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

Review on butylparaben: exposure, toxicity and risk assessment

With a focus on endocrine disrupting properties and cumulative risk assessment

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Synopsis

Review of butylparaben: exposure, toxicity and risk assessment
With the focus on endocrine-disrupting properties and cumulative risk assessment

Butylparaben is used as a preservative because it inhibits the growth of fungi and bacteria in, for example, personal care products. However, butylparaben, just like other parabens, is suspected of having endocrine-disrupting properties or, to put it another way, of being an endocrine disruptor. Endocrine disruptors can compromise the hormonal system in the human body.

As yet, RIVM has been unable to determine whether butylparaben must actually be considered an endocrine disruptor. Because of the likely limited extent to which consumers are exposed to butylparaben and the information currently available about its effect on health, there does not appear to be any reason for concern. Additional research is needed to reduce any uncertainties in this conclusion.

Personal care products are the most significant source of the total calculated amount of butylparaben with which consumers come into contact. For safety's sake, this calculation is based on worst-case scenarios. There are also indications that such products are far less likely to contain parabens these days. There is no relevant information available for estimating exposure via medicines. Intake via food does not play a role in exposure because, among other reasons, butylparaben’s use as an additive in foods or in food contact materials, such as packaging, is forbidden in Europe.

Many studies on the properties of butylparaben show that it has an endocrine-related action or suggest that it is an endocrine disruptor. Experts will have to discuss further whether the data in question yields sufficient evidence to actually classify butylparaben is an endocrine disruptor. They will test whether the substance meets the criteria recently drawn up for endocrine disruptors. Additional evidence may also be necessary.

The risk assessment of butylparaben entails uncertainties. It is highly likely that the current calculated exposure is higher than is actually the case and, moreover, it is possible that the differences between the effects on humans and the effects on laboratory animals are not being taken sufficiently into account.

Keywords: butyl paraben, parabens, exposure, hazard, endocrine disruption, cosmetics

Publiekssamenvatting

Review over butylparabeen: blootstelling, toxiciteit en risicobeoordeling
Met een focus op hormoonverstorende eigenschappen en cumulatieve risicobeoordeling

Butylparabeen wordt als conserveermiddel gebruikt omdat het de groei van schimmels en bacteriën tegengaat, bijvoorbeeld in persoonlijke verzorgingsproducten. Maar butylparabeen wordt, net als andere parabenen, ervan verdacht een hormoonverstorende werking te hebben. Hormoonverstorende stoffen kunnen de hormoonhuishouding in het menselijk lichaam in de war brengen.

Het RIVM kan nog niet bepalen of butylparaben daadwerkelijk als hormoonverstorende stof moet worden beschouwd. Vanwege de waarschijnlijk geringe mate waarin consumenten worden blootgesteld en de huidige informatie over gezondheidseffecten, lijkt er geen reden tot bezorgdheid te zijn. Aanvullend onderzoek is nodig om de onzekerheden in deze conclusie te verkleinen.

Persoonlijke verzorgingsproducten zijn de belangrijkste bron van de totale berekende hoeveelheid butylparaben waar consumenten in aanraking mee komen. Bij deze berekening is veiligheidshalve uitgegaan van ongustige situaties. Ook zijn er aanwijzingen dat deze producten tegenwoordig veel minder vaak parabenen bevatten. Er is geen relevante informatie beschikbaar om de blootstelling via geneesmiddelen te kunnen schatten. De inname via voedsel speelt geen rol, onder andere omdat het gebruik als toevoeging in levensmiddelen of in materialen waar voedsel mee in contact kan komen, zoals verpakkingen, in Europa is verboden.

Veel studies over de eigenschappen van butylparabeen laten zien dat het een hormoongerelateerde werking heeft, of duiden erop dat butylparabeen een hormoonverstorende stof is. Of deze gegevens voldoende bewijs leveren dat butylparabeen daadwerkelijk een hormoonverstorende stof is, zullen experts verder moeten onderzoeken. Zij toetsen dan of de stof aan criteria voldoet die recentelijk voor hormoonverstorende stoffen zijn opgesteld. Het kan ook zijn dat aanvullend bewijs nodig is.

Voor de risicobeoordeling van butylparaben zijn er onzekerheden. De huidige berekende blootstelling is zeer waarschijnlijk te hoog. Daarnaast wordt mogelijk onvoldoende rekening gehouden met de verschillen tussen effecten bij mensen en bij proefdieren.

Kernwoorden: butyl parabeen, parabenen, blootstelling, toxiciteit, hormoonverstoring, cosmetica

Contents

Summary

Chemical substances that potentially have an effect on the endocrine system have attracted increasing attention in recent years. For that reason, the Netherlands Food and Consumer Product Safety Authority (NVWA) asked 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 and non-food products. Previously, we have reported on methylparaben, ethylparaben and propylparaben. The present report is on the fourth most-used paraben: butylparaben.

The aim of this report is to provide an overview of the exposure, hazard and risk assessments performed on butylparaben, and to assess whether any potential ED effects are included in these risk assessments and in the derivation of the existing toxicological reference value(s). The report describes and summarizes the information on exposure, hazard and risk assessments with respect to butylparaben present in the literature.
In addition, it includes a statement on whether a cumulative exposure assessment of methyl-, ethyl-, propyl- and butylparaben together is justified. Recommendations for further research have also been formulated.

Use of and exposure to butylparaben
Like other parabens, butylparaben can be used as a preservative in various consumer products. An aggregate exposure assessment includes an assessment for a single substance that takes into account several exposure routes (inhalation, dermal and oral), as well as several exposure sources. As in the report on methyl-, ethyl- and propylparaben, three major sources of butylparaben exposure are considered: personal care products, food and medicinal products. Data on exposure assessments conducted for non-food consumer products other than personal care products are virtually absent. However, this source of exposure is regarded to be minor compared with the exposure via personal care products. Exposure assessments for the different sources vary greatly in approach, the 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 the exposure to butylparaben via personal care products. According to the studies that are most relevant to the situation found in the Netherlands and/or Europe, a higher-tier estimation (97.5th percentile value) of an internal exposure to butylparaben equalled about 0.1 mg/kg bw/day for adults. Based on another higher-tier study, 0.20 mg/kg bw/day (95th percentile value) has been estimated for infants and toddlers.
Several factors within the exposure estimation could have resulted in an overestimation of the exposure to butylparaben via personal care products. These factors include:

- the method of aggregating exposure from different products;
- assumptions regarding the frequency at which these products were used and the amount of product applied;

- the assumed concentration of butylparaben in personal care products;
- the fraction of available products in which butylparaben is present;
- a possibly unrealistic high estimation of the fraction of product that remains on the skin after application;
- the estimated extent to which butylparaben is absorbed from the skin into the internal system is possibly unrealistically high.

The present report reviewed existing exposure assessments. Additional relevant, available data with respect to several of the factors above (including recent product use and concentration data) could be used to refine the exposure assessment of butylparaben via personal care products in the Netherlands and/or Europe.

Exposure via food
In the EU, butylparaben, as opposed to methyl- and ethylparaben, is not allowed to be used as a food preservative or in the manufacture of plastic materials and articles intended for contact with food.

Two studies, one from China and one from the USA, reported realistic concentrations from actual measurements of butylparaben in food, which were used to assess exposure to butylparaben via food. No relevant studies into the actual intake of butylparaben via food in Europe were available. The highest mean butylparaben concentrations reported in the food products considered were 0.059 ng/g in grain products in the USA study, and 1.75 ng/g in vegetables in the Chinese study. The highest (95^{th} percentile) exposure to butylparaben was reported for adults in China: 0.09 µg/kg bw/day. For children (<1 year), the estimated daily exposure was 0.005 µg/kg bw/day (95^{th} percentile) in the USA. It should be emphasized that these studies were performed in China and the USA, where regulation of the use of parabens in food is different from regulation 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 only give an impression of the actual level of exposure.

In these studies, the sources of butylparaben in food were not identified (preservative or migration from food packaging material). However, the migration of parabens into food via food packaging material was shown not to be an important source of exposure in both the Chinese and the USA study. In the EU this source of exposure is expected to be negligible.

Exposure via medicinal products
Exposure to butylparaben via medicinal products may occur concurrently via various administration routes, but few data are available. As no relevant information on exposure to butylparaben via medicinal products was available, the exposure to butylparaben via medicinal products in the Netherlands could not be estimated. There are only nine medicinal products on the Dutch market containing butylparaben (compared with 263 products containing methylparaben and 183 containing propylparaben). Most of these nine products are intended for use for a short period of time.

A probabilistic exposure assessment for this source would be very valuable as the exposure to parabens via medicinal products can be temporary, long-term or (for a large part of the population) non-existent. The necessary data for such an exposure assessment are, however, not publicly available.

Summary of exposure assessment

The aggregation of exposure to butylparaben via personal care products, food and medicinal products, as considered in this report, was difficult to achieve because of the varying levels of information quality and uncertainties concerning the different sources, or even a lack of information (on the contribution by medicinal products). Overall, uncertainties suggest an overestimation of the total exposure. The aggregated butylparaben exposure values can be estimated by adding the estimates from personal care products and food, ignoring differences in the levels of information detail. The overall aggregate exposure estimate for butylparaben is ~0.1 mg/kg bw/day for adults, and ~0.2 mg/kg bw/day for children. These values consist almost entirely of the estimated contribution made by personal care products, as the contribution by food is less than 1%.

The worst-case character of the (aggregate) exposure estimate, essentially the estimated exposure via personal care products, was supported by three biomonitoring studies (in several specific populations of mostly non-Europeans, and using different calculation methods). In these studies, 95th percentile values of urine metabolite concentrations were back-calculated to internal exposure or daily intake levels. These internal exposure values and daily intake levels are 1 up to 2 orders of magnitude lower than the estimated internal (compared with back-calculation to internal exposure estimates) or external (compared with back-calculated daily intake levels) exposure estimates of butylparaben in the present report. However, it is unclear how accurately these exposure estimates represent the current situation in the Netherlands. At most, the estimations give only an impression of an actual level of exposure.

Toxicity of butylparaben

Interspecies differences in metabolites indicate that parabens are not as effectively metabolized in humans as they are in rats, at least after dermal exposure. Therefore, rats might not sufficiently represent the biotransformation of butylparaben as it occurs in humans. This difference is important because the availability of un-metabolized parabens is expected to determine their biological activity and toxicity, including any potential ED activity.

With regard to the hazard of butylparaben, available studies on all toxicological endpoints are summarized in this report. Butylparaben has low acute toxicity, but may cause irritation in the gastrointestinal tract after high oral doses. In humans, inhalational exposure to butylparaben may cause irritation to the respiratory tract. Butylparaben was irritating to the skin and corrosive to the eye in animal tests and may cause eye and skin irritation in humans. Animal tests have indicated that butylparaben is non-sensitizing. But human studies indicate a low sensitization potential. Butylparaben is non-genotoxic *in vitro* and *in vivo* and non-carcinogenic according to the available studies in rats and mice. However, this review

is mainly focused on studies of developmental and reproductive toxicity, and on ED effects.

There are no OECD TG studies available on the reproductive and developmental toxicity of butylparaben. However, there are peer-reviewed studies that have investigated the potential developmental toxicity of butylparaben. A study conducted by Fisher et al. (1999) looked at the postnatal developmental toxicity of butylparaben, with respect to reproductive parameters. In that study, juvenile rats were subcutaneously exposed to butylparaben for 17 days (only one dose group), and a NOEL of 2 mg/kg bw/day was identified, based on the absence of testicular effects. The subcutaneous route bypasses dermal absorption and skin metabolism. Nevertheless, this NOEL was selected as a “conservative” effect level for the risk assessment of cosmetics (i.e. personal care products) by the Scientific Committee on Consumer Products (SCCP) and its successor, the Scientific Committee on Consumer Safety (SCCS). Other reproductive toxicity studies have several shortcomings, as described in this report, including five recent studies not taken into account by the SCCS which describe the effects on sperm counts and related parameters. Overall, much more information is now available about the developmental and reproductive effects of butylparaben than were available at the time of the latest SCCS opinion (2013). However, because of major differences in the methodologies used and the endpoints assessed, a direct comparison of the effect levels derived from these studies is impeded. With regard to the effects on testosterone concentrations and sperm parameters, a LOAEL of 10 mg/kg bw/day is obvious from these studies. With the current information available, RIVM agrees with the NOEL on 2 mg/kg bw/day for developmental and reproductive toxicity as the critical endpoint used by the SCCS and it considers this effect level to be not very conservative.

ED properties

Concerns have been raised about the ED potential of butylparaben, an issue which has also been addressed by the SCCS in its latest opinion on butylparaben (2013). The present report evaluates new *in vitro* and *in vivo* studies on butylparaben with regard to ED properties. Such studies can be used to determine the ED mode of action (MoA) as needed for the identification of butylparaben as an endocrine disruptor. Altogether, several, particular *in vitro* studies support the ED MoA by butylparaben. ED effects have also been observed *in vivo* studies, though some of these studies lack a clear dose-response relationship because of the dosing regime (limited doses). The available data provides many indications that butylparaben has endocrine-disrupting effects via the estrogenic, androgenic and steroid pathways. However, according to the ED criteria, the question of whether the adverse effects are a consequence of the ED MoA needs to be answered and, therefore, a biologically plausible link between adverse effects and endocrine MoA must be demonstrated. The question of whether the available data presents a level of evidence that is high enough to properly identify whether butylparaben is an endocrine disruptor based on the ED criteria and the EFSA-ECHA guidance, or whether additional functional assays are necessary, should also be discussed. If additional *in vivo* experiments are to be conducted, these should be well-designed by

taking into account the potential, much more effective metabolism in experimental animals as compared with humans, at least with regard to the relevant route of human exposure (dermal).

