could help to confirm this indicative conclusion.

6.2 Recommendations for further research

Taking into account the uncertainties present in the available data and methodology with regard to exposure, hazard and risk assessment, the following issues should be addressed in (future) studies:

- With regard to the uncertainties in the NOAEL of methyl- and ethylparaben, and the lack of an established toxicological effect level for propylparaben, the a more realistic exposure estimation might be necessary with regard to exposure via non-food consumer products other than personal care products, and especially medicinal products, in order to be able to refine the exposure assessment.
- Better information on (toxico)kinetics, including dermal absorption and metabolic interspecies differences, is required to help set more realistic toxicological reference values, as metabolic inactivation is likely more effective in rats than in humans, which could affect the relevance of animal studies.
- In order to derive an actual level of exposure with which to compare the calculated aggregate exposure estimate, more realistic biomonitoring data representative of the current situation in the Netherlands or Europe are needed – provided the toxicokinetics of parabens will be further clarified; this will either confirm the existing exposure assessments or provide an alternative.
- When the final EFSA-ECHA guidance becomes available, additional *in vivo* data on developmental/reproductive toxicity (e.g. according to OECD TG 443) with special attention to hormone-related parameters might be needed in order to facilitate a weight-of-evidence decision as to whether methyl-, ethyl- and propylparaben are endocrine disruptors.
- Further studies are needed into the toxicological mechanism of the parabens in order to contribute to the decision whether a cumulative exposure assessment is justified.

7 Acknowledgements

The authors would like to thank Coen Graven, Betty Hakkert, Joke Herremans and Aldert Piersma for their critical reading and reviewing of the draft report.

8 References

1. Soni, M.G., I.G. Carabin, and G.A. Burdock, *Safety assessment of esters of p-hydroxybenzoic acid (parabens).* Food Chem Toxicol, 2005. **43**(7): pp. 985–1015.
2. Csiszar, S., et al., *Stochastic modeling of near-field exposure to parabens in personal care products.* Journal of Exposure Science and Environmental Epidemiology, 2017. **27**: pp. 152–9.
3. Guo, Y., and K. Kannan, *A survey of phthalates and parabens in personal care products from the United States and its implications for human exposure.* Environmental Science & Technology, 2013. **47**(24): pp. 14442–9.
4. Guo, Y., L. Wang, and K. Kannan, *Phthalates and parabens in personal care products from China: concentrations and human exposure.* Archives of Environmental Contamination & Toxicology, 2014. **66**(1): pp. 113–9.
5. Cowan-Ellsberry, C.E., and S.H. Robison, *Refining aggregate exposure: example using parabens.* Regul Toxicol Pharmacol, 2009. **55**(3): pp. 321–9.
6. Gosens, I., et al., *Aggregate exposure approaches for parabens in personal care products: a case assessment for children between 0 and 3 years old.* Journal of Exposure Science & Environmental Epidemiology, 2014. **24**(2): pp. 208–14.
7. Gosens, I., et al., *Aggregate exposure assessment of chemicals in consumer products. Exposure to parabens in cosmetics in children as a case study.* 2011, RIVM.
8. Danish EPA, *Survey and health and environmental assessment of preservatives in cosmetic products*, in *Survey of chemical substances in consumer products* 2015, The Danish Environmental Protection Agency: Copenhagen.
9. Loretz, L., et al., *Exposure data for personal care products: Hairspray, spray perfume, liquid foundation, shampoo, body wash, and solid antiperspirant.* Food and Chemical Toxicology, 2006. **44**: pp. 2008–18.
10. Loretz, L.J., et al., *Exposure data for cosmetic products: Facial cleanser, hair conditioner, and eye shadow.* Food and Chemical Toxicology, 2008. **46**: pp. 1516–24.
11. Loretz, L.J., et al., *Exposure data for cosmetic products: lipstick, body lotion, and face cream.* Food and Chemical Toxicology, 2005. **43**: pp. 279–91.
12. USEPA, *Exposure Factors Handbook*. 2011, U.S. Environmental Protection Agency: Washington, DC.
13. Garcia-Hidalgo, E., et al., *Use-patterns of personal care and household cleaning products in Switzerland.* Food & Chemical Toxicology, 2017. **99**: pp. 24–39.
14. SCCS, *The SCCS's Notes Of Guidance For The Testing Of Cosmetic Substances And Their Safety Evaluation 8th Revision*. 2012, Scientific Committee on Consumer Safety.
15. SCCNFP, *Notes of guidance for testing of cosmetic ingredients for their safety evaluation*. 2000, Scientific Committee On Cosmetic Products And Non-Food Products Intended For Consumers.

16. SCCS, *SCCS Notes of guidance for the testing of cosmetic ingredients and their safety evaluation 9th revision, 29 September 2015, SCCS/1564/15, revision of 25 April 2016*. 2016.
17. EC, *Regulation (EC) No 1223/2009 of the European Parliament And of the Council of 30 November 2009 on Cosmetic Products.* Official Journal of the European Union, 2009.
18. Baranowska, I., et al., *Determination of preservatives in cosmetics, cleaning agents and pharmaceuticals using fast liquid chromatography.* J Chromatogr Sci, 2014. **52**(1): pp. 88–94.
19. VWA, *Cosmetische producten voor kinderen: Inventarisatie van de markt en de veiligheidsborging door producenten en importeurs.* 2007.
20. NVWA, *Babylotions en -cremes. Onderzoek aanwezigheid parabenen.* 2016.
21. Rastogi, S.C., et al., *Contents of methyl-, ethyl-, propyl-, butyl-, and benzylparaben in cosmetic products.* Contact Dermatitis, 1995. **32**: pp. 28–30.
22. EFSA, *Guidance on dermal absorption.* EFSA Journal, 2017. **15**(6):4873.
23. Harville, H.M., R. Voorman, and J.J. Prusakiewicz, *Comparison of paraben stability in human and rat skin.* Drug Metab Lett, 2007. **1**(1): pp. 17–21.
24. Hussein, S.E., et al., *Assessment of principal parabens used in cosmetics after their passage through human epidermis-dermis layers (ex-vivo study).* Exp. Dermatol., 2007. **16**: pp. 830–6.
25. Ishiwatari, S., et al., *Effects of methyl paraben on skin keratinocytes.* J. Appl. Toxicol. , 2007. **27**: pp. 1–9.
26. Janjua, N.R., et al., *Systemic uptake of diethyl phthalate, dibutyl phthalate, and butyl paraben following whole-body topical application and reproductive and thyroid hormone levels in humans.* Environ Sci Technol, 2007. **41**(15): pp. 5564–70.
27. Hall, B., et al., *European consumer exposure to cosmetic products, a framework for conducting population exposure assessments.* Food and Chemical Toxicology, 2007. **45**: pp. 2097–108.
28. McNamara, C., et al., *Probabilistic modelling of European consumer exposure to cosmetic products.* Food and Chemical Toxicology, 2007. **45**: pp. 2086–96.
29. Ford, R.A., *The human safety of the polycyclic musks AHTN and HHCB in fragrances— A review.* Deutsche Lebensmittel-Rundschau, 1998. **94**(8): pp. 268-75.
30. OECD, *Occupational and consumer exposure assessment*. 1993, Organization for Economic Cooperation and Development.
31. Cross, S.E. and M.S. Roberts, *The effect of occlusion on epidermal penetration of parabens from a commercial allergy test ointment, acetone and ethanol vehicles.* J Invest Dermatol, 2000. **115**(5): pp. 914–8.
32. Jewell, C., et al., *Hydrolysis of a series of parabens by skin microsomes and cytosol from human and minipigs and in whole skin in short-term culture.* Toxicol Appl Pharmacol, 2007. **225**(2): pp. 221–8.
33. SCCS, *Opinion on parabens.* 2010.

34. Mancini, F.R., et al., *Dietary exposure to benzoates (E210-E213), parabens (E214-E219), nitrites (E249-E250), nitrates (E251-E252), BHA (E320), BHT (E321) and aspartame (E951) in children less than 3 years old in France.* Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 2015. **32**(3): pp. 293–306.
35. Liao, C., L. Chen, and K. Kannan, *Occurrence of parabens in foodstuffs from China and its implications for human dietary exposure.* Environment International, 2013. **57–58**: pp. 68–74.
36. Liao, C., F. Liu, and K. Kannan, *Occurrence of and dietary exposure to parabens in foodstuffs from the United States.* Environmental Science & Technology, 2013. **47**(8): pp. 3918–25.
37. SCOOP, *Report from the Commission on dietary food additive intake in the European Union. Scientific Cooperation (SCOOP) Task Report.* http://ec.europa.eu/food/fs/scoop/index_en.html. 2001, European Commission, DG Health and Consumers: Brussels.
38. EMA, *Reflection paper on the use of methyl- and propylparaben as excipients in human medicinal products for oral use*. 2015.
39. Rowe, R.C., P.J. Sheskey, and M.E. Quinn, *Handbook of pharmaceutical excipients. Sixth edition.* 2009: Pharmaceutical press and American Pharmacists Association.
40. Moreta, C., M.T. Tena, and K. Kannan, *Analytical method for the determination and a survey of parabens and their derivatives in pharmaceuticals.* Environ Res, 2015. **142**: pp. 452–60.
41. Bouwman-Boer, Y., V. Fenton-May, and P. Le Brun, *Practical pharmaceutics.* 2015: KNMP and Springer International Publishing Switzerland.
42. Ma, W.L., et al., *A survey of parabens in commercial pharmaceuticals from China and its implications for human exposure.* Environ Int, 2016. **95**: pp. 30–5.
43. Dodge, L.E., et al., *Medications as a source of paraben exposure.* Reproductive Toxicology, 2015. **52**: pp. 93–100.
44. Gabb, H.A., and C. Blake, *An informatics approach to evaluating combined chemical exposures from consumer products: a case study of asthma-associated chemicals and potential endocrine disruptors.* Environ Health Perspect, 2016. **124**(8): pp. 1155–65.
45. Danish EPA, *Survey of parabens. Part of the LOUS -review.* 2013.
46. Berger, E., et al., *Effect-directed identification of endocrine disruptors in plastic baby teethers.* J Appl Toxicol, 2015. **35**(11): pp. 1254–61.
47. Asimakopoulos, A.G., M. Elangovan, and K. Kannan, *Migration of parabens, bisphenols, benzophenone-type UV filters, triclosan, and triclocarban from teethers and its implications for infant exposure.* Environ Sci Technol, 2016. **50**(24): pp. 13539–47.
48. Liao, C., and K. Kannan, *Concentrations and composition profiles of parabens in currency bills and paper products including sanitary wipes.* Science of the Total Environment, 2014. **475**: pp. 8–15.
49. Wang, L., et al., *Occurrence and human exposure of p-hydroxybenzoic acid esters (parabens), bisphenol A diglycidyl ether (BADGE), and their hydrolysis products in indoor dust from the United States and three East Asian countries.* Environ Sci Technol, 2012. **46**(21): pp. 11584–93.

50. Mitro, S.D., et al., *Consumer Product Chemicals in Indoor Dust: A Quantitative Meta-analysis of U.S. Studies.* Environ Sci Technol, 2016. **50**(19): pp. 10661–72.
51. Ye, X., et al., *Parabens as urinary biomarkers of exposure in humans.* Environ Health Perspect, 2006. **114**(12): pp. 1843–6.
52. Guo, J., et al., *Urinary paraben concentrations and their associations with anthropometric measures of children aged 3 years.* Environ Pollut, 2017. **222**: pp. 307–14.
53. Kang, H.S., et al., *Urinary concentrations of parabens and their association with demographic factors: A population-based cross-sectional study.* Environ Res, 2016. **146**: pp. 245–51.
54. Ma, W.L., et al., *Urinary concentrations of parabens in Chinese young adults: implications for human exposure.* Archives of Environmental Contamination & Toxicology, 2013. **65**(3): pp. 611–8.
55. Moos, R.K., et al., *Daily intake and hazard index of parabens based upon 24 h urine samples of the German Environmental Specimen Bank from 1995 to 2012.* J Expo Sci Environ Epidemiol, 2016.
56. Kang, S., et al., *Urinary paraben concentrations among pregnant women and their matching newborn infants of Korea, and the association with oxidative stress biomarkers.* Science of the Total Environment, 2013. **461–462**: pp. 214–21.
57. Moos, R.K., et al., *Parabens in 24 h urine samples of the German Environmental Specimen Bank from 1995 to 2012.* Int J Hyg Environ Health, 2015. **218**(7): pp. 666–74.
58. Biesterbos, J.W., et al., *Usage patterns of personal care products: important factors for exposure assessment.* Food Chem Toxicol, 2013. **55**: pp. 8–17.
59. Ficheux, A.S., et al., *Consumption of cosmetic products by the French population second part: Amount data.* Food Chem Toxicol, 2016. **90**: pp. 130–41.
60. Ficheux, A.S., et al., *Consumption of cosmetic products by the French population. First part: frequency data.* Food Chem Toxicol, 2015. **78**: pp. 159–69.
61. Delmaar, C., et al., *Validation of an aggregate exposure model for substances in consumer products: a case study of diethyl phthalate in personal care products.* J Expo Sci Environ Epidemiol, 2015. **25**(3): pp. 317–23.
62. Dudzina, T., et al., *The probabilistic aggregate consumer exposure model (PACEM): validation and comparison to a lower-tier assessment for the cyclic siloxane D5.* Environ Int, 2015. **79**: pp. 8–16.
63. Boberg, J., et al., *Possible endocrine disrupting effects of parabens and their metabolites.* Reproductive Toxicology, 2010. **30**(2): pp. 301–12.
64. Abbas, S., et al., *Metabolism of parabens (4-hydroxybenzoic acid esters) by hepatic esterases and UDP-glucuronosyltransferases in man.* Drug Metabolism & Pharmacokinetics, 2010. **25**(6): pp. 568–77.
65. Janjua, N.R., et al., *Urinary excretion of phthalates and paraben after repeated whole-body topical application in humans.* Int J Androl, 2008. **31**(2): pp. 118–30.