Risk assessment
When comparing the aggregate exposure estimate of ~0.1-0.2 mg/kg bw/day for butylparaben with the NOEL of 2 mg/kg bw/day, the margin of exposure may not be protective enough. However, because the exposure estimate is very likely overly conservative, a refinement of the exposure assessment will probably sufficiently increase this margin of safety to a level that is sufficiently protective. In general, most risk assessments conclude there is no risk presented by the use of butylparaben in personal care products. Overall, the extent to which people appear to be exposed to butylparaben and the current information on health effects do not seem to present a reason for concern.

Up to 2010, according to the SCCS, methyl-, ethyl-, propyl- and butylparaben could be safely used as a preservative up to a maximum concentration of 0.4% in the final product for an individual paraben, and up to 0.8% for a mixture of parabens. In 2010, the SCCS lowered the maximum safe concentration of propyl- and butylparaben to 0.19% for the sum of their individual concentrations because of a lack of scientifically sound data with regard to the dermal absorption for the exposure estimation and because of interspecies differences with regard to metabolism. In 2011, Denmark banned propyl- and butylparaben from cosmetic products for children up to three years of age. In reaction, the SCCS concluded that a risk could not be excluded for leave-on cosmetic products designed for application on the nappy area for children below the age of six months in the light of both their immature metabolism and the possibility of damaged skin in this area. Based on this opinion, the Cosmetic Regulation for propyl- and butylparaben prohibits the use of propyl- and butylparaben in these products designed for children under three. Additionally, in 2013, the SCCS questioned the relevance of the animal studies concerning parabens to human risk assessment due to the more rapid and effective metabolism of parabens in rats in contrast to humans.

Cumulative paraben exposure, toxicity and risk assessment
There are several approaches to performing cumulative exposure and risk assessments. A prerequisite for a cumulative risk assessment is sufficient proof of a common mechanism of action. Based on the current *in vivo* and *in vitro* studies available, there are indications that methyl-, ethyl-, propyl- and butylparaben have a common mechanism of toxicity via an estrogenic, androgenic mechanism. In most studies, different endpoints were affected by different parabens and no consistent effects on one or two specific endpoints could be identified. The absence of standard TG studies for developmental and reproductive toxicity hampers the comparison of the (mechanism of) toxicity between the different parabens. It will take further discussion about whether the present data provide a sufficient level of evidence to conclude whether (or not) a common mechanism of action for (certain) parabens is present, or to decide whether additional studies are necessary.

Conclusions

- Exposure to butylparaben via personal care products has been examined in some detail. Butylparaben is not allowed as a food additive or in food contact materials; the estimated exposure via food outside the EU is very limited. An estimation of the exposure to butylparaben via medicinal products and other consumer products could not be performed. A refined exposure assessment will contribute to a more realistic aggregate exposure assessment;
- Studies demonstrate that the metabolism of butylparaben in rats is more effective than in humans, especially during dermal uptake, apparently. No official OECD guideline studies (TGs) have been performed for the reproductive toxicity of butylparaben. But the relevance of such a study should be questioned because of interspecies differences with regard to metabolism. No effects were observed for butylparaben in a developmental toxicity study. From a postnatal developmental toxicity study, which also studied male reproductive parameters, a NOEL of 2 mg/kg bw/day was used by the SCCS as a toxicological reference value. Several recent non-guideline reproductive toxicity studies indicate that a LO(A)EL of 10 mg/kg bw/day is evident.
- The available data provide many indications that butylparaben has endocrine-disrupting effects via the estrogenic and androgenic and steroid pathways. Further discussion is needed concerning whether the available data identify butylparaben as an ED substance according the ED criteria and the EFSA-ECHA guidance. ED properties were discussed and have been taken into account (to the extent possible) by SCCS (2013) in setting the toxicological reference value for butylparaben. It is, however, not possible to say with certainty that this reference value completely covers possible ED effects.
- As the present calculated exposure values of ~0.1-0.2 mg/kg bw/day for butylparaben are very likely overly conservative, it is expected that a refinement of the exposure assessment would contribute to more realistic values and increase the margin of safety to a level that is sufficiently protective. Overall, the extent to which consumers appear to be exposed to butylparaben and the current information on health effects do not seem to present a reason for concern.
- With regard to cumulative exposure, there are indications that methyl-, ethyl-, propyl- and butylparaben share a common mechanism of toxicity via an estrogenic and/or androgenic mechanism. However, more mechanistic studies are needed to conclude whether (or not) a common mechanism of action exists and justifies a cumulative exposure and risk assessment of parabens.

Recommendations for further research

- To further discuss whether butylparaben is an endocrine disruptor based on the ED criteria and the EFSA-ECHA guidance, or whether additional functional assays are necessary;
- To obtain improved information about (toxico)kinetics and metabolic interspecies differences in order to assist with setting more realistic toxicological reference values (this also accounts for other parabens);

- To obtain improved information about dermal absorption, including metabolism because metabolic inactivation is possibly more effective in rats than it is in humans, in order to facilitate more realistic exposure estimates (this also accounts for other parabens);
- A market surveillance to study whether butylparaben is still being used as a preservative in personal care products, as well as in other non-food consumer products, in order to identify whether further exposure studies are still relevant and should be conducted (this also accounts for other parabens);
- Realistic biomonitoring data representative of the current situation in the Netherlands as an alternative to or conformation of the (possibly limited) current consumer exposure to butylparaben (this also accounts for other parabens);
- A new (probabilistic) exposure assessment for butylparaben via personal care products focused on actual products used, concentration and presence data that represents the current situation in the Netherlands (this also accounts for other parabens);
- When relevant, the performance of an additional exposure study with regard to the exposure via non-food consumer products other than personal care products, and especially medicinal products, might be necessary in order to establish a more realistic aggregate exposure estimation (this also accounts for other parabens);
- The performance of specific mechanistic studies that address several parabens simultaneously in order to conclude whether (or not) a common mechanism of action exists and consequently whether a cumulative exposure and risk assessment of parabens is justified.

1 Introduction

There is concern about the effects of substances with possible endocrine-disrupting properties on humans and the environment. However, the causal relationship between exposure, an endocrine mechanism and the occurrence of specific diseases is often uncertain. This is partly due to complicating factors, which include an often fluctuating or temporary exposure. In addition, in practice people will be exposed to a combination of substances that have endocrine-disrupting properties.

The issue of substances with endocrine-disrupting properties has received attention in relation to products relevant to the daily life of people. Some substances received specific attention, including parabens, a group of possible Endocrine-Disrupting Chemicals (EDCs), some of which are generally used as a preservative in consumer products. People may therefore be exposed to parabens via various sources, including personal care products, food (including migration from food contact materials) and medicinal products.

Previously, we reported on the exposure (taking into account all possible sources), toxic effects (with a focus on endocrine-disrupting properties) and risk assessments for methyl-, ethyl- and propylparaben based on information from scientific literature [1]. The present report aims to investigate these aspects for butylparaben, a fourth paraben generally used. In addition, this report pays attention to the question of whether a common mechanism of toxicity exists for these four parabens and whether a cumulative exposure and risk assessment, in which the four parabens are to be added up, is justified. This investigation includes:

- an inventory and discussion of estimates of exposure to butylparaben for consumers via consumer products and food, taking into account actual exposure scenarios at certain life stages (i.e. childhood) based on available information;
- a description of the toxicity of butylparaben, with a focus on endocrine-disrupting properties, including the current toxicological reference values;
- making a statement about the risks related to the exposure to butylparaben and how it relates to the current toxicological reference value, and about whether the possible endocrine-disrupting effect is included in the derivation of this reference value; and
- identifying uncertainties present in the available data and the methodology used with regard to exposure, toxicity and risk assessments, and making proposals for reducing these uncertainties by additional research, whenever relevant.
- In addition, with regard to the other parabens, an inventory of the methods and practices of cumulative exposure; and
- a statement on a potentially common mechanism of action for methyl-, ethyl-, propyl- and butylparaben.

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). Previously we published and exploratory report focusing on methyl-, ethyl- and propylparaben, the most frequently used parabens [1]. The present additional report focuses on butylparaben (Figure 1). In this report, butylparaben refers to n-butylparaben and not to its isobutylparaben isomers.

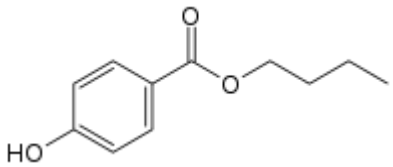

Figure 1. Structural formula of n-butylparaben (CAS 94-26-8).

1.2 Aggregated and cumulative exposure

Because products containing parabens can be applied on the skin or hair, and can also be used in products for oral consumption, both dermal and oral exposure routes are relevant and should be combined in exposure assessment. Inhalation is considered less relevant with regard to parabens. Because people may be exposed to parabens via various sources, including personal care products, food (including migration from food contact materials) and medicinal products, it is relevant to perform an aggregate exposure assessment. The meaning of the term, 'aggregated' and 'cumulative' exposure are used differently across organizations, working groups and in (scientific) literature, and therefore they are defined in this chapter, at least for the purpose of this report, according to the definitions used regularly by RIVM [2].

When **aggregate** exposure assessment is used in this report, it means *the total combined exposure to a single chemical (a paraben) via different routes of exposure (oral, dermal, respiratory) from different sources (i.e. products).* Therefore aggregated exposure automatically includes the exposure via different exposure routes. Aggregated exposure assessments can be performed according to different approaches (see Chapter 2 and our previous report [1]).

Parabens as a group consist of similar chemical substances (with an apparent similar hazard profile), therefore it might be necessary as well to add up the exposure to different paraben substances, i.e. methyl-, ethyl-, propyl- and/or butylparaben. In addition, this could be relevant because often different parabens are used simultaneously. Methyl- and propylparaben, especially, are often used in combination as a commercial mixture in personal care products and medicinal products. Such a cumulative exposure assessment can be performed by different approaches (see Chapter 5). However, it should be justified by (a) similar mechanism(s) of action or toxic effects. So **cumulative** exposure assessment, as used here, means *the exposure to two or more chemicals (parabens) that share a common mechanism of toxicity or the likelihood for the cumulation of a common toxic effect resulting from all sources and routes of exposure.*

1.3 Set–up of the report

The set-up of this report is as follows:
A study was conducted with regard to exposure to and the toxicity of butylparaben based on the existing literature and previously performed reviews and assessments, such as those conducted by the Scientific Committee on Consumer Safety (SCCS).

Exposure to butylparaben
The exposure via different sources (personal care products, food additives, food contact materials, medicinal products and other consumer products) for butylparaben is examined. An overview is provided of available exposure estimates for butylparaben for both adult and child populations. For the sake of a comparison, data from biomonitoring studies that address back-calculated exposure estimates for butylparaben are briefly presented. The exposure estimates are presented, together with the exposure estimates for the three other parabens from our previous report. This is presented in Chapter 2.

Toxicity of butylparaben
An overview of the known hazard characteristics of butylparaben is provided. A summary of studies and outcomes are presented in a table, together with those for the three other parabens. Information on the endocrine-disrupting effects of butylparaben are described and assessed. The toxicological reference values that have been derived by others are described and discussed, and a statement is made as to whether the possible endocrine-disrupting effects are included in the derivation of this reference value. This is presented in Chapter 3.

Reviews and risk assessments
In Chapter 4, an overview of the available reviews and risk assessments conducted by others on butylparaben is provided. Also, a statement about the risks related to the estimated exposure to butylparaben (Chapter 2) and how it relates to the current toxicological reference value (Chapter 3) is given.

Cumulative exposure to parabens, toxicity and risk assessment
A brief description of several approaches used to address cumulative exposure and risk assessment are provided. The potentially common toxicological mechanism of butylparaben in relation to methyl-, ethyl-, and propylparaben is described. A statement is made about whether the cumulative exposure and risk assessment of these four parabens is justified. This is presented in Chapter 5.

Regulatory framework
Chapter 6 provides the framework for cosmetics, food additives, food contact materials and REACH relevant for butylparaben.

Conclusions and recommendations for further research
In Chapter 7, the conclusions are presented and recommendations for further work are provided.

It should be noted that the various components of the assessment (exposure, toxicity, endocrine-disrupting properties, toxicological

reference values, uncertainties, cumulative exposure and risk assessment, etc.) are described as an inventory in an exploratory manner. Only literature (scientific publications as well as existing opinions) is used and no exposure assessments are carried out as part of this study. Also, an extensive review of available biomonitoring studies is not included. This report is therefore not exhaustive *and does not provide a definitive answer about the possible health risks* related to the presence of butylparaben or other parabens in consumer products. Altogether, this report is an overview and discussion of the exposure, toxicity and risk assessments of butylparaben in light of its potential endocrine-disrupting properties and potential cumulative toxicity.

2 Exposure to butylparaben

2.1 Introduction

Parabens are effective, stable, and sufficiently water soluble preservatives [3]. As the chain length of the ester group of the paraben increases, antimicrobial activity increases, but water solubility decreases [3]. Their properties as preservatives make them suitable for use in a variety of products. Subsequently, exposure can occur via many different sources. The exposure to methyl-, ethyl- and propylparaben was recently reported by RIVM [1]. The present report expands this selection with butylparaben.

The main exposure sources considered are: 1) personal care products, 2) food and 3) medicinal products. These sources of exposure are described below in Sections 2.2 up to 2.4. Section 2.5 addresses the exposure via other consumer products. In Section 2.6 exposure estimates recalculated from urine measurements in biomonitoring studies are briefly addressed. Section 2.7 summarizes the exposure estimations via the different sources and adds up (aggregates) the exposure to butylparaben via the different sources to come to an aggregate exposure estimate. A brief comparison is made with the recalculated exposure estimates taken from biomonitoring studies. A discussion on the exposure estimations, conducted in the light of uncertainties, is added.