66. SCCS, *Opinion on parabens. Updated request for a scientific opinion on propyl- and butylparaben. COLIPA No. P82.* 2013.
67. Sandanger, T.M., et al., *Plasma concentrations of parabens in postmenopausal women and self-reported use of personal care products: the NOWAC postgenome study.* Journal of Exposure Science & Environmental Epidemiology, 2011. **21**(6): pp. 595–600.
68. EFSA, *Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a Request from the Commission related to parahydroxybenzoates (E214-219), Question number EFAS-Q-2004-063, adopted on 13 July 2004.* The EFSA Journal, 2004. **83**: 1–26.
69. NICNAS, *Inventory multi-tiered assessment and prioritisation (IMPA). Human health Tier II assessment for parabens.* 2015.
70. Anderson, F., *Cosmetic Ingredient Review. Final amended report on the safety assessment of methylparaben, ethylparaben, propylparaben, isopropylparaben, butylparaben, isobutylparaben, and benzylparaben as used in cosmetic products.* Int J Toxicol, 2008. **27**: pp. 1–82.
71. Oishi, S., *Lack of spermatotoxic effects of methyl and ethyl esters of p-hydroxybenzoic acid in rats.* Food Chem Toxicol, 2004. **42**(11): pp. 1845–9.
72. Hoberman, A.M., et al., *Lack of effect of butylparaben and methylparaben on the reproductive system in male rats.* Birth Defects Res B Dev Reprod Toxicol, 2008. **83**(2): pp. 123–33.
73. Lemini, C., et al., *In vivo and in vitro estrogen bioactivities of alkyl parabens.* Toxicol Ind Health, 2003. **19**(2–6): pp. 69–79.
74. Vo, T.T., et al., *Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model.* Reprod Toxicol, 2010. **29**(3): pp. 306–16.
75. Gazin, V., E. Marsden, and F. Marguerite, *Oral propylparaben administration to juvenile male Wistar rats did not induce toxicity in reproductive organs.* Toxicological Sciences, 2013. **136**(2): pp. 392–401.
76. Oishi, S., *Effects of propyl paraben on the male reproductive system.* Food Chem Toxicol, 2002. **40**(12): pp. 1807–13.
77. Khanna, S., and P.D. Darbre, *Parabens enable suspension growth of MCF-10A immortalized, non-transformed human breast epithelial cells.* J Appl Toxicol, 2013. **33**(5): pp. 378–82.
78. Khanna, S., P.R. Dash, and P.D. Darbre, *Exposure to parabens at the concentration of maximal proliferative response increases migratory and invasive activity of human breast cancer cells in vitro.* Journal of Applied Toxicology, 2014. **34**(9): pp. 1051–9.
79. Wrobel, A.M., and E.L. Gregoraszczuk, *Action of methyl-, propyl- and butylparaben on GPR30 gene and protein expression, cAMP levels and activation of ERK1/2 and PI3K/Akt signaling pathways in MCF-7 breast cancer cells and MCF-10A non-transformed breast epithelial cells.* Toxicology Letters, 2015. **238**(2): pp. 110–6.

80. Wrobel, A.M., and E.L. Gregoraszczuk, *Actions of methyl-, propyl- and butylparaben on estrogen receptor-alpha and -beta and the progesterone receptor in MCF-7 cancer cells and non-cancerous MCF-10A cells.* Toxicology Letters, 2014. **230**(3): pp. 375–81.
81. Wrobel, A.M., and E.L. Gregoraszczuk, *Differential effect of methyl-, butyl- and propylparaben and 17beta-estradiol on selected cell cycle and apoptosis gene and protein expression in MCF-7 breast cancer cells and MCF-10A non-malignant cells.* Journal of Applied Toxicology, 2014. **34**(9): pp. 1041–50.
82. Vo, T.T., et al., *Estrogen receptor alpha is involved in the induction of Calbindin-D(9k) and progesterone receptor by parabens in GH3 cells: a biomarker gene for screening xenoestrogens.* Steroids, 2011. **76**(7): pp. 675–81.
83. Yang, H., et al., *Synergistic effects of parabens on the induction of calbindin-D(9k) gene expression act via a progesterone receptor-mediated pathway in GH3 cells.* Hum Exp Toxicol, 2012. **31**(2): pp. 134–44.
84. Lillo, M.A., et al., *Methylparaben stimulates tumor initiating cells in ER+ breast cancer models.* J Appl Toxicol, 2017. **37**(4): pp. 417–25.
85. Wrobel, A., and E.L. Gregoraszczuk, *Effects of single and repeated in vitro exposure of three forms of parabens, methyl-, butyl- and propylparabens on the proliferation and estradiol secretion in MCF-7 and MCF-10A cells.* Pharmacological Reports: PR, 2013. **65**(2): pp. 484–93.
86. Dagher, Z., et al., *p-Hydroxybenzoate esters metabolism in MCF7 breast cancer cells.* Food & Chemical Toxicology, 2012. **50**(11): pp. 4109–14.
87. Zhang, Z., et al., *Inverse antagonist activities of parabens on human oestrogen-related receptor gamma (ERRgamma): in vitro and in silico studies.* Toxicology & Applied Pharmacology, 2013. **270**(1): pp. 16–22.
88. Kjaerstad, M.B., et al., *Mixture effects of endocrine disrupting compounds in vitro.* Int J Androl, 2010. **33**(2): pp. 425–33.
89. Ma, D., et al., *Assessment of combined antiandrogenic effects of binary parabens mixtures in a yeast-based reporter assay.* Environmental Science & Pollution Research, 2014. **21**(10): pp. 6482–94.
90. Hu, P., et al., *Effects of parabens on adipocyte differentiation.* Toxicol Sci, 2013. **131**(1): pp. 56–70.
91. Pereira-Fernandes, A., et al., *Evaluation of a screening system for obesogenic compounds: screening of endocrine disrupting compounds and evaluation of the PPAR dependency of the effect.* PLoS One, 2013. **8**(10): e77481.
92. Sun, L., et al., *The estrogenicity of methylparaben and ethylparaben at doses close to the acceptable daily intake in immature Sprague-Dawley rats.* Sci Rep, 2016. **6**: 25173.
93. Manservisi, F., et al., *Effect of maternal exposure to endocrine disrupting chemicals on reproduction and mammary gland development in female Sprague-Dawley rats.* Reprod Toxicol, 2015. **54**: pp. 110–9.