2.2 Exposure to butylparaben via personal care products

2.2.1 Exposure estimates

The evaluation describes the estimation of aggregated exposure to butylparaben across different personal care products with three different tiers: (1) simple summation, (2) summation per use pattern, and (3) probabilistic model simulations (Table 1). Higher tiers represent more refined approaches in aggregating exposure, yielding less conservative estimates. More details on the different tiers can be found in our previous report [1].

Exposure estimates for butylparaben presented in Table 1 from the Tier 1 studies referring to adults all fall within the same order of magnitude (0.006-0.047 mg/kg bw/day). The Tier 2 and 3 studies yield lower exposure doses, estimated as medians (0.0016-0.02 mg /kg bw/day), but the 97.5th percentile, representing the dose for the highly exposed individuals within the population in Tier 3, is estimated to be considerably higher (0.1 mg /kg bw/day). For infants and toddlers, there is also little agreement between the aggregated exposure estimates across personal care products by Guo & Kannan (2013) [4] and Gosens et al. (2011, 2014) [5, 6]; differing as they do by four orders of magnitude (Table 1). Guo & Kannan (2013) estimated butylparaben exposure from personal care products for infants and toddlers in the USA as 0.015 x10^{-3} and 0.009 x10^{-3} mg /kg bw/day, respectively [4], whereas for Dutch toddlers and infants internal exposure was estimated to be 0.20 mg/kg bw/day by Gosens et al. (2011, 2014) [5, 6]. Details about four key studies (Cowan-Ellsberry & Robison (2009) and Csiszar et al. (2017) for adults, and Guo & Kannan (2013) and Gosens et al. (2011, 2014) for children) are provided in the text below.

Table 1. Overview of studies on the aggregate exposure estimation of butylparaben across different personal care products (PCPs) taken from several studies for different populations across different tiers. Exposure values could represent external or internal exposure, taking into account dermal absorption values (see column with Remarks).

Tier	Population	Exposure estimates		Reference
		(mg/kg bw/day)	**Remarks**	
1	Adult females, USA	0.016	**External** dermal exposure; simply summed exposure	Cowan-Ellsberry & Robison (2009) [7]
1	Adult females, USA	0.047	**Internal** dermal exposure (40% absorption); summed exposure to leave-on and rinse-off PCPs with the highest concentration values	Guo & Kannan (2013) [4]
1	Infants (0-1 year), US	0.015×10^{-3}	**Internal** dermal exposure (80% absorption); idem	Guo & Kannan (2013) [4]
1	Toddlers (2-3 year), USA	0.009×10^{-3}	**Internal** dermal exposure (80% absorption); idem	Guo & Kannan (2013) [4]
1	Adults, China	0.006	**External** dermal exposure; simply summed exposure to PCPs with the highest concentration values	Guo et al. (2014) [8]
1	0-3 year olds, the Netherlands	0.47	**External** dermal and oral exposure	Gosens et al. (2011, 2014) [5, 6]
1	0-3 year olds, the Netherlands	0.20	**Internal** dermal exposure (42% absorption) and oral exposure (100% absorption)	Gosens et al. (2011, 2014) [5, 6]
2	Adult females, USA	0.016	**External** dermal exposure; based on co-use patterns for five PCPs	Cowan-Ellsberry & Robison (2009) [7]
2	Adult females, USA	0.007	**External** dermal exposure; based on co-use patterns for nine PCPs	Cowan-Ellsberry & Robison (2009) [7]
2	Adult females, USA	0.002	**External** dermal exposure; based on co-use patterns for nine PCPs, refined using extend of use data	Cowan-Ellsberry & Robison (2009) [7]
2	Adult females, USA	0.0016	**Internal** dermal exposure (80% absorption); based on co-use patterns for nine PCPs, refined using extent-of-use data	Cowan-Ellsberry & Robison (2009) [7]
3	Adult females, USA	0.02 (0-0.1)	**Internal** exposure (dermal absorption probabilistically derived from skin permeation coefficients); mean values (2.5-97.5th percentiles)	Csiszar et al. (2017) [9]

2.2.2 *Key studies on/for the exposure to butylparaben*

Cowan-Ellsberry & Robison (2009)

Based on reported product concentrations by Steinberg (2002, 2006, 2008) and Elder (1984) [7], Cowan-Ellsberry & Robison (2009) first determined a simply summed aggregate exposure across personal care products using data from a survey conducted among 360 women ages 19-65 years in the USA, which was performed by Loretz et al. in 2005 [10-13]. The exposure assessment was further refined by analysed data on patterns of use from a company survey of 3,297 women in the USA [7]. 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 exposure for the products in the survey [7]. However, the survey included only nine different personal care products, whereas the inventory used for the simply summed exposure assessment included 23 different personal care products. Cowan-Ellsberry & Robison (2009) therefore conservatively assumed that the remaining products were all used and the sum of the exposure via the products not included in the survey was added in a refined exposure calculation. This resulted in an external aggregate exposure estimate across personal care products of 0.007 mg/kg bw/day for butylparaben, with co-use data for nine products (Table 1) [7]. These exposure values were further refined by using extent-of-use data for the less frequently used butylparaben relative to methylparaben. This resulted in an aggregate external exposure estimate across personal care products of 0.002 mg/kg bw/day for butylparaben (Table 1) [7]. The study considered dermal exposure from personal care products only and used a dermal absorption factor of 80%. This resulted in an internal exposure estimate of 0.0016 mg/kg bw/day for adults (Table 1) [7].

Csiszar et al. (2017)

In the study conducted by Csiszar et al. (2017), product use is expressed as a uniform distribution for which the minima and maxima are taken from the survey conducted by Loretz et al. from 2005, representing a female population in the USA [10-12]. The concentrations of the parabens in the products are also represented with a uniform distribution, but the minima and maxima were taken from the same data sources described in the simple, summed exposure estimation paragraph by Cowan-Ellsberry & Robison (2009) 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 [14]. Furthermore, Csiszar et al. (2017) use the weight fraction data presented in Guo et al. (2014) and Guo & Kannan (2013) [4, 8, 9]. Csiszar et al. (2017) derived lognormal distributions for the 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 [9]. The stochastic simulation itself was performed with the Monte Carlo approach, consisting of 10,000 iterations. For each iteration a value is randomly taken from the given input distributions for which the exposure is calculated. However, not all samples of the products listed in the inventory necessarily contain the parabens. Therefore Csiszar et al. (2017) adjusted their simulations by adding an appropriate number of zeros, representing such non-exposure in the 10,000 iterations performed [9]. The study considered dermal exposure from personal care products

only and a mean internal exposure of 0.02 mg/kg bw/day was calculated and a 97.5th percentile value of 0.1 mg/kg bw/day (Table 1).

Guo & Kannan (2013)

In addition to the exposure of adult females, Guo & Kannan (2013) also calculated the exposure of USA infants (0-1 year) and toddlers (2-3 years) to butylparaben. Data on use from Wormuth et al. (2006) and the US-EPA exposure factors handbook (2011), which also refers to the studies conducted by Loretz et al. (2005, 2008) [11-13, 15], were coupled to analysed concentrations in ~20 baby care products: shampoo, lotion and oil, diaper cream, and powder. Sunscreen products were excluded. Butylparaben was only detected in lotions and oil (a relatively low weight fraction of only 0.07 mg/kg), which contributed to the exposure estimate for butylparaben. The study considered dermal exposure from personal care products only and used a dermal absorption factor of 80%, which resulted in an internal exposure estimate of 0.015 x 10^{-3} and 0.009 x 10^{-3} mg/kg bw/day for infants and toddlers, respectively (Table 1) [4]. This difference is determined by the difference in body weight between infants (7.8 kg) and toddlers (12.6 kg) in the calculation only.

Gosens et al. (2011, 2014)

The study by Gosens et al. (2011, 2014) [5, 6] was actually performed for the Dutch infant population. The product use data were used in their worst-case approach from the Cosmetics Fact Sheet (from 2006; using European data) and in their worst-case approach 75th percentile values were taken from the Cosmetics Fact Sheet. More detailed information on the amount of product and use frequency was obtained from a very small pilot survey (number of respondents was 28) [6].

This was combined with analysed concentrations of butylparaben (maximum amounts) in 13 categories of baby products (sunscreen, aftersun, shampoo, hair lotion, 2 in 1 shampoo, liquid soap, shower/bath soap, bath oil, body lotion, baby salve, baby wipes, and toothpaste). In all product categories relevant for dermal exposure, butylparaben was detected with maximum amounts varying from 247 to 1473 mg/kg, which is four orders of magnitude higher than seen in the study conducted by Guo & Kannan (2013). In toothpaste, which is relevant for oral exposure, no butylparaben was found. The study considered oral (100% absorption) and dermal exposure from personal care products only and used a dermal absorption factor of 42%, which together resulted in an internal exposure estimate of 0.20 mg/kg bw/day (Table 1) [5, 6]. The main contribution originated from the use of baby wipes and, to a lesser extent, from sunscreen, body lotion and baby salve [5, 6]. The results from the probabilistic modelling approach by Gosens et al. (2011, 2014) were presented in cumulative probability plots, which are used in a risk assessment to compare whether the complete calculated exposed population was below a toxicological reference value for the respective parabens, and results were presented graphically [5, 6].

2.3 Exposure to butylparaben via food

2.3.1 Presence in food

The exposure to methyl-, ethyl- and propylparaben from food has been evaluated previously [1]. Here, the exposure to butylparaben is evaluated. Methyl- and ethylparabens may be intentionally added to food as preservatives in Europe according to Regulation (EC) No.

1333/2008 and 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. However, butylparaben is not allowed for both of these uses in Europe. Furthermore, smaller chain-length 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, botrytised wine, and Bourbon vanilla (Ali et al. (1998) as cited in [3]). Soni et al. (2005) however report that the intake of parabens from natural sources is negligible [3].

2.3.2 Studies into the exposure to butylparaben via food

The literature search yielded two studies by Liao et al. (2013ab) in which the intake of butylparaben via food was assessed. One of these was conducted in an adult population in China [16], and one was conducted in the general population in the USA [17]. Below, these studies are described in detail.

Adult population in China

In 2013, a study into the occurrence of six parabens, including benzyl-, butyl-, ethyl-, heptyl-, methyl- and propylparaben, in food in China was published [16]. In this study, paraben concentrations were found in 282 foodstuffs belonging to 13 food groups, including cereals and cereal products, meat, fish and seafood, eggs, dairy products, bean products, fruits, vegetables, cookies, beverages, cooking oils, condiments, and other things, collected from nine cities in China. The food samples were collected during the summer (July-September) of 2012. The majority of the food samples were purchased from large retail stores and a few samples were purchased from local grocery stores. Brands were chosen to represent available varieties that are commonly consumed by the Chinese, including national, store and specialty brands. For the data analysis, samples with a level below the limit of quantification (LOQ: 0.01 ng/g) were assumed to contain the parabens at a level equal to LOQ divided by 2.

Occurrence

Butylparaben was detected in all 13 food groups that were analysed. Mean concentrations ranged from 0.005 ng/g in the food group ‘others’ (n=13; e.g., jelly, black sesame powder, lotus root starch, milk, tea powder and coffee powder) to 1.75 ng/g in vegetables (n=60; e.g. mushrooms, peanuts, peppers, seaweed, bamboo shoots, potatoes, edible tree fungus, Chinese cabbage and salted mustard). The overall mean concentration of butylparaben in the analysed foods was 0.607 ng/g. Butylparaben accounted for <5% of the total paraben concentrations in the analysed foods (e.g. methylparaben accounted for 59%). The source for the presence of parabens in the foods analysed (natural occurrence, preservative or via migration from food packaging material) was not specified.

To establish whether the 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 or plastic). The results suggested that there was no association between paraben concentrations in foods and the

packaging materials used [32]. The concentrations of parabens in canned foods were low.

Exposure

Based on the analysed concentrations, a mean and high exposure level was calculated for butylparaben. For this purpose, the mean and 95th percentile concentration levels per food group were combined with the mean consumption per food group by adult men and women derived from the literature. The resulting mean and high exposure per sex were divided by a fixed body weight of 62.7 kg for men and 54.8 kg for women. Table 2 lists the calculated exposures to butylparaben.

Table 2. Estimated mean and high (95th percentile) daily exposure (µg/kg bw/day) to butylparaben via food by adults in China (taken from Liao et al. (2013a) [16]).

Population	**Exposure** *(µg/kg bw/day)*	
	Mean	High
Men	0.023	0.087
Women	0.023	0.090

Butylparaben accounted for only 2% of the total exposure to the six parabens in China [16]. For comparison, methylparaben accounted for 69% of the total exposure, ethylparaben 16% and propylparaben 12%. The remaining parabens, benzyl- and heptylparaben, accounted for 0.2% and 0.02% of total exposure, respectively.

General population of the USA

A study similar to the one in China was performed in the USA [17]. In this study, 267 foods belonging to eight food groups, including beverages, dairy products, fats and oils, fish and shellfish, grain products, meat, fruits and vegetables, were analysed for five parabens, including 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 a variety of available manufacturers and most of the foods were of USA origin. For the data analysis, samples with a level below the limit of quantification (LOQ; 0.01 ng/g) were assumed to contain the parabens at a level equal to LOQ divided by 2.