94. Ahn, H.J., et al., *Parabens inhibit the early phase of folliculogenesis and steroidogenesis in the ovaries of neonatal rats.* Mol Reprod Dev, 2012. **79**(9): pp. 626–36.
95. Lee, J.H., et al., *Parabens Accelerate Ovarian Dysfunction in a 4-Vinylcyclohexene Diepoxide-Induced Ovarian Failure Model.* Int J Environ Res Public Health, 2017. **14**(2): e161.
96. Gopalakrishnan, K., et al., *Changes in mammary histology and transcriptome profiles by low-dose exposure to environmental phenols at critical windows of development.* Environ Res, 2017. **152**: pp. 233–43.
97. Costa, J.R., et al., *Endocrine-disrupting effects of methylparaben on the adult gerbil prostate.* Environ Toxicol, 2017. **32**(6): pp. 1801–12.
98. Snodin, D., *Regulatory risk assessments: Is there a need to reduce uncertainty and enhance robustness? Update on propylparaben in relation to its EU regulatory status.* Hum Exp Toxicol, 2017. **36**(10): pp. 1007–14.
99. Fisher, J.S., et al., *Effect of neonatal exposure to estrogenic compounds on development of the excurrent ducts of the rat testis through puberty to adulthood.* Environ Health Perspect, 1999. **107**(5): pp. 397–405.
100. WHO, G*lobal assessment of the state-of-the-science of endocrine disruptors. WHO/PCS/EDC/02.2.* Geneva: World Health Organisation. 2002.
101. EC, *COMMISSION DELEGATED REGULATION (EU) 2017/2100 of 4 September 2017 setting out scientific criteria for the determination of endocrine-disrupting properties pursuant to Regulation (EU) No 528/2012 of the European Parliament and Council.* 2017.
102. Graven, C., et al., *Endocrine disrupting substances in the EU legal frameworks. Human health perspectives.* RIVM Briefrapport (2016-0137), 2016.
103. Oishi, S., *Effects of butylparaben on the male reproductive system in rats.* Toxicol Ind Health, 2001. **17**(1): pp. 31–9.
104. Oishi, S., *Effects of butyl paraben on the male reproductive system in mice.* Arch Toxicol, 2002. **76**(7): pp. 423–9.
105. Soni, M.G., et al., *Evaluation of the health aspects of methyl paraben: a review of the published literature.* Food Chem Toxicol, 2002. **40**(10): pp. 1335–73.
106. SCCP, *Extended Opinion on the Safety Evaluation of Parabens*. 2005.
107. SCCP, *Extended Opinion on parabens, underarm cosmetics and breast cancer*. 2005.
108. SCCP, *Opinion on parabens.* 2006.
109. SCCP, *Opinion on parabens.* 2008.
110. SCF, *Opinion on p-hydroxybenzoic acid alkyl esters and their sodium salts expressed on 25 February 1994. European Commission, Reports of the Scientific Committee for Food (Thirty-fifth series)*. 1994. pp. 9–12.
111. Danish EPA, *Survey and health Assessment of the exposure of 2 year-olds to chemical substances in consumer products. Survey of Chemical Substances in Consumer Products, No. 102.* 2009.

112. Soni, M.G., et al., *Safety assessment of propyl paraben: a review of the published literature.* Food Chem Toxicol, 2001. **39**(6): pp. 513–32.
113. EC, *Regulation (EU) No 1004/2014 amending Annex V to Regulation (EC) No 1223/2009 of the European Parliament and the Council on cosmetic products.* Official Journal of the European Union, 2014.
114. ECHA. *Registration dossier Methyl 4-hydroxybenzoate (https://echa.europa.eu/registration-dossier/-/registered-dossier/14310) accessed 8/11/2017.* 2017.
115. ECHA, *Registration dossier Ethyl 4-hydroxybenzoate (https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/13843/7/3/3). Accessed on 8/11/2017.* 2017.
116. ECHA, *Registration dossier Propyl 4-hydroxybenzoate (https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/13890/7/3/3) Accessed on 8/11/2017.* 2017.
117. ECHA, *Methyl 4-hydroxybenzoate. Justification for the selection of a candidate CoRAP substance.* 2013.
118. ECHA, *Decision on substance evaluation pursuant to article 46(1) of Regulation (EC) No 1907/2006 for methyl 4-hydroxybenzoate, CAS No 99-76-3 (EC No 202-785-7). https://echa.europa.eu/documents/10162/8fc435b8-73ab-6340-441f-dedb23dfdf2f.* 2016.
119. ECHA, *Ethyl 4-hydroxybenzoate. Justification document for the slection of a CoRAP substance.* 2017.
120. ECHA, *Decision on substance evaluation of propyl 4-hydroxybenzoate. https://echa.europa.eu/documents/10162/d7f1a077-1d21-ea36-cfb2-c304990c4944.* 2017.

9 Appendices

9.1 Literature search details

The literature discussed in Section 3.7 was based on the following search details.

Databases

- Embase
- Medline

Search terms

Searched for Mesh heading Parabens and/or Paraben in title of publication, in combination with:

- consumer / household products and/or environmental exposure;
- pharmaceutical preparations;
- diet / food / food additives;
- toxicity / poisoning and/or endocrine disruptors, etc.

Inclusion criteria

- Published between January 2010 and May 2017
- English language
- Methyl paraben study *in vitro*
- Methyl paraben study *in vivo*
- Reviews on methylparaben

Exclusion criteria

- Ecotoxicity study
- Mixture studies
- No clear description of the study design
- No relevant ED endpoints
- Other parabens studies *in vitro*
- Other parabens studies *in vivo*
- Human epidemiology studies

Number of literature studies selected on the toxicity of methylparaben

- *In vivo* studies (n=7)
- *In vitro* studies (n=14)

9.2 Overview of *in vitro* and *in vivo* data of endocrine parameters and toxicity of parabens

Table A1. In vitro data on endocrine parameters and toxicity of parabens published after 2010. MeP = methylparaben, EtP = ethylparaben, PrP = propylparaben, BtP = butylparaben, iBtP = isobutylparaben, and BzP = benzylparaben.

Test substances	Test system	Test principle(s)	Results	Ref.
In vitro assays – Estrogenic activity				
MeP, PrP, BtP	non-transformed MCF-10A cells, breast tissue cell line.	Aim: Investigate the potential for parabens on suspension growth of the immortalized but non-transformed MCF-10A human breast epithelial cells. Compounds and concentration: 17 nM 17β-estradiol (positive control), $5x10^{-4}$ M MeP, $1x10^{-5}$ M PrP, or $1x10^{-5}$ M n-BtP. Endpoints tested: Number of colonies, average size of colonies.	Parabens can induce anchorage-independent growth of MCF-10A immortalized but non-transformed human breast epithelial cells. In semi-solid methocel suspension culture, MCF-10A cells produced very few colonies and only of a small size. Addition of $5x10^{-4}$ M MeP, $1x10^{-5}$ M PrP, or $1x10^{-5}$ M BtP resulted in a greater number of colonies per dish ($P<0.05$ in each case) and an increased average colony size ($P<0.001$ in each case). When 17 nM 17β - oestradiol was added the number of colonies per dish also increased ($P<0.05$) and their relative size was also greater ($P<0.001$).	[77]
MeP, PrP, BtP	MCF-7, T-47-D, ZR-75-1, estrogen-responsive human breast cancer cell lines	Aim: Investigate the effects of exposure to parabens at concentrations of maximal proliferative response on migratory and invasive properties using three estrogen-responsive human breast cancer cell lines (MCF-7, T-47-D, ZR-75-1). Compounds and concentration: $1x10^{-8}$ M 17β-estradiol (positive control), with 5×10^{-4} M (MCF-7/T-47-D cells) or 1x10–4 M (ZR-75-1 cells) MeP, with 1x10–5 M PrP or with $1x10^{-5}$ M n-BtP. Exposure was 7 days or 20 weeks. Endpoints tested: mortality after 24 hours, cumulative length moved 24 hours after scratch.	Long-term exposure (20 ± 2 weeks) of MCF-7 cells to MeP, PrP or BtP increased migration. Increased migratory activity was also demonstrated in long-term paraben-exposed T-47-D and ZR-75-1 cells using a scratch assay and time-lapse microscopy at the tested concentrations. Estradiol showed comparable results.	[78]

Test substances	Test system	Test principle(s)	Results	Ref.
MeP, PrP, BtP	MC-7 cancer cells and noncancerous MCF-10A cells	Aim: Examined the effects of methyl-, propyl- and butylparaben on the mRNA and protein expression of estrogen receptor (ER)-α (ESR1) and -β (ESR2) and the progesterone receptor (PGR). Compounds and concentration: 20 nM MeP, PrP or BtP, or 10 nM 17β-estradiol (positive control). Endpoints tested: ESR1 (estrogen receptor-α), ESR2 (estrogen receptor-β) and progesterone receptor (PGR) gene and protein expression.	Both PrP and BtP stimulated PGR mRNA expression in MCF-7 cells, whereas MeP and PrP stimulated PGR protein expression. In MCF-10A cells, PrP and BtP increased only PGR mRNA expression. All parabens increased ESR1 gene and protein expression in MCF-7 and, with the exception of BtP, in MCF-10A cells. All parabens significantly increased ESR2 mRNA and protein expression in MCF-7 cells, but in MCF-10A cells only ESR2 protein expression. Estradiol showed comparable results.	[80]
MeP, PrP, BtP	MC-7 cancer cells and noncancerous MCF-10A cells	Aim: Examine cAMP levels and activation of the MAPK/ERK1/2 and PI3K/Akt signalling pathways in response to the actions of parabens on GPR30 in MCF-7 and MCF-10A cells. Compounds and concentration: Cells were exposed to MeP, PrP, BtP at dose of 20 nM or 17β-estradiol (positive control) at dose 10 nM for 6 and 24 h. Endpoints tested: GPR30, cAMP, pERK1/2, ERK1/2 gene and protein expression.	17β-estradiol and all tested parabens increased GPR30 gene and protein expression in MCF-7 and MCF-10A cells. No parabens affected cAMP levels in either cell line, with the exception of PrP in MCF-10A cells. 17β-estradiol, PrP and BtP increased phosphorylation of ERK1/2 in MCF-7 cells, whereas 17β-estradiol, MeP and BtP, but not PrP, increased phosphorylation of ERK1/2 in MCF-10A cells. Akt activation was noted only in MCF-7 cells and only with PrP treatment.	[81]

Test substances	Test system	Test principle(s)	Results	Ref.
MeP, PrP, BtP	MC-7 cancer cells and noncancerous MCF-10A cells	Aim: The present study determines that MeP, PrP, BtP, even at low doses, stimulate the proliferation of MCF-7 breast cancer cells and non-transformed MCF-10A breast epithelial cells and whether this represents a direct effect on cell cycle and apoptotic gene and protein expression. Compounds and concentration: MeP, PrP, BtP were added at a concentration of 20 nM for 24 h and 17β-estradiol (positive control) was added at a concentration of 10 nM. Endpoints tested: expression of cell cycle or apoptosis regulatory genes and proteins (e.g. Cyclin B1, D, E, A, P21, BCL2).	Upregulation of Bcl-xL and downregulation of caspase 9 was observed in MCF-7, while upregulation of Bcl-xL, BCL2L2 and caspase 9 was noted in MCF-10A. Cyclins in MCF-7 cells were not affected by any of the parabens. MeP BtP had no effect on the expression of selected apoptotic genes in MCF-7. In MCF-10A, all parabens tested increased the expression of G1/S phase genes, and downregulated cell cycle inhibitors. MeP increased pro-survival gene. BtP increased BCL2L1 gene, as did 17β-estradiol, while PrP upregulated both the extrinsic and intrinsic apoptotic pathways. There are differences in cell cycle and apoptosis gene expression between parabens and 17β-estradiol in MCF-7 cells. In MCF-10A cells, most of the genes activated by parabens were comparable to those activated by 17β-estradiol.	[79]
MeP, EtP, PrP, BtP, BzP	human ERRγ coactivator recruiting assay	Aim: In this study, an *in vitro* nuclear receptor coactivator recruiting assay was developed and used to evaluate the binding activities of parabens via antagonist competitive binding on the human estrogen-related receptor γ (ERRγ), which is known as both a diagnostic biomarker and a treatment target of breast cancer. Compounds and concentrations: MeP, EtP, PrP, BtP, BzP ($1x10^{-10}$ M, $1x10^{-9}$ M, $1x10^{-8}$ M, $1x10^{-7}$ M, $1x10^{-6}$ M, $1x10^{-5}$ M, $1x10^{-4}$ M). Endpoints tested: antagonist activity on ERRγ: 50% relative effective concentrations (REC50).	In silico molecular docking analyses showed that parabens fitted well into the active site of ERRγ, with hydrogen bonds forming between the p-hydroxyl group of parabens and the Glu275/Arg316 of ERRγ. The REC50 values of MeP, EtP, PrP, BuP and BzP were 4.79×10^{-7} M, 3.73×10^{-7} M, 3.45×10^{-7} M, 3.09×10^{-7} M and 5.88×10^{-7} M, respectively. The results were not compared with a positive control such as 17β-estradiol.	[87]