Occurrence

In total, 24% of the analysed foods contained butylparaben at levels above the LOQ, with percentages ranging from 12.1% in beverages ((n=33; e.g. bottled water, carbonated, soft drinks, wine, beer and juice) to 37% in grain products (n=54; e.g. wheat flour, bread, rice, noodles, pie, pasta, pizza, corn products, cookies and cakes). The mean concentrations ranged from 0.006 ng/g in fats and oils (n=5; e.g. salad and cooking oil) to 0.059 ng/g in grain products. The overall mean concentration was 0.030 ng/g.

As in the Chinese study, the source for the presence of butylparaben in the foods (natural occurrence, preservative or via migration from food packaging material) was not specified. Also as in the Chinese study, there was no association between paraben concentrations found in foods and the packaging materials used [17]. The concentrations of parabens in canned foods were low.

Exposure

The analysed concentrations were used to assess the exposure to the five analysed parabens in the USA population. For this purpose, for each paraben and food group, the mean analysed concentration was multiplied by the average per capita consumption rate according to the USA Environmental Protection Agency (EPA) Exposure Factors Handbook and summed over food groups to obtain an estimate of the daily exposure. Calculations were performed for five age groups (Table 3). A high level exposure was calculated by combining the 95th percentile per capita food consumption per age group from the same Handbook with a mean concentration for each food group. Body weights to express the mean and high intakes per kg of body weight were also obtained from the Handbook.

The mean and high exposures to butylparaben are listed in Table 3. The highest exposures were estimated for infants (<1 year) and young children (1 to <6 years), and the lowest in persons aged 11 and above.

Table 3. Estimated mean and high (95th percentile) daily exposure (µg/kg bw/day) to butylparaben via food by five age groups in the USA (taken from Liao et al. (2013b) [17]).

Population	**Exposure** *(µg/kg bw/day)*	
	Mean	High
Infants (< 1 year)	0.001	0.005
Young children (1 to <6 years)	0.002	0.005
Children (6 to < 11 years)	0.001	0.003
Teenagers (11 to <21 years)	0.001	0.002
Adults (≥21 years)	0.001	0.001

Butylparaben accounted on average, across the five age groups, for only 0.2% of the total exposure to the five parabens in the USA [17]. For comparison, methylparaben accounted for 46%, ethylparaben for 38% and propylparaben for 15% of total exposure. The remaining paraben, benzylparaben, also accounted for 0.2% of the total exposure to the five parabens.

The authors observed that several food items that may contain parabens were not included in the current assessment – for example eggs, condiments, fast food, and breast milk [17]. Due to this, the estimated intakes of the parabens may underestimate the actual exposures. Furthermore, the number of analysed samples was low and the paraben concentrations varied within food groups.

2.4 Exposure to butylparaben via medicinal products

2.4.1 Occurrence

Butylparaben is used as preservative in medicinal products, but to a lesser extent than methyl- and propylparaben (Table 4), and it is predominantly used in pharmaceutical formulations for the cutaneous route [18]. A

search in the Medicines Information Bank of the Dutch Medicines Evaluation Board (CBG-MEB) revealed nine approved medicinal products containing butylparaben[1]. The majority of these products (six products) are topical formulations. There are also liquid oral formulations (two products) and a rectal formulation. The concentration of butylparaben 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 therefore not publicly known. However, handbooks on pharmaceutical excipients indicate concentration ranges of 0.006 to 0.05% for oral suspensions and 0.02 to 0.4% for topical preparations [19].

Table 4. Information on the application of methyl- (MeP), ethyl– (EtP), propyl- (PrP) and butylparaben (BtP) in medicinal products.*

	MeP	**EtP**	**PrP**	**BtP**
Number of products in CBG-MEB database*	263	6	183	9
	(mg/kg bw/day)			
Maximum exposure**	2.3	n.d.	0.83	n.d.

*Search performed on June 21, 2018. ** Maximum exposure values estimated very roughly and worst-case by the European Medicines Agency (EMA); n.d. = exposure has not been determined.

Moreta et al. (2015) performed measurements on parabens in several medicinal products in the USA [20]. Butylparaben was only detected in liquids or creams and four of the 32 analysed liquid or creams contained butylparaben. According to this study, the maximum detected value for butylparaben in several medicinal products in the USA was 0.14 mg/g in a liquid or cream [20].

2.4.2 Exposure

The literature review conducted by the National Toxicology Program (NTP) from 2005 on butylparaben contains a conservative exposure assessment from medicinal products [21]. This is based on the long-term use of a product for the relief of gastric distress. The consumption of 25 ml/day of such a product, made up of 0.0067% butylparaben (concentration found in Fosamax), would lead to an exposure of 1.71 mg/day. A teaspoon dose (~5 g) of a preparation that is made up of 0.018% butylparaben intended for short-term use by children would amount to 0.9 mg butylparaben per serving.

The search in the Medicines Information Bank of CBG-MEB revealed two approved liquid oral formulations containing butylparaben. But the butylparaben concentration of these products is unknown. Because the exposure assessment by the NTP is likely outdated and possibly not relevant for the European situation, no relevant exposure calculation for the exposure to butylparaben from medicinal products is available.

2.5 Exposure to butylparaben via other sources

In addition to exposure via personal care products, food and medicinal products, exposure to parabens could occur via other consumer products as well. Though other parabens (especially methyl- and propylparaben)

[1] https://www.Geneesmiddeleninformatiebank.nl (21/06/2018)

are used more often, butylparaben can also be present. Unfortunately, there are no exposure studies with regard to such specific sources, only studies mentioning the presence (or concentration) in specific products, such as household pesticides [22], baby teethers [23], and paper products [24].

A recent study by Pasto-Nieto et al. (2017) analysed the ingredients of 2,300 products representing different categories (including household cleaning products, n=209) bought in the first half of 2015 in Spain. The presence of various preservatives listed on the label was analysed [25]. No methyl-, ethyl-, propyl- or butylparaben were found in these household cleaning products (isothiazolinones such as MI, CMIT, BIT and/or OIT (present in 64% of these products), or bronopol (in 17% of the products) were used as preservatives in household cleaning products instead) [25].

In addition, we consulted the Database of chemicals in consumer products[2] ("*Database over kemiske stoffer i forbrugerprodukter*") kept by the Danish EPA, where products containing butylparaben are recorded. Mentions of the following products, other than personal care products, containing butylparaben were found: carnival and theatre make-up, pleasure gel, slimy toys, and animal care products.

Exposure to butylparaben via other additional, environmental sources can occur as well, such as from indoor dust [26, 27]. In a study conducted by Wang et al. (2012), 158 indoor dust samples from China, South Korea, Japan and the USA were collected (period 2006-2012) and the concentrations of six parabens and their common metabolite p-hydroxybenzoic acid (PHBA) were detected [26]. Methyl- and propylparaben were the most predominant substances detected in the samples, with methylparaben accounting for 42 to 73% of the total paraben concentration (mean concentrations per country ranging from 226 to 1,670 ng/g), and propylparaben accounting for 12 to 46% of the total paraben concentration (mean concentrations per country ranging from 123 to 761 ng/g) [26]. The concentration of butylparaben (like ethylparaben) was minor, with a mean concentration per country ranging from 1.5 to 42 ng/g. The estimated 95th percentile daily intake values of butylparaben in this study via dust ingestion was 5-10 times higher for children than it was for adults, and were the highest in South Korea (0.11 ng/kg bw/day) and Japan (0.10 ng/kg bw/day) [26].

Altogether, though less present than methyl- and propylparaben, exposure to butylparaben can potentially occur through a great variety of sources and products other than personal care products, food and medicinal products. Although only limited information is available on the content and concentration within different products and from different sources, and so subsequently very limited information is available on the exposure via these additional sources, the exposure is usually regarded to be minor in comparison with the exposure via personal care products.

[2] http://mst.dk/kemi/kemikalier/fokus-paa-saerlige-produkter/database-over-kemiske-stoffer-i-forbrugerprodukter/ (21/02/2018)

2.6 Exposure estimates recalculated from biomonitoring data

Butylparaben is mainly excreted via the urine. Metabolites and conjugates (mainly glycine, glucuronic acid and sulfuric acid conjugates), but also a small fraction of the free parent substance, can therefore be detected in the urine of humans [3, 28]. The major part of butylparaben is excreted as metabolites of p-hydroxybenzoic acid (PHBA), which cannot be directly used to discriminate metabolites from other parabens (e.g. methyl-, ethyl- or propylparaben). As the length of the alkyl chain increases, the rate of urinary excretion of p-hydroxybenzoic acid decreases [3, 28].

There are numerous studies that report on biomonitoring data collected on parabens in urine, often in combination with epidemiological findings. As mentioned in the introduction, an extensive review of those available biomonitoring studies has, however, been excluded. 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 [7, 28-30]. For that reason, the topic is addressed briefly here in order to provide insight into the relevance of the aggregated estimated modelled exposure. Total exposure estimates for butylparaben, back-calculated from metabolites in urine in different studies, are described below [7, 28-30] and summarized in Table 5. Because of the (specific) study populations and periods, their representativeness for the current situation in the general population in the Netherlands and/or Europe is likely very limited. For details on the calculation methods used, reference is made to the respective publications.

Table 5. 95th percentile values of back-calculated daily intake values (µg/kg bw/day) for butylparaben from selected biomonitoring studies.

Reference	**Study population (size) and period**	**Exposure, 95th percentile** *(µg/kg bw/day)*
Cowan-Ellsberry & Robison (2009) [7], based on Ye et al. (2006) [28] *	Adults (demographically diverse group of males and females), USA (n=100), 2003-2005	0.86*
Moos et al. (2017) [30]	Students (age 20-30 years), Germany (n=660), 1995-2012	4.6
	- male (n=330)	2.2
	- female (n=330)	7.0
Guo et al. (2017) [29]	Children (age 3 years), Agricultural region in China (n=436), 2012-2013	0.64
	- male (n=221)	0.53
	- female (n=215)	0.98

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

Ye et al. (2006) and Cowan-Ellsberry & Robison (2009)
In a USA study performed by Ye et al. (2006), urinary concentrations of different parabens and their conjugates were examined in a demographically diverse group of 100 adults with no known exposure to parabens [28]. The urine samples were collected from 2003 to 2005. In 69% of the urine samples, butylparaben (n-butylparaben and

isobutylparaben combined) or their metabolites (conjugates of the respective parabens) were detected [28]. In the study conducted by Cowan-Ellsberry & Robison (2009), the 95th percentile values from these measurements were used in order to estimate internal exposure concentration, of 0.86 µg/kg bw/day using a steady-state toxicokinetic model.

Moos et al. (2017)
In a study published by Moos et al. (2017) that was conducted among German male and female students at Muenster University (20-30 years old), 660 24-hr urine samples were collected from 60 subjects (30 female, 30 male) per year, from 1995 to 2012 [30]. By using a calculated urinary excretion factor of 5.6% for butylparaben, oral equivalent daily intake values were back-calculated from urinary levels [30, 31]. This resulted in a median daily intake level of 0.2 µg/kg bw/day for butylparaben [30]. The 95th percentile is 4.6 µg/kg bw/day. There was a difference between the male and female sub-population, with respective median values of 0.1 and 0.5 µg/kg bw/day, and with respective 95th percentile values of 2.2 and 7.0 µg/kg bw/day for males and females [30]. The exposure of women was significantly higher than that of men.

Guo et al. (2017)
In a recent publication by Guo et al. (2017), urinary concentrations were detected in children (n=436) three years of age, during the 2012-2013 period in an agricultural region in Jiangsu province, China [29]. In 98% of the samples, butylparaben was detected (free + deconjugated glucuronide and sulphate metabolites). A median concentration of 0.03 ng/ml total butylparaben was detected. The 95th percentile value reported was 0.42 ng/ml. From this, a median daily intake of 0.06 µg/kg bw/day was estimated [29]. The 95th percentile value for the total, male and female (sub)populations are 0.64, 0.53 and 0.98 µg/kg bw/day respectively (Table 5).

2.7 Summarizing butylparaben exposure estimations

Three major product sources were included for the exposure estimation: personal care products, food and medicinal products. Table 6 (for adults) and Table 7 (for children) present the most relevant estimates for butylparaben, added to those for methyl-, ethyl- and propylparaben from our previous report [1]. Exposure to parabens, including butylparaben, can potentially also occur from a great variety of other consumer products and environmental sources such as toys, animal care products and indoor dust. 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 challenging because of differences in the quality of information and uncertainties in the different sources. The exposure estimates for butylparaben are summarized below (for more details on the exposure estimates for the other parabens, see our previous report [1]).

2.7.1 Exposure via personal care products

The exposure to butylparaben from personal care products has been estimated in different studies, in different tiers, for different populations

(Table 1). The internal exposure to butylparaben is conservatively estimated for adults to be 0.0016 mg/kg bw/day by Cowan-Ellsberry & Robison (Tier 2) (2009) [7]. A ~tenfold higher mean exposure value of 0.02 mg/kg bw/day was estimated stochastically by Csiszar et al. (2017) [9]. The 97.5th percentile representing the dose for the highly exposed individuals in this study was 0.1 mg /kg bw/day, a factor ~60 higher than the exposure estimate by Cowan-Ellsberry & Robison (2009). This is remarkable because, as for the other parabens (methyl-, ethyl- and propylparaben), the exposure estimates by Cowan-Ellsberry & Robison (2009) where very similar to the 97.5th percentile of the exposure estimate by Csiszar et al. (2017) (Table 6). This can be largely explained by the fact that Csiszar et al. (2017) take into account more products containing butylparaben (stay-on products with relative high concentrations of butylparaben) than do Cowan-Ellsberry & Robison (2009). As there is no ground to decide which product and weight fraction selection is more representative for the current situation in the Netherlands with respect to the adult exposure to butylparaben via personal care products, the highest value of 0.1 mg /kg bw/day by Csiszar et al. (2017) has been chosen for the aggregate exposure, as has been done for methyl-, ethyl- and propylparaben in our previous report (Table 6).