Test substances	Test system	Test principle(s)	Results	Ref.
MeP, BtP, BzP	MCF-7 cells	Aim: to gain insight into the metabolism of parabens in breast cancer cells (MCF7) since they have demonstrated estrogenic activity towards these cells and have been detected in breast cancer tissues. Compounds and concentration: 0, 5, 10, 15, 20, 25, 50, 100, 250, 500, 1000 µM (MeP); 2.5, 5, 10, 15, 20, 25, 50, 100, 250 µM (BtP, BzP). Tested endpoints: cell viability, Hydrolysis of methyl-, butyl and benzyl-paraben to p-hydroxybenzoic acid was analysed in cultured MCF7 cells and in cellular homogenates. Glucuronidation and sulfoconjugation were studied in MCF7 homogenates.	MeP was shown to be far less toxic than BtP and BzP. Parabens were completely stable in MCF7 homogenates whereas p-nitrophenyl acetate, a substrate type, underwent hydrolysis. MCF7 cell homogenates did not express glucuronidation and sulfoconjugation activities toward parabens.	[86]
MeP	MCF-7 human breast cancer cells and MDA-MB-231 cells; Mammosphere assay: MDA-MD-231 cells, HCI-7-Luc2 PDX tumours (*in vivo*)	Aim: To determine if MeP, which is one of the highest paraben found in the breast, affects breast cancer tumour-initiating cells *in vitro* and tumour proliferation *in vivo.* Compounds and concentration: 10 nM estradiol or 10 nM MeP . Tested endpoints: tumour size (*in vivo*), size of mammospheres, nanog expression (gene and protein).	MeP does not cause MCF-7 cells to proliferate, nor do they increase canonical estrogen-responsive genes; MeP induces ALDH1 and increases mammosphere size *in vitro*; MeP increases stem cell marker expression in mammospheres and tumours; MeP does not affect MDA-MB-231 mammosphere size.	[84]
MeP, EtP, PrP, iPrP, BtP, iBtP	GH3 rat pituitary cancer celline	Aim: Induction of an estrogenic biomarker gene, Calbindin-D9k (CaBP-9k), to investigate the xenoestrogenic activity of a panel of parabens (MeP, EtP, PrP, iPrP, BtP, iBtP) in GH3 rat pituitary cancer cell line. Compounds and concentration: $1x10^{-7}$ M, $1x10^{-6}$ M, $1x10^{-5}$ M, $1x10^{-4}$ M for all parabens, $1x10^{-9}$ M estradiol. Endpoints tested: gene and protein expression (CaBP-9k, CaBP-9k/1A, ERα, PR (progesterone receptor)) .	A significant increase in CaBP-9k and PR expression of transcript and protein was dependent on the concentration treated as well as the linear length of the alkyl chain from MeP to iBtP.	[82]

Test substances	Test system	Test principle(s)	Results	Ref.
MeP, EtP, PrP, iPrP, BtP, iBtP	GH3 rat pituitary cancer celline	Aim: The estrogenic biomarker gene calbindin-D9k (CaBP-9k) was measured in the rat pituitary lactosomatotrophic GH3 cells. Estrogen receptors (ERs) and progesterone receptors (PRs) expression were investigated. Compounds and concentrations: $1x10^{-5}$ M each paraben and for 17β -estradiol. Endpoints tested: gene and protein CaBP-9K, ERα, progesteron receptor (PR).	After 24 h of treatment, a significant increase in CaBP-9k expression was observed. This was dependent upon the length of the paraben alkyl chains (shortest in MeP and longest in iBtP). The expression of ERa mRNA and protein were not significantly different. The expression patterns of CaBP-9k and PR-B genes appeared to be similar in response to paraben treatments.	[83]
In vitro assays – Androgenic activity				
MeP, EtP, PrP, BtP, iBtP	AR-transfected Chinese Hamster Ovary (CHO) cells	Aim: AR reporter gene assay in the AR-transfected Chinese Hamster Ovary (CHO) cells after exposure to single parabens or a mixture of these parabens. Compounds and concentration: The test compounds were added to the cells with or without 0.01 nm of the AR agonist R1881 and were tested in eleven or twelve concentrations within the range of 0.03–30 or 0.025–50 μM, respectively. Endpoints tested: The ability of the test compounds to activate the AR and to inhibit androgen-induced activation of the AR was tested using a reporter gene assay based on AR-transfected Chinese Hamster Ovary (in luminescence units).	Of the five selected parabens, only iBtP paraben antagonized the AR in the assay used. BtP and PrP inhibited the R1881 induced response, but only at cytotoxic concentrations. The effect of iBtP was statistically significant at concentrations of 25 μM and above.	[88]
PrP, iPrP, MeP	A yeast-based human androgen receptor assay (YAS)	Aim: A yeast-based human androgen receptor assay (YAS) was applied to assess the antiandrogenic activities of PrP, iPrP, MeP compared with dihydrotestosterone. Compounds and concentration: range between $10x10^{-6}$ and $10x10^{-3}$ M. No positive control is tested. Endpoints tested: half maximal inhibition effective concentration (IC50) calculations of individual antiandrogens.	All compounds tested exhibited significant and concentration-dependent antiandrogenic properties. The antiandrogenic potencies of PrP were higher than those of MeP. Conclusion: IC50 MeP: 2.3x10-4M, IC50 PrP: 3.9×10^{-4} M.	[89]