An exposure estimate of 0.2 mg/kg bw/day was determined by Gosens et al. (2011, 2014) for children aged 0-3 years in the Netherlands [5, 6] using analysed concentrations of butylparaben in baby products in the Netherlands [32]. The exposure calculation, however, was very much worst-case using the Cosmetics Fact Sheet's 75th percentile values and additional data from a limited survey. The main contributor to the estimated exposure consisted of the exposure via baby wipes [5, 6]. Nowadays, however, parabens seem to be used to a very limited degree in baby wipes. In a recent exploration conducted in the context of Waarzitwatin.nl (a webpage intended to provide consumers with current, reliable and scientifically based information on the presence and content of chemicals in consumer products), parabens were indicated (both methyl- and ethylparaben) on the labels of only two products out of 37 different baby wipe products. Propyl- and butylparaben were not found. This estimated exposure of children to 0.20 mg/kg bw/day is ~ten thousand fold higher than the Guo & Kannan (2013) estimated butylparaben exposure from personal care products for infants and toddlers in the USA: 0.015 x10^{-3} and 0.009 x10^{-3} mg /kg bw/day, respectively [4]. Their exposure estimate is solely dependent on the analysed concentration in two products in the category 'lotion and oil' and is therefore likely an underestimation of the actual exposure, as the authors mention themselves [4]. Therefore, the exposure estimate from Gosens et al. (2011, 2014) has been chosen for the aggregate exposure calculation for children to butylparaben, as has been done for the aggregate exposure to methyl-, ethyl- and propylparaben in our previous report (Table 7).
The representativeness of these values with regard to the present situation in the Netherlands remains largely uncertain, whereas the underlying product use data in the available aggregate exposure studies may be outdated (> 10 years ago) and/or refer to the US. At least with regard to the European situation, more recent product use datasets are

available which potentially could be used for new exposure estimation for parabens via personal care products [33-36].

2.7.2 *Exposure via food*

The exposure to butylparaben via food was studied in two exposure studies (in China and the USA) based on actual analysed concentrations found in food products. There were no relevant studies into the actual intake of butylparaben via food in Europe. The highest mean butylparaben concentrations reported in the food products considered were 0.059 ng/g in grain products in the USA study and 1.75 ng/g in vegetables in the Chinese study. In both studies, the sources for the presence of butylparaben in food were not identified (preservative, natural occurrence and/or migration from food packaging material) [16, 17].

Based on analysed concentrations and mean consumption patterns, the 95th percentile estimated daily exposure to butylparaben for adults was 0.001 µg/kg bw/day in the USA study (Table 3) [17]. For children (<1 year), the 95th percentile estimated daily exposure was 0.005 µg/kg bw/day (Table 3) [17]. In the Chinese study, the 95th percentile daily exposure estimates for men and women were 0.087 and 0.090 µg/kg bw/day, respectively (Table 2) [16].

The approach to assess the exposure via food using analysed concentrations represents the potential exposure the best, although this information was only available for China and the USA, where the regulation of parabens used in foods is likely to be different from that in the EU. Consumption patterns also differ. It is therefore unclear how well these exposures represent the situation in the Netherlands and/or Europe. At most, the estimations may only provide an impression of the actual level of exposure. Nevertheless, the highest exposure values were chosen for the aggregate exposure calculation (Table 6 and 7).

2.7.3 *Exposure via medicinal products*

As there are no data on product concentrations for butylparaben, as is the case with ethylparaben, an analogous calculation as has been performed by EMA for methyl- and propylparaben cannot be made. Because no other, relevant information on exposure to butylparaben via medicinal products is available, the exposure to butylparaben via medicinal products in the Netherlands cannot be estimated (Table 4). There are only a limited number of medicinal products on the Dutch market containing butylparaben (9), compared to products containing methylparaben (263) or propylparaben (183). Most of these nine products are intended for use for a short period of time, i.e. from a few days or weeks up to three months. However, chronic exposure could potentially also occur. As the exposure to parabens via medicinal products can be long-term or for a short time, or even absent in a large part of the population, a probabilistic exposure assessment for this source especially would be very valuable.

2.7.4 *Aggregated butylparaben exposure*

Compared with the three parabens considered previously, the exposure to butylparaben was the lowest, at least within the adult populations. The aggregated butylparaben exposure for adults (Table 6) and children (Table 7) could be estimated by adding the estimates from personal care

products and food, although the level of detail for the estimates differed greatly between the two sources. As no relevant estimate for butylparaben exposure via medicinal products was available, an aggregated exposure value which is comparable with those for methyl- and propylparaben could not be calculated because this source is left out for the internal exposure estimate of butylparaben (as is the case for ethylparaben previously). The overall aggregate exposure estimate for butylparaben was ~0.1 mg/kg bw/day for adults and ~0.2 mg/kg bw/day for children. These estimates were almost entirely determined by exposure via personal care products.

Table 6. The exposure estimates of adults to methyl- (MeP), ethyl- (EtP), propyl- (PrP) and butylparaben (BtP) through three sources from different studies (with very different qualities of estimation). Where there are multiple values per source, the value in bold was used for TOTAL. The exposure estimates for MeP, EtP and PrP have been described previously [1], the exposure estimates for BtP are described in the present report.

Source	**Estimated external exposure** *(mg/kg bw/day)*				**Estimated internal exposure** *(mg/kg bw/day)*				**Route**	**Quality of estimate + Reference**
	MeP	**EtP**	**PrP**	**BtP**	**MeP**	**EtP**	**PrP**	**BtP**		
Personal care products, US	**1.61**	**1.70**	**0.80**	**0.016**	0.79	0.13	0.34	0.0016	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 [7].
Personal care products, US	-	-	-	**-**	**0.8**	**0.2**	**0.3**	**0.1**	Dermal only	P97.5 values by stochastic modelling; more realistic. Dermal absorption fraction probabilistically derived from skin permeation coefficients [9].
Food, China	1.49 $x10^{-3}$	0.89 $x10^{-3}$	0.43 $x10^{-3}$	0.09 $x10^{-3}$	1.49 $x10^{-3}$	0.89 $x10^{-3}$	0.43 $x10^{-3}$	0.09 $x10^{-3}$	Oral	P95 value for male population based on analysed concentrations and mean consumption patterns [16].
Food, China	**1.56 $x10^{-3}$**	**0.92 $x10^{-3}$**	**0.45 $x10^{-3}$**	**0.09 $x10^{-3}$**	**1.56 $x10^{-3}$**	**0.92 $x10^{-3}$**	**0.45 $x10^{-3}$**	**0.09 $x10^{-3}$**	Oral	P95 value for female population based on analysed concentrations and mean consumption patterns [16].

Source	Estimated external exposure *(mg/kg bw/day)*				Estimated internal exposure *(mg/kg bw/day)*				Route	Quality of estimate + Reference
	MeP	**EtP**	**PrP**	**BtP**	**MeP**	**EtP**	**PrP**	**BtP**		
Food, US	0.36 $x10^{-3}$	0.40 $x10^{-3}$	0.10 $x10^{-3}$	0.00 $x10^{-3}$	0.36 $x10^{-3}$	0.40 $x10^{-3}$	0.10 $x10^{-3}$	0.00 $x10^{-3}$	Oral	P95 value based on analysed concentrations and mean consumption patterns [17].
Medicinal products, EU	**2.3**	**?**	**0.83**	**?**	**2.3**	**?**	**0.83**	**?**	Oral, no dermal	Maximum exposure estimated very roughly and worst-case [18, 19].
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	P95 value for male population based on measured concentrations daily ingestion rates [37].
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	P95 value for female population based on measured concentrations daily ingestion rates [37].
TOTAL	**~3.9**	**~1.7**	**~1.6**	**~0.0**	**~3.1**	**~0.2**	**~1.2**	**~0.1**		

Table 7. The exposure estimates of children to methyl- (MeP), ethyl- (EtP), propyl- (PrP) and butylparaben (BtP) through three sources from different studies (with very different qualities of estimation). Where there are multiple values per source, the value in bold was used for TOTAL. The exposure estimates for MeP, EtP and PrP have been described previously [1], the exposure estimates for BtP are described in the present report.

Source	**Estimated external exposure** *(mg/kg bw/day)*				**Estimated internal exposure** *(mg/kg bw/day)*				**Route**	**Quality of estimate + Reference**
	MeP	**EtP**	**PrP**	**BtP**	**MeP**	**EtP**	**PrP**	**BtP**		
Personal care products	**2.32**	**0.36**	**1.05**	**0.47**	**1.01**	**0.20**	**0.41**	**0.20**	Dermal/ oral	Simple summed exposure, worst-case deterministic estimate from several products. Internal exposure calculated with dermal absorption fraction of 36%, 55%, 37% and 42% for MeP, EtP, PrP and BtP, respectively [5].
Food, France	0		-	-	0		-	-	Oral	P90 value for children aged 1-6 months estimated on food records, and MPLs [38].
Food, France	0.07 x10^{-3}		-	-	0.07 x10^{-3}		-	-	Oral	P90 value for children aged 7-12 months estimated on food records, and MPLs. Therefore very conservative [38].
Food, France	0.9 x10^{-3}		-	-	0.9 x10^{-3}		-	-	Oral	P90 value for children aged 13-36 months estimated on food records, and MPLs. Therefore very conservative [38].
Food, US	**1.38 x10^{-3}**	**1.74 x10^{-3}**	0.39 x10^{-3}	**0.01 x10^{-3}**	**1.38 x10^{-3}**	**1.74 x10^{-3}**	0.39 x10^{-3}	**0.01 x10^{-3}**	Oral	P95 value for children aged <1 year based on analysed concentrations and mean consumption patterns in USA [17].

Source	**Estimated external exposure** *(mg/kg bw/day)*				**Estimated internal exposure** *(mg/kg bw/day)*				**Route**	**Quality of estimate + Reference**
	MeP	**EtP**	**PrP**	**BtP**	**MeP**	**EtP**	**PrP**	**BtP**		
Food, US	1.03 $x10^{-3}$	0.68 $x10^{-3}$	**0.45 $x10^{-3}$**	0.01 $x10^{-3}$	1.03 $x10^{-3}$	0.68 $x10^{-3}$	**0.45 $x10^{-3}$**	0.01 $x10^{-3}$	Oral	P95 value for children aged 1 to 6 years based on analysed concentrations and mean consumption patterns in USA [17].
Medicinal products	**2.3**	**?**	**0.83**	**?**	**2.3**	**?**	**0.83**	**?**	Oral, no dermal	Maximum exposure estimated very roughly and worst-case [18, 19].
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	P95 value based on measured concentrations daily ingestion rates [37].
TOTAL	**~4.0**	**~0.4**	**~1.9**	**~0.5**	**~3.3**	**~0.2**	**~1.2**	**~0.2**		

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 (Table 5) can be compared to the aggregate exposure for the exposure via personal care products, food and medicinal products. Only the back-calculated *internal* exposure values by Cowan-Ellsberry (2009) based on Ye et al. (2006) can be compared to the calculated aggregated *internal* exposure estimates [7, 28].

For adults, the 95th percentile values for the back-calculated internal exposure to butylparaben of 0.86 µg/kg bw/day from urine metabolite levels as calculated by Cowan-Ellsberry & Robison (2009) for a general USA population [5] is over a factor of 100 lower than the calculated aggregated internal exposure of ~0.1 mg/kg bw/day from personal care products and food (the exposure to butylparaben via medicinal products could not be estimated) in the present report (Table 6).

If we consider the back-calculated internal exposure to butylparaben or the estimated daily intake values from biomonitoring studies based on 95th percentile values of urinary concentrations of parabens, as calculated in different studies in several specific populations according to different methods (Table 5), as a proper estimation of the actual exposure, then the difference of one up to two orders of magnitude illustrates the worst-case character of the model calculations of the aggregate exposure estimate. However, it is unclear how well these exposure estimates represent the current situation in the Netherlands. At most, the estimations may provide only an impression of an actual level of exposure [39].

2.7.6 *Uncertainties*

The calculated aggregate exposure to butylparaben (Tables 6 and 7) was based on several studies with a wide variety in set-up, type and level of detail, and the assumptions made. To better illustrate the uncertainties present in the current (worst-case) aggregated exposure estimation, Table 8 presents a summary of the main uncertainties and their implications for the exposure value. Overall, uncertainties point in the direction of an overestimation of the total exposure, as indicated in the comparison with the back-calculated exposure levels from biomonitoring studies (Section 2.7.5).

Table 8. Overview of the main factors within the sources of exposure to butylparaben and the effect of their possible uncertainty on the exposure assessment (↑ = increases exposure estimate, ↓ = decreases exposure estimate, - = no effect on exposure estimate due to uncertainty, ? = unknown).