In vitro assays – adipocyte differentiation				
MeP, EtP, PrP, BtP	Murine 3T3-L1 cells, celline for adipocyte differentiation	Aim: the adipogenic effect of parabens on murine 3T3-L1 cells differentiation was investigated. Compounds and concentration: 0, 1, 100, 1000 µM (gene expression); 1000 µM for differentiation. Endpoints tested: Oil Red O (ORO)-stained adipocyte morphology, lipid accumulation, and mRNA expression of specific adipocyte marker genes (PPARγ, C/EBPα, FAS, FABP4, Adiponectin, Leptin).	parabens promote adipogenesis (or adipocyte differentiation) in murine 3T3-L1 cells, as revealed by adipocyte morphology, lipid accumulation and mRNA expression of adipocyte-specific markers; the adipogenic potency of parabens is increased with increasing length of the linear alkyl chain in the following potency ranking order: MeP-< EtP- < PrPl- < BtP.	[90]
MeP, EtP, PrP, BtP	Murine 3T3-L1 fibroblasts and PPARγ CALUX celline (Chemically Activated LUciferase eXpression assay)	Aim: to develop a reproducible, standardized protocol for the adipocyte differentiation assay / this adipocyte differentiation assay was further evaluated by screening different compounds including parabens. Compounds and concentration: concentration of all parabens 25, 50, 100, 200 µM adiposite differentiation, 3, 10, 30, 100 µM PPARγ activity in the CALUX cell line. Endpoints tested: adipocyte differentiation, ap2 mRNA expression, PPARγ activity in the CALUX cell line.	The paraben compounds EtP-(200 µM), PrP-(100 and 200 µM), and BtP (100 and 200 µM) strongly induced the differentiation of the 3T3-L1 cells, whereas MeP did not. PPARγ activation was associated with adipogenesis for parabens. Concerning paraben compounds, they all activated the PPARγ receptor and as for the differentiation, this activation increased with increasing alkyl chain length PrP and BtP were strong activators whereas MeP and EtP were weak.	[91]

Table A2. In vivo data of endocrine parameters and toxicity of parabens published after 2010. MeP = methylparaben, EtP = ethylparaben, PrP = propylparaben, and BtP = butylparaben.

Test substances	Test system	Test principle(s)	Results	Ref.
In vivo assays				
MeP, EtP	Sprague Dawley rats	The uterotrophic activities of MeP and EtP (at doses 0.8; 4 and 20 mg/kg bw/day) were investigated in immature Sprague Dawley (n=8) rats by intragastric administration from day 21 to day 24. Uterotrophic activities were measured. Expression of estrogen-responsive biomarker genes were studied in uteri of the rats by and urinary concentrations the parabens were measured.	Gene expression was affected: Icabp, Itmap1, CaBP-9k, Pgr. (0.8, 4 and 20 mg/kg bw/day). Relative uterine weight was increased (EtP: 4 and 20 mg/kg bw/day) and MeP (20 mg/kg bw/day).	[92]
MeP	Sprague Dawley rats	The aim of the study is to determine whether low doses of EDCs affect the development and proliferative activity of the mammary glands (MGs). Doses: 0.1050 mg/kg bw/day MeP (n=10 per group). F1 generation: exposure GD20-PND 181. Two groups: nulliparous (rats not given birth), parous (rats given birth). Morphology and histology and gene expression were measured. Gene expression.	No effect on fertility rate. Treatment with MeP resulted in a significantly higher number of pups per litter at delivery with a litter size of 15.2. Increased mortality in all treated groups at PND 7 and onwards. Compared with the average mortality of about 3% in the control group (+/- 22 %, n=3). Dams at end of lactation effects: treated animals showed evident histological differences from controls: the alveoli were not always milk-filled and an increase in adipose tissue was noted. The collapsed alveolar and duct structures showed residual secretory content. Gene expression affected.	[93]
PrP	Wistar rats	In the pharmacokinetic study, Wistar male rats received a single dose of PrP at 3, 10, 100, or 1000 mg/kg bw, orally on postnatal day (PND). PrP was orally administered by gavage to 20 Wistar male rats at doses of 3, 10, 100, or 1000 mg/kg bw/day for 8 weeks starting on PND21. This study was undertaken to resolve the conflicting published evidence on the *in vivo* effects of PrP on the male reproductive tract in juvenile rats and to investigate other parameters, including low-dose exposure, recovery, and hormone analysis.	No effect was observed on the weight of the reproductive organs (epididymis, prostate and seminal vesicle, and testis). PrP did not affect mean testicular spermatid counts nor epididymal sperm counts or mean motility parameters. PrP had no effect on hormone levels (LH, FSH, and testosterone) at the end of the treatment period. There was no evidence of an effect of propylparaben on the weight of the male reproductive organs, epididymal sperm parameters, hormone levels or histopathology.	[75]*

Test substances	Test system	Test principle(s)	Results	Ref.
MeP, PrP, BtP	Sprague-Dawley rats	55 female pups were given daily subcutaneous injections of MeP, PrP, and BtP at doses of 62.5, 250 or 1000 mg/kg bw/day or 17β-estradiol (40 µg/kg bw/day) (E2) during neonatal days 1–7 (n=5 per group). Organ weight was measured, the ovaries were excised on postnatal day 8, then fixed and stained with hematoxylin and eosin for histological analysis. The follicles were counted and classified as being in the primordial, early primary or primary stages and gene expression was measured.	The data show that PrP and BtP stimulated AMH mRNA expression and consequently inhibited the early phase of folliculogenesis in the ovaries of neonatal female rat. PrP and BtP interrupted ovarian follicle development by increasing the number of primordial follicles and decreasing early primary follicle numbers. MeP did not. These results suggest that estrogenic parabens suppress the transformation of primordial follicles into early primary follicles in the rat ovaries.	[94]
MeP, PrP, BtP	Sprague-Dawley rat	The influence of parabens on ovarian folliculogenesis and steroidogenesis was investigated. Female 8-week-old Sprague Dawley rats were orally exposed to 100 mg/kg bw/day for 5 weeks (n=6 per group). Ovarian follicle development and steroid synthesis were investigated through real-time PCR and histological analyses. A disruptor of ovarian small pre-antral follicle 4-vinylcyclohexene diepoxide (VCD, 40 mg/kg bw/day), was used to induce premature ovarian failure (POF).	Diestrus phases in the PrP and BtP groups were longer than those in the vehicle and MeP groups. MeP treatment showed a regular estrous cycle; treatment with PrP and BtP shortened the interval of the estrous cycle. No effect on number of primary follicles; secondary follicles show a decrease in total number in all treated groups. Parabens induced an increase in FSH levels in serum and significantly decreased the total number of follicles. Diestrus phases in the VCD, PrP and BtP groups were longer than those in the vehicle group; VCD, PrP and BtPdecreased mRNA level of folliculogenesis-related genes (Foxl2, Kitl and Amh). Parabens induced an increase in FSH levels in serum and significantly decreased the total number of follicles. Increased FSH implies impairment in ovarian function due to VCD or parabens. Increased plasma FSH implies impairment in ovarian function due to parabens.	[95]

Test substances	Test system	Test principle(s)	Results	Ref.
MeP	Sprague Dawley rat	Study effects of MeP on histology and transcriptome profiles of normal (noncancerous) mammary glands at doses mimicking human exposure (0.105 mg/kg bw/day, orally). Animals were exposed across several key developmental stages (n=number per group (compound and treated)) including perinatal (GD1–GD20, n=10 or PND1–PND21, n=10), prepubertal (PND21–PND42, n=5) and pubertal (PND42-PND63, n=5) windows as well as long-term exposures from birth to lactation (PND1–PND146, n=3).	Perinatal MeP exposure decreases amounts of adipose tissue and increases expansion of the ductal tree within the fat pad. Prepubertal MeP treatment was associated with a significant reduction in adipose tissue and more abundant glandular tissue. Pubertal MeP exposure elevated the amounts of glandular tissue compared to control, visible as a higher degree of branching relative to the total gland area. Long-term MeP treatment from birth to lactation did not result in significant histological changes. In the pubertal window gene expression changes were observed.	[96]
MeP	Gerbils	The aim was to evaluate the effects of oral exposure to methylparaben (500 mg/kg bw/day) for 3, 7, and 21 days on male and female adult gerbil prostate with biometrical, morphological, and immunohistochemical analyses.	MeP caused morphological changes in gerbil prostates in all experimental groups. These animals displayed similar alterations such as prostate epithelial hyperplasia, increased cell proliferation, and a higher frequency of AR-positive cells. However, the prostate of the female gerbil showed additional changes such as stromal inflammatory infiltration, intraepithelial neoplasia foci, and an increase in AR-positive frequency.	[97]

**Data already available and taken into account by the SCCS.*

9.3 Paraben entries in the Cosmetics Regulation

Table A3. Entries 12 and 12a from Annex V 'List of preservatives allowed in cosmetic products' in the Cosmetics Regulation (EC) No. 1223/2009[3]

	Substance Identification				Conditions			
Reference number	**Chemical name / INN**	**Name of common ingredients glossary**	**CAS number**	**EC number**	**Product type, Body parts**	**Maximum concentration in ready for use preparation**	**Other**	**Wording of conditions of use and warnings**
12	4-Hydroxybenzoic acid and its Methyl- and Ethyl-esters, and their salts	4-Hydroxybenzoic acid methylparaben potassium ethylparaben potassium paraben sodium methylparaben sodium ethylparaben ethylparaben sodium paraben potassium methylparaben calcium paraben	99-96-7 99-76-3 36457-19-9 16782-08-4 5026-62-0 35285-68-8 120-47-8 114-63-6 26112-07-2 69959-44-0	202-804-9 202-785-7 253-048-1 240-830-2 225-714-1 252-487-6 204-399-4 204-051-1 247-464-2 274-235-4		0.4% (as acid) for single ester 0.8% (as acid) for mixtures of esters		
12a	Butyl 4-hydroxy-benzoate and its salts Propyl 4-hydroxy-benzoate and its salts	Butylparaben propylparaben sodium propylparaben sodium butylparaben potassium butylparaben potassium propylparaben	94-26-8 94-13-3 35285-69-9 36457-20-2 38566-94-8 84930-16-5	202-318-7 202-307-7 252-488-1 253-049-7 254-009-1 284-597-5		0.14% (as acid) for the sum of the individual concentrations 0.8% (as acid) for mixtures of substances mentioned in entry 12 and 12a, where the sum of the individual concentrations of butyl- and propylparaben and their salts does not exceed 0.14%.	Not to be used in leave-on products designed for application on the nappy area of children under 3 years of age.	For leave-on products designed for children under 3 years of age: 'Do not use on the nappy area'

[3] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02009R1223-20160812&qid=1482148361835&from=EN

Table A4. Entries 1374–1378 in Annex II 'List of substances prohibited in cosmetic products' in the Cosmetics Regulation (EC) No. 1223/2009[4]

Reference number	Substance identification		
	Chemical name / INN	CAS number	EC number
1374	Isopropyl 4-hydroxybenzoate (INCI: Isopropylparaben) Sodium salt or Salts of Isopropylparaben	4191-73-5	224-069-3
1375	Isobutyl 4-hydroxybenzoate (INCI: Isobutylparaben)	4247-02-3	224-208-8
	Sodium salt or Salts of Isobutylparaben	84930-15-4	284-595-4
1376	Phenyl 4-hydroxybenzoate (INCI: Phenylparaben)	17696-62-7	241-698-9
1377	Benzyl 4-hydroxybenzoate (INCI: Benzylparaben)	94-18-8	
1378	Pentyl 4-hydroxybenzoate (INCI: Pentylparaben)	6521-29-5	229-408-9

[4] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02009R1223-20160812&qid=1482148361835&from=EN

9.4 List of abbreviations

ADI	Acceptable Daily Intake
BPR	Biocidal Products Regulations
CBG-MEB	Medicines Evaluation Board
CIR	Cosmetic Ingredient Review
COLIPA	Cosmetic Toiletry and Perfumery Association
CoRAP	Community Rolling Action Plan
DTU	Danish National Food Institute
DNEL	Derived No Effect Level
ECHA	European Chemicals Agency
ED	endocrine-disrupting
EDC	Endocrine Disrupting Chemical
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EPA	Environmental Protection Agency
FCM	food contact material
LOAEL	Lowest Observed Adverse Effect Level
LOQ	limit of quantification
MOA	Mode/Mechanism Of Action
MOS	Margin Of Safety
MPL	maximum permitted level
NICNAS	Australian National Industrial Chemicals Notification and Assessment Scheme
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NVWA	Netherlands Food and Consumer Product Safety Authority
OECD	Organisation for Economic Co-operation and Development
PACEM	Probabilistic Aggregate Consumer Exposure Model
PHBA	*p*-hydroxybenzoic acid
PPPR	Plant Protection Products Regulation
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SCF	Scientific Committee for Food
SCOOP	Scientific Cooperation
SML	Specific Migration Limit
TG	(OECD) Test Guidance
WHO	World Health Organization

RIVM
Committed to *health and sustainability* -