Source	Description	Effect on Exposure estimate
Factor		
Personal care products		
Aggregation method	Method of aggregation of exposure across different products - dependent on tier	↑ / -
Product use data	Frequency of products used and amount of product applied	↑ / ↓
Concentration data	Concentration of parabens in personal care products is possible currently lower, because of the suspicion of ED properties and public attention, and/or legislation	↑ (?)
Presence data	The fraction of products in which parabens are present are possible currently lower, because of the suspicion of ED properties and public attention, and/or legislation	↑ (?)
Retention factor	The fraction of product that stays on the skin after application are chosen pragmatically, not based on investigation	↑ / ↓
Dermal absorption	There is uncertainty about the extent to which the parabens are taken up from the skin to the internal system - in most assessments a worst-case percentage is chosen	↑
Food		
Coverage of sources	Not all sources of exposure were covered	↓
High exposure estimates	Mean consumption x 95th percentile concentration per food group summed over food groups	↑
Populations	Representativeness of the studies for the Netherlands	?
Medicinal products		
Concentration data	No data for butylparaben - therefore not taken into account	↓
Other consumer products		
Exposure data	Virtual no information on use of and exposure to butylparaben from other sources - therefore excluded	↓

2.8 Discussion on the exposure assessment with regard to butylparaben, and parabens in general

With regard to personal care products, it seems that the application of butylparaben and other parabens as a preservative is decreasing. The uncertainties in the present exposure assessments of butylparaben (Section 2.7.6) could be reduced by performing a more relevant exposure assessment using more recent products use, concentration and presence data that better represents the current situation in the Netherlands or

Europe. This also accounts for the methyl-, ethyl- and propylparaben [1]. More recent data are present on product use and, by using these, a more realistic estimation for the exposure to parabens from personal care products would be possible, for example, with the probabilistic aggregate consumer exposure model (PACEM) [40, 41]. However, as model input, actual and relevant (i.e. from the Netherlands) concentration data in non-food consumer products are also needed.

For food, the high exposure levels estimated for populations in China and the USA also overestimate the exposure based on the available data due to the assumption that all analysed food groups contain the parabens at the 95th percentile of the analysed samples. However, the aggregate exposure estimates showed that the contribution via food to the total exposure is small (<1%) for all parabens and, therefore, further refinement of the exposure assessment of parabens from food is likely not relevant. Especially for butylparaben, which is not allowed as a food additive in the EU, nor as a food contact material.

For medicinal products, no exposure to butylparaben was estimated, such as previously no exposure to ethylparaben from medicinal products could be estimated [1]. Data with respect to product content and concentration related to product use would be helpful to (better) estimate this exposure for all parabens. Because the exposure via medicinal products can be chronic, though is usually for a short period of time or even absent in a part of the population, a probabilistic exposure assessment for this source would be especially valuable. This is, however, not possible to execute as long as data on product concentration and use are not adequately available.

Exposure to parabens can occur through a great variety of other sources and products, other than personal care products, food and medicinal products. However, in literature, quantitative exposure estimations for such exposure are virtually absent due to the absence of concentration data as well as survey data on use of consumer products other than personal care products. Nevertheless, exposure via these other sources is usually regarded to be very minor compared with the exposure via personal care products, food and medicinal products.

3 Toxicity of butylparaben

3.1 Introduction

This chapter provides an overview of the available information on toxicokinetics (Section 3.2) and hazard identification (Sections 3.3-3.9) for butylparaben. Studies on reproductive and developmental toxicity and endocrine-disrupting (ED) properties are discussed in greater detail because the focus of this review is on the possible ED potential of butylparaben. In Section 3.10, the endocrine activity of butylparaben is discussed in relation to the EU ED criteria and EFSA-ECHA guidance on endocrine disruption for biocides and pesticides [42, 43].

3.2 Toxicokinetics

3.2.1 Kinetics

Butylparaben can be absorbed via both oral and dermal exposure routes. Absorption from the gastrointestinal tract in rats is rapid, followed by extensive metabolism (both in the gastrointestinal tract and in the liver) by non-specific esterases to para-hydroxybenzoic acid (PHBA) with free butylparaben not detectable in plasma. Elimination primarily takes place via urine [44, 45]. Extensive metabolism (primarily hydrolysis to PHBA) following oral absorption is also found in rabbits and dogs [3]. Recently, similar results were shown in human volunteers who were given butylparaben orally, PHBA being the primary metabolite in urine (approximately 60% of the excreted dose). Free and conjugated butylparaben in urine made up respectively 0.06 and 5.5% of the excreted dose. Other minor metabolites were p-hydroxyhippuric acid (PHHA) and 3-hydroxybutylparaben [46]. Hydrolysis of butylparaben was observed *in vitro* in human plasma, as well as pooled human liver microsomes. Upon the addition of UDP-glucuronic acid to human liver microsomes, butylparaben-glucuronide was also found [47].

Butylparaben is rapidly and relatively well absorbed after dermal administration to rats (around 50% of the applied dose remained unabsorbed). As with oral administration, elimination was primarily via urine and free butylparaben was not detectable in plasma. The only metabolite identified in plasma was PHBA, suggesting minimal or no systemic exposure to butylparaben [45]. Hydrolysis of butylparaben was shown to be three orders of magnitude lower in human skin fractions than in rat skin fractions. Furthermore, hydrolysis was 300-fold lower in human skin microsomes than it was in human liver microsomes, while hydrolysis was in similar order in rat skin and liver microsomes [48]. In human volunteers, free butylparaben was detectable in plasma and urine following daily whole-body topical application, for one week, of a cream containing 2% butylparaben, diethyl phthalate and dibutyl phthalate. In the same study, no effects were found humans with regard to hormone levels (thyroid-stimulating hormone, TSH; luteinizing hormone, LH; estradiol; Inhibin B; thyroxine, T_4; free thyroxine, FT_4) [49, 50]. It should be noted that this scenario exceeds the worst case of normal use, as was calculated by the SCCS in 2013. Furthermore, the study was confounded because the combination of the three substances at high doses may have saturated metabolism or may have hampered

dermal absorption, thereby either increasing or decreasing the absorption of intact esters [51]. In a recent publication, permeation of butylparaben was detected in hairless mouse full skin and human skin epidermis in an *in vitro* method according to OECD TG 428. Only parent concentrations were measured. The permeability coefficient (K_p) was 0.56, 0.40 and 0.37 cm/h x 10^{-1} for 0.1, 0.4 and 2% butylparaben, respectively [52].

These results indicate that, in contrast to rats, butylparaben escapes complete hydrolysis following oral or dermal exposure in humans. This is supported by several biomonitoring studies that identified conjugated and free butylparaben in human urine [53-55]. Furthermore, due to differences in esterase activity in human skin and liver, a greater proportion of butylparaben could be systematically available following dermal exposure, compared with oral exposure. This effect is essential with regard to the interpretation or design of animal toxicity studies. Therefore, more data on toxicokinetics are needed to clarify interspecies differences in order to obtain insight into the human relevance of animal studies for butylparaben.

3.2.2 Dermal absorption values

The SCCS (2010) has considered three *in vitro* dermal absorption studies [56-58] to derive a dermal absorption value of 3.7% for butylparaben as a conservative estimate [59]. This value was derived from a mean dermal absorption of 37%, as determined in split-thickness human skin (which lacks major skin metabolism). This value was corrected using a conservative factor of 10 to account for the extensive metabolism (resulting in butylparaben concentrations 65 to 140 times lower than PHBA) observed in full-thickness human skin [59]. The SCCS has questioned the relevance of rat models to derive toxicokinetic values for parabens, as the available data suggests there are substantial differences in the systemic availability of butylparaben in rodents or humans following dermal exposure [59, 60]. The SCCS argues that, for a sound risk assessment, relevant human data regarding metabolism is missing [7]. Other risk assessment studies have used higher (conservative) factors for dermal absorption, ranging from 40 to 80%, to account for the high variability in reported factors [4, 7]. Gosens et al. (2014) have used a dermal absorption value of 42% for deterministic modelling with conservative assumptions, but a range of 1-55% (the lowest and highest value reported) for a probabilistic (person-oriented) approach [5].

3.2.3 Oral absorption values

The study in human volunteers indicates extensive metabolism, as only 0.06% of the excreted dose was recovered as free butylparaben [46]. Unfortunately, plasma concentrations were not detected, thereby leaving uncertainties with regard to the actual systemic availability of butylparaben. Hence, in the absence of substance-specific data on absorption, a default absorption factor of 100% is used by the SCCS, EFSA and others.

3.2.4 Conclusions on toxicokinetics

Studies demonstrate that the metabolism of butylparaben in rats is more effective than it is in humans, especially during dermal uptake. Therefore, the relevance of rat studies are currently under debate, since

they potentially underestimate the effects in humans. More data on toxicokinetics are needed to clarify these interspecies differences in order to gain insight into the human relevance of animal studies for butylparaben.

3.3 Acute toxicity

Butylparaben displayed low acute toxicity in mice (oral LD_{50} value >5000 mg/kg bw) and rabbits (dermal LD_{50} value >2000 mg/kg bw). Following intraperitoneal (i.p.) injection, the LD_{50} value in mice was 230 mg/kg bw [21]. In humans, inhalational exposure to butylparaben may cause irritation to the respiratory tract (coughing and shortness of breath). Large doses of butylparaben taken orally may cause irritation in the gastrointestinal tract [21].

3.4 Irritation / corrosion / sensitization

Butylparaben was a mild irritant when dermally applied to guinea pigs (5%) and rabbits (0.3% in product formulation). Moderate irritation was indicated when applied to the skin of rabbits (0.2% in product formulation) [21]. Products containing 0.1-0.8% butylparaben did not cause eye irritation in rabbits [3]. In humans, butylparaben may cause eye and skin irritation [21].
Animal tests indicate that butylparaben is non-sensitizing. Butylparaben (0.1%) was non-sensitizing in guinea pigs when injected intracutaneously. Allergic lesions were observed in 2/20 animals when 5% was applied to the skin of guinea pigs under occlusive conditions. Human studies indicate a low sensitization potential when up to 15% butylparaben was applied [21].

3.5 Repeated dose toxicity

The available repeated dose toxicity studies following short-term or subchronic oral exposure in mice or rats indicate target organ toxicities (atrophy of lymphoid tissue and liver toxicity) at high doses only (NOAEL and LOAEL of 900 and 1,900 mg/kg bw/day, respectively). Based on these high effect levels, these data do not indicate serious effects to human health. No repeated dose toxicity studies following dermal or inhalational exposure are available [21].

3.6 Genotoxicity / Carcinogenicity

Butylparaben was not genotoxic in an Ames assay (tested up to 1,000 mg/plate) and in Chinese hamster CHO-KI ovary cells. A 1-3% increase in polypoid cell production was found in Chinese hamster cells at 0.06 mg/mL (only dose tested), however no indications for chromosomal aberrations were found in Chinese hamster fibroblasts when butylparaben was tested at 60 mg/ml. An *in vivo* comet assay, in which animals were dosed with 2,000 mg/kg butylparaben, did not indicate treatment-related DNA damage. Taking all these data into account, butylparaben is not considered genotoxic [21, 61].
Carcinogenic effects were investigated in mice (0.15, 0.3, or 0.6% in diet) after oral administration for up to 102 weeks. There were no statistically relevant findings that could be related to the treatment. However, as tumour incidences and mortality was high in both control and treatment groups, the reliability of the study was put into doubt by the EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids

and Materials in Contact with Food (AFC) in 2004 [Cited in 21, 62]. In a similar study in which mice were treated with the same doses for 106 weeks, no carcinogenic effects were identified. In rats, oral administration of butylparaben (0.6 or 1.2% in the diet) did not reveal carcinogenic potential [21].

3.7 Developmental and reproductive toxicity

The focus of this report is on the known endocrine modifying effects of butylparaben mainly as regards the reproductive system. For this reason, studies on reproductive and developmental toxicity relevant to ED-related endpoints are discussed in more detail.
The EU Scientific Committee on Consumer Products (SCCP), the predecessor of the Scientific Committee on Consumer Safety (SCCS), published a scientific opinion in 2005 on parabens, which was updated several times up to 2013 [51, 59, 63-66]. Of the available data, the following studies were considered most relevant with respect to the developmental and reproductive toxicity of butylparaben.

Fisher et al. (1999)
In a developmental toxicity study, male Wistar rats were treated by subcutaneous injection with 2 mg/kg bw/day butylparaben on postnatal days 2 to 18 in order to investigate alterations in the structure of the testicular excurrent ducts [67]. Animals were sacrificed four hours after the last injection and testis weight was recorded. Histopathology and immunochemistry (i.e. immunoexpression of aquaporin-1) of the testes and epididymides were assessed as well. Treatment with the potent estrogenic compounds diethylstilbestrol and ethinylestradiol affected each of these parameters. None of the parameters was affected by treatment with butylparaben. Therefore, 2 mg/kg bw/day was considered as the NOEL and can additionally be regarded as a NOAEL.

Oishi (2001)
To investigate potential reproductive effects in male Wistar rats, three-week-old rats were fed a diet containing 0.01, 0.10 or 1.00% butylparaben (actual intake was respectively 10.4, 103 and 1,026 mg/kg bw/day) *ad libitum* for 8 weeks [68]. Treatment did not affect the weight of the testes, ventral prostates, preputial glands and seminal vesicles with coagulation glands. The weight of the epididymides and serum testosterone concentrations were decreased at the mid and high doses. Sperm count in both testes and epididymides was affected in all dose groups, resulting in a LOAEL of 10.4 mg/kg bw/day.

Oishi (2002a)
The potential reproductive effects of butylparaben were also investigated in Crj:Cd-1 mice. Four-week-old mice were fed 0.01, 0.10 or 1.00% butylparaben through the diet (*ad libitum*), resulting in an average intake of 14.4, 146 or 1,504 mg/kg bw/day, respectively [69]. Treatment did not affect the weight of the liver, ventral prostates, seminal vesicles or preputial glands. The weight of the epididymides and serum testosterone concentrations were decreased at the high dose. Treatment affected spermatogenesis: the number of round spermatids was decreased at the high dose and the number of elongated spermatids was decreased at,

respectively, the high dose and the full dose range. Based on these results, 14.4 mg/kg bw/day was considered the LOAEL.

Daston (2004)
The developmental toxicity of butylparaben was investigated in a study similar to OECD TG 414. Pregnant Sprague-Dawley rats were treated orally with butylparaben on Gestation Day (GD) 6-19 at doses of 10, 100 or 1,000 mg/kg bw/day. Body weights and feed consumption were assessed at three-day intervals. Dams were sacrificed and Caesarion-sectioned on GD20. Maternal investigations included gravid uterus weight, the number and distribution of implantation sites, corpora lutea, live and dead foetuses and early and late resorptions. Foetal investigations consisted of sex determinations, gross external alterations, body weights, soft tissue alterations and skeletal alterations. Maternal weight gain and food consumption was decreased in the high dose group. None of the developmental parameters were altered, hence the NOAEL for developmental toxicity was 1,000 mg/kg bw/day [70].

Hoberman et al. (2008)
In order to replicate the studies by Oishi, 22-day-old male Wistar rats were fed 100, 1,000 or 10,000 ppm butylparaben through diet (*ad libitum*) [71].These doses corresponded to a mean daily intake of 10.9, 109.3 or 1,087.6 mg/kg bw/day, respectively. During the study, blood was collected to determine reproductive hormone levels. Following sacrifice, animals were subjected to gross pathology and (reproductive) organs were weighted and prepared for histopathology. Sperm concentrations and motility were evaluated as well. None of the evaluated parameters was affected by treatment. Testosterone concentrations were significantly lowered in the mid and high dose groups after three weeks of dosing. The authors attributed this effect to two high outliers in the control group. However, upon removal of these outliers, the decrease remained significant [60]. The authors designated the high dose, 1,087.6 mg/kg bw/day, as the NOAEL. The RIVM considers that, based on the decreased testosterone concentrations, 10.9 mg/kg bw/day was the NOEL.

SCCP/SCCS (2005, 2006, 2008, 2010, 2011, and 2013)
The SCCP concluded that the developmental study performed by Daston (2004) suggests that butylparaben does not have a strong estrogenic potential to cause developmental toxicity when administered to pregnant Sprague-Dawley rats. With regard to developmental reproductive parameters, a NO(A)EL with respect to male developmental parameters *in vivo* was provided only by Fisher et al. (1999), who identified a NOEL at 2 mg butylparaben/kg bw/day. Although this NOEL represented the only dose tested, the SCCP initially considered the Oishi studies (which identified a LOAEL of approximately 10 mg/kg bw/day) as supportive to proposing a NOEL of 2 mg/kg bw/day [63]. In later opinions, the doubts expressed by the industry with regard to the quality of the Oishi studies were acknowledged by the SCCS, however the quality of these studies could not be properly assessed because the full test protocols and raw data were not available [59].

In an attempt to repeat the Oishi study with a more robust study design, the industry applicant performed the Hoberman et al. (2008)

study. Although this study refuted findings in the Oishi study [51], some significant shortcomings precluded the scientific acceptance of the Hoberman et al. (2008) study. The following comments could not be refuted by industry: (1) the raw data provided were considered insufficient (e.g. which pups were born from the same dam), (2) a large variation range was noted in the body weights of the animals, (3) large standard deviations were noted in the hormone levels, while exact sampling times for blood collection were not included in the raw data, (4) general animal husbandry was put into doubt as 26% of the animals displayed unexpected clinical signs such as chromorhinorrhea and chromodacryorrhea, and (5) too many statistically significant adverse effects were dismissed based on arguments such as a lack of dose-dependency and abnormally high values in control animals [59].

The SCCS has evaluated newly published *in vivo* studies up to 2013, some of which identified potential endocrine effects. However, these effects were found at relatively high dosage levels. The SCCS notes that for changes in hormone levels or endocrine functions it is difficult to make a distinction between adverse and non-adverse effects. Furthermore, in some studies animals were exposed to butylparaben by subcutaneous application, which bypasses dermal absorption and skin metabolism and is therefore not an adequate reflection of human exposure. Considering these points, the SCCS concludes that it is difficult to derive a NO(A)EL which can be used for risk assessment of developmental effects. Hence, in 2010 the SCCS selected the study by Fisher et al. (1999) for the determination of the effect level, even though it represents a conservative effect level due to the subcutaneous application, as the 2 mg/kg bw/day was a clear NOEL instead of a NOAEL [51, 59].

RIVM conclusions on developmental and reproductive toxicity
There are no OECD TG studies available on the reproductive and developmental toxicity of butylparaben. Available relevant peer-reviewed studies are summarized in this section. Both Daston (2004) and Fisher et al. (1999) investigated the potential developmental toxicity of butylparaben. However, while the former investigated prenatal developmental toxicity (e.g. embryo/foetal viability, foetal weight, malformations, or variations), Fisher et al. (1999) looked at the postnatal developmental toxicity of butylparaben (histopathology of the testes and epididymides of neonatal rats). Considering the accompanying differences in design and endpoints, the NOEL of 2 mg/kg bw/day, as identified by Fisher et al. (1999), represents a developmental NOEL with respect to reproductive parameters.

RIVM agrees that the reliability of the Oishi studies cannot be properly assessed in the absence of the full test description and the complete raw data package. However, in line with the SCCS, RIVM notes that decreased testosterone concentrations in the industry repeat study conducted by Hoberman et al. (2008) cannot be easily dismissed due to abnormally high values in control animals. This is especially important considering that it is not entirely clear whether fluctuations in hormone levels have an adverse effect on the organism or not. Hence, RIVM does not agree with the NOAEL of 1,087.6 mg/kg bw/day as proposed by the authors of the Hoberman et al. (2008) study. However, the relevance of

the fluctuations in hormone levels is difficult to assess, as the blood was withdrawn using retro-orbital bleeding [59]. This method, which is no longer accepted, can result in increased hormone level. Furthermore, the RIVM agrees that the variation in the body weight of the animals is an important factor to consider, especially since this leads to a high difference (by at least factor 2) in final dosages. Considering these points, the RIVM agrees that the Fisher et al. (1999) study was the only study available to the SCCP/SCCS that described a true NOEL.

3.8 Endocrine-disrupting activity

3.8.1 Update on the hormonal (estrogenic/anti-androgenic) properties of butylparaben after the SCCS opinion of 2010/2013

The SCCS opinions of 2010 and 2013 were (partly) dedicated to the potential *in vitro* and *in vivo* ED effects of parabens and the identification of a NO(A)EL to be used in risk assessment. For this purpose, the available *in vitro* and *in vivo* studies on the ED properties of parabens, with a special focus on reproductive and developmental toxicity, were evaluated. Following the most recent SCCS opinion, several *in vitro* and *in vivo* studies on the ED effects of butylparaben were published. These are identified by a literature search and evaluated for their relevance on the ED properties of butylparaben and possible implications for the SCCS conclusion. The selected *in vitro* and *in vivo* studies are summarized in Tables A1 and A2, respectively (Appendix 10.1) and briefly described below.

3.8.2 In vitro studies on ED properties

The general results or conclusions from recent *in vitro* studies on ED properties are described below.

Estrogenic activity

The SCCS concluded that *in vitro* studies show the potential of the endocrine-modifying effects of parabens, with estrogenic activity as a function of chain length. PHBA, the common metabolite, does not seem to exhibit endocrine-modifying effects. They also found that parabens induce proliferation of MCF-7 cells based on an estrogenic-related mode of action [51, 59].

Since 2013, a number of articles have investigated the estrogenic activity of butylparaben *in vitro.* Butylparaben was shown to be a clear inverse antagonist (i.e. able to offset the antagonistic activity of 4-hydroxy-tamoxifen) of the human estrogen-related receptor γ (ERRγ) [72]. Butylparaben displayed estradiol-mediated activation of an ER-dependent reporter in T47D-Kbluc cells [73]. With regard to estrogenic activity, increased cell proliferation was shown in non-transformed MCF-10A human breast epithelial cells and/or MCF-7 human breast cancer cells [74-76]. A recent study additionally showed the promotion of a proliferation of MCF-7 and T47D cells by the butylparaben metabolite 3-hydroxy-*n*-butyl-4-hydroxybenzoate [77]. Butylparaben promoted the proliferation of MCF-7 at low µM concentrations (Table A1, Appendix 10.1). The mechanism of proliferative action seems to differ between MCF-10A and MCF-7 cells, as butylparaben-induced proliferation was related to estradiol secretion and aromatase activity in MCF-7, but not in MCF-10A cells [76]. In further work, it was shown that the proliferative

effect of butylparaben in MCF-7 cells did not result from a direct effect on cell cycle or apoptosis gene expression, in contrast to the mechanism of action of 17β-estradiol. In MCF-10A cells, apoptosis and cell cycle regulatory gene and protein expression changes by 17β-estradiol was paralleled by butylparaben [78].

Furthermore, acute exposure of primary rat Sertoli cells to butylparaben leads to the disruption of vimentin filaments, which may result in a release of spermatogenic cells and subsequent apoptosis [79]. Butylparaben treatment resulted in developmental toxicity in zebrafish embryos, leading to deformities, including intestinal effusion, pericardial oedema, and accelerated yolk utilization. Furthermore, redox conditions were altered and the endocrine pancreas was identified as a sensitive target [80].

Androgenic activity
Androgenic activity was investigated using reporter assays. Antagonism of AR by butylparaben was only observed at cytotoxic concentrations (higher than 10 µM) [81].

Other ED-related mechanisms
Some recent articles discussed the adipogenic activity of butylparaben *in vitro*. At 10 µM and below, adipogenic activity was not observed in 3T3-LI cells [82]. However, at higher concentrations, butylparaben was shown to promote the adipogenic differentiation of 3T3-LI preadipocytes, human adipose-derived multipotent stromal cells (hADSC) and multipotent stem cells C3H10T1/2. Differentiation of 3T3-LI and C3H10T1/2 cells was attenuated by the antagonism of PPARγ or GR and knockdown of PPARγ, respectively. Butylparaben activated both PPARγ and GR in 3T3-LI cells, but only PPARγ in the C3H10T1/2 cells. This latter observation may be related to the low endogenous expression of GR in C3H10T1/2 cells [83, 84]. In MDA-kb2 human breast cancer cells, which contain high levels of endogenous GR, butylparaben displayed glucocorticoid-like activity with an EC_{50} of 1.75 µM [85]. The common paraben metabolite 4-hydroxybenzoic acid did not promote the adipogenic differentiation of 3T3-LI cells [84]. Butylparaben suppressed the chondrogenic and osteogenic differentiation of C3H10T1/2 cells, the latter effect being suppressed by PPARγ or GR knockdown [83].

Summary
The published *in vitro* studies after the SCCS opinion of 2013 complement the existing *in vitro* evidence for the ED potential of butylparaben. Estrogenic activity was shown in various models as already highlighted by the SCCS [51, 59]. Also, possible modes of actions that might lead to reprotoxic or developmental effects of butylparaben were further investigated *in vitro*. The mode of action of potential adipogenic activity of butylparaben was further unravelled. For both androgenic and adipogenic activity, *in vitro* studies underscored the complexity of effects following exposure to mixtures. These *in vitro* studies can be used to determine an endocrine mode of action as one of the aspects needed to establish whether butylparaben is an endocrine disruptor.

3.8.3 *In vivo studies on ED properties*

Following the last update published by the SCCS in 2013, a few new *in vivo* studies on the reproductive and developmental toxicity of butylparaben were published. These are summarized below and in Table A2 (Appendix 10.1).

Ali et al. (2013)

Developmental neurotoxicity was studied in the male offspring of rats. Butylparaben was administered orally or subcutaneously at 200 mg/kg bw/day to pregnant female albino rats from GD1 to lactation day 21 [86]. Treatment with valproic acid was included as a positive control. Results on behaviour indicated a reduced social approach to foreigner rats and disturbances in learning and memory abilities. Furthermore, alterations were found in the monoamine content in different sections of the brain, free amino acids content in the frontal cortex and brain-derived neurotrophic factor (BDNF) content in the entire brain. Similar effects were noted in offspring from valproic acid-treated dams. Oral administration had a greater effect than subcutaneous administration. Based on these results, 200 mg/kg bw/day can be considered a LOAEL for the developmental neurotoxicity of butylparaben.

Comments RIVM*:* The study was well conducted and provides insight into the mechanism of potential neurotoxicity. However, the study would have benefited from the inclusion of multiple doses. Nevertheless, the data indicate some possible neuro-developmental toxicity, so further research is needed for the confirmation and derivation of a NOAEL.

Alam & Kurohmaru (2014)

Male prepubertal rats were treated orally with 1,000 mg/kg bw and sacrificed within 24 hours [79]. Treatment disrupted the Sertoli cell vimentin filaments and microfilaments. No changes were found in the microtubule network. Spermatogenic cells were detached from Sertoli cells and sloughed into the lumen of the tubules. The single dose of 1,000 mg/kg bw represents the LOAEL.

Comments RIVM: These data suggest an ED mode of action. However, considering that only one dose was tested and only a limited set of endpoints was investigated, the study is not sufficient to derive a NOAEL and is instead considered as being supportive to other studies.

Zhang et al. (2014)

Pregnant Wistar dams were treated by oral gavage from GD7 to Postnatal Day (PND) 21 with butylparaben at doses of 64, 160, 400 or 1,000 mg/kg bw/day [87]. Litters were culled down to eight pups (preferably all male) per litter on PND4. Treatment reduced anogenital distance (AGD) and delayed preputial separation (PPS) at 400 and 1,000 mg/kg bw/day. At these doses, hormone levels (testosterone, 17β-estradiol, progesterone, luteinizing hormone, follicle-stimulating hormone) were altered at selected ages, and epididymal sperm counts and daily sperm production were decreased and histopathological observations were made. The weight of the testis was affected from 160 mg/kg bw/day. No effects were found on the timing of testicular descent. Therefore, 64 mg/kg bw/day can be considered as the NOAEL.

Comments RIVM: This was a well conducted study and relevant endpoints are assessed. The data is described clearly in the publication and accompanying supplemental material.

Boberg et al. (2016)
To investigate potential endocrine disrupting effects in male and female offspring, pregnant Wistar rats were treated with 10, 100 or 500 mg/kg bw/day of butylparaben by oral gavage from GD7 to GD21 and from PD 1 to 22 [88]. Treatment with the high dose resulted in decreased prostate weights and alterations in prostate histology. Both the mid and high doses resulted in reduced AGD and ovary weights, and an increased mammary gland outgrowth. Sperm counts were reduced and testicular gene expression (i.e. *CYP19a1* and *Nr5a1*) was reduced at all doses. Therefore, 10 mg/kg bw/day was considered a LOAEL.

Comments RIVM: The study was well conducted with relevant endpoints. The findings suggest an ED mode of action, yet including a lower dose in the current study design would have been valuable in this study.

Goswami & Kalita (2016)
In order to investigate effects on the uterus, Swiss albino mice were exposed subcutaneously to 10, 50 or 100 mg/kg bw/day of butylparaben for seven days [89]. Treatment resulted in an increased uterine weight and changes in uterine histology at mid and high doses. At high dose the thickness of the endometrium and myometrium and total uterine tissue were increased. Treatment resulted in an increase in the number of uterine glands at all doses. The LOAEL is 10 mg/kg bw/day.

Comments RIVM: Although only a limited number of endpoints were assessed, these are relevant and not often investigated. Although it is recognized that subcutaneous administration is a very uncommon route of exposure, it is considered informative in this specific case because the differences in kinetics between rodents and humans, as potential ED activity is likely determined by the systemic availability of un-metabolized butylparaben (see Section 3.2.1) [59]. It is further noted that the description of the methods is limited.

Hu et al. (2016)
The potential of butylparaben to promote adipogenesis *in vivo* was investigated by the exposure of obesity-prone C57BL/6J mice to 100 mg/kg bw/day by oral gavage for 12 weeks [90]. Mice were fed either chow or a high-fat diet. Butylparaben treatment did not increase adiposity and serum leptin levels, but it did decrease serum procollagen type 1 N-terminal propeptide (related to bone formation) and did induce changes in gene expression in white adipose tissue and the liver that can be related to adipocyte differentiation and lipogenesis. 100 mg/kg bw/day cannot be considered as a LOAEL because the serum procollagen type 1 N-terminal propeptide decrease is not an adverse effect.

Comments RIVM: Only one dose was studied. However, considering the mechanistic information derived from the gene expression analysis, this study can only be considered as being supportive to evaluating the

mechanism of the possible adipogenic effects of butylparaben, but no adverse effects were observed.

Roberts et al. (2016)
Pregnant Sprague Dawley rats were exposed to 1,500, 5,000 or 15,000 ppm butylparaben through diet form GD6 to PND28 in order to investigate the internal exposure to butylparaben in foetuses and pups [91]. Doses translate to an average butylparaben exposure of 106.6-339.2, 360.3-1224.5 or 1217.8-3493.8 mg/kg bw/day, respectively, throughout the dosing period. Based on total (hydroxylated) butylparaben concentrations in amniotic fluid compared with maternal plasma (<1%) and in pup plasma compared with maternal plasma (<5%), a limited placental transfer and low lactational transfer was suggested. Butylparaben conjugation was the primary metabolic route in dams (>99%), but it was age-dependent in pups (26 to 53% conjugation at PNDs 4 and 10, >99% at PND21 to PND28). Based on pup body weights, which decreased at the high dose (persistent effects at PND28, 16.5% and 13.7% in male and female pups, respectively), the NOAEL is 360.3-1224.5 mg/kg bw/day.

Comments RIVM: This well-conducted study provides mechanistic information regarding foetal exposure to butylparaben and is consequently complementary to interpreting the results of developmental studies.

Zhang et al. (2016)
This study was designed as a follow-up to Zhang et al. (2014) [87] in order to investigate the possible mechanisms of endocrine and reproductive disorders [92]. Pregnant Wistar rats were exposed by oral gavage to 64, 160, 400 or 1,000 mg/kg bw/day from GD7 to PND21. Male pups were sacrificed on PND21 or PND90. At 400 and 1,000 mg/kg bw/day, observations included increased testosterone and estradiol levels in plasma, increased aromatase CYP19 and ERα expression in testes, and decreased expression of cytochrome cholesterol side-chain cleavage (P450scc), AR, steroidogenic acute regulatory protein (StAR) and estrogen sulfotransferase (SULT1E1). Effects on ERα, P450scc and AR expression were also detected in the 160 mg/kg bw/day group. At 1,000 mg/kg bw/day, DNA methylation of the estrogen receptor was decreased and Dnmt3b mRNA expression was increased. An increased and decreased expression of respectively CYP19 and SULT1E1 can be linked to increased estradiol concentrations, which in turn, together with epigenetic hypomethylation of ERα, can promote ERα expression. In the 400 and 1,000 mg/kg bw/day group, reduced and loosely arranged germ cells, as well as decreased layers of germinal epithelium, were observed in the testes. Based on the histopathological observations and changes in serum hormone concentrations, the NOAEL was 160 mg/kg bw/day.

Comments RIVM: The data from this study is relevant to obtaining mechanistic information regarding possible endocrine and reproductive disorders. Considering the similarity with the study previously conducted by the authors, the study is supportive of the findings reported before [87].

Garcia et al. (2017)
Six-week-old Sprague-Dawley rats were treated subcutaneously with 150, 300 or 600 mg butylparaben/kg bw/day for 57 days in order to investigate reprotoxicity following exposure through a complete spermatogenic cycle [93]. No effects were found in haematology, biochemistry and hormonal analysis. The prostate weight was increased at the high dose. Treatment resulted in decreased spermatozoa counts and increased abnormal spermatozoa in the testis, accompanied by histopathological observations in seminal vesicles at all doses. The LOAEL is 150 mg/kg bw/day.

Comments RIVM: For the exposure route, interspecies differences are important to consider because potential ED activity is likely determined by the systemic availability of un-metabolized butylparaben (see Section 3.2.1) [59] and the metabolism in humans is much slower than that in rats. Therefore, the subcutaneous route is considered relevant to assessing the effects on unmetabolized butylparaben in rats. However, relevant endpoints were assessed in a well-defined exposure period. The study is described clearly. The study could have benefitted from additional dose groups to establish a NOAEL.

Guerra et al. (2017a)
Developmental toxicity in male foetuses was investigated by treating pregnant Wistar rats at doses of 10, 100 or 200 mg/kg bw/day from GD12 until GD20 or with corn oil (diet) subcutaneously [94]. No treatment-related effects were observed following a histopathological investigation of the uterus and ovaries of the female foetuses and the testes of male foetuses. For the *in utero* exposure of male foetuses, the NOAEL is ≥200 mg/kg bw/day.

Developmental toxicity in male pups was investigated by treating pregnant Wistar rats at doses of 10, 100 or 200 mg/kg bw/day from GD12 until PND22 [94]. Treatment resulted in an increased number of adult Leydig Cells at the mid and high doses. At the high dose, hormone levels in blood were altered (testosterone levels increased, LH and FSH levels decreased). Spermatogenesis kinetics were affected at low and high doses and sperm motility was impaired at low dose. Sperm head abnormalities were observed at all doses. For *in utero* and lactational exposure, 10 mg/kg bw/day can be considered the LOAEL.

Comments RIVM: An extensive and well-described study in which relevant endpoints were assessed. However, the exposure window is relatively late. The OECD TG 414 (prenatal developmental toxicity study) ascribes administration at least from implantation. In this study, important periods are missed, for example the period of organogenesis (days 5-15 in rodents).

Guerra et al. (2017b)
The rodent uterotrophic assay was performed to investigate the estrogenicity of butylparaben [95]. Immature, weaned female Wistar pups were treated at doses of 10, 100 or 200 mg/kg bw/day from PND20 to PND22. No effects on uterus weight were found, the NOAEL was 200 mg/kg bw/day.

Developmental toxicity in female foetuses was investigated by treating pregnant Wistar rats at doses of 10, 100 or 200 mg/kg bw/day from GD12 until GD20 [95]. No treatment-related effects were observed following a histopathological investigation of the uterus and ovaries and the ovaries of female foetuses. For *in utero* exposure, the NOAEL is 200 mg/kg bw/day.
Developmental toxicity in female pups was investigated by treating pregnant Wistar rats at doses of 10, 100 or 200 mg/kg bw/day from GD12 until PND22 [95]. No treatment-related effects were found, although impaired sexual behaviour in the high dose group (not significant) suggests that brain sexual development might be a more sensitive endpoint for future studies. Based on these results, the NOAEL for *in utero* and lactational exposure is 200 mg/kg bw/day.

Comments RIVM: An extensive and well-described study in which results are clearly presented. The assessment of multiple endpoints, which may be relevant for possible endocrine-disrupting effects, within one study allows the comparison of several effects with each other.

Lee et al. (2017)
Ovarian failure was investigated by orally exposing female rats to 100 mg butylparaben/kg bw/day for five weeks [96]. Treatment resulted in alterations in the estrous cycle and follicular depletion. mRNA expression of genes associated with ovarian steroidogenesis was effected in the ovaries and FSH concentrations were increased. These results suggest that butylparaben induces premature ovarian failure by disrupting folliculogenesis and steroidogenesis. The (only) dose of 100 mg/kg bw/day represents a LOAEL.

Comments RIVM: This study was described well and provides a clear description of results and the effects seem relevant.

Summary
Several recent *in vivo* studies published after the latest SCCS opinion were found. These peer-reviewed studies have employed a diverse range of methods to investigate the estrogenic effects or developmental toxicity of butylparaben. Consequently, a wide range of affected dose levels were observed, based on a wide range of endpoints/effects, ranging from a LOAEL of 10 mg/kg bw/day to a NOAEL of 1224.5 mg/kg bw/day. Testicular toxicity led to a NOAEL of 64 mg/kg bw/day in the recently conducted study by Zhang et al. (2016) [87] and two follow-up studies found comparable effects on testicular toxicity [92, 93]. In two studies, the testicular toxicity resulted in a LOAEL of 10 mg/kg bw/day [88, 94]. Thus, especially in male offspring, developmental toxicity was found in *in vivo* studies. Other effects that may be related to ED activity were found as well, such as effects on uterus, ovaries, AGD distance, adipogenesis and hormone levels (other than testosterone). One study indicated developmental neurotoxicity following butylparaben exposure of 200 mg/kg bw/day, but dose groups in the lower levels were not always included in the studies, so a NO(A)EL could not be determined[86]. The overall conclusion of the *in vivo* studies is that, despite the limited placental transfer suggested by Roberts et al. (2016), serious effects were identified after butylparaben exposure in rodents [91].

3.9 Conclusions on hazard characteristics

The conclusions drawn on the regular toxicological endpoints for butylparaben are summarized in Table 9. Although no guideline studies are available on the reproductive or developmental toxicity of butylparaben, RIVM is of the opinion that, by including the recently conducted studies, much information is available about the developmental and reproductive effects. It should be considered, however, that the studies have major differences in methodologies and the endpoints assessed, thereby impeding direct comparison of the effect levels.

Taking all studies into account, the most investigated endpoint is testicular toxicity, particularly with regard to steroidogenesis and spermatogenesis. Each of the four studies previously assessed by the SCCS included testicular toxicity as an endpoint [59]. The Oishi studies identified LOAELs of around 10 mg/kg bw/day, while the Hoberman et al. (2008) study found decreased testosterone concentrations at the mid and high doses, leading to a NOEL of 10.9 mg/kg bw/day [68, 69, 71]. However, these studies had methodological shortcomings, thus hampering their scientific acceptance. The RIVM agrees with the SCCP/SCCS that for some endocrine effects (in particular changes in hormone levels) it is currently difficult to assess whether these effects are adverse or non-adverse on the organism. The SCCP/SCCS selected the NOEL of 2 mg/kg bw/day from the study conducted by Fisher et al. (1999) as a conservative effect level [59, 67]. Five recently conducted studies describe effects in sperm counts or related parameters [87, 88, 92-94], two of which establish a LOAEL of 10 mg/kg bw/day [88, 94]. Considering the LOAELs of all these studies together suggests that a LOAEL of 10 mg/kg bw/day is obvious. Based on the data of all these studies and the LOAELs of 10 mg/kg bw/day as identified, the NOEL of 2 mg/kg bw/day used by the SCCS is not very conservative. In conclusion, several *in vitro* and *in vivo* studies support the endocrine potential of butylparaben.

Table 9. Summary of hazard characteristics for the main toxicological endpoints for methyl- (MeP), ethyl- (EtP), propyl- (PrP) and butylparaben (BtP). For the overview, the hazard characteristics for MeP, EtP and PrP are copied from the previous report [1].

Toxicological Endpoint	**MeP**	**EtP**	**PrP**	**BtP**
Acute toxicity	Low in OECD TG 401	Low in OECD TG 401	Low in OECD TG 401	Low (TG not specified)
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	Irritating to skin in OECD TG 439 or TG 435; corrosive to eye in OECD TG 437.
Repeated dose toxicity	Negative	Negative	Negative	Negative
Genotoxicity	Some positive *in vitro*, negative *in vivo*	Negative *in vitro* and *in vivo*	Negative *in vitro* and *in vivo*	Negative *in vitro* and *in vivo*
Carcinogenicity	Negative	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 in reproductions in screening study (OECD TG 422)	Negative in study similar to OECD TG 414 (Daston et al. (2004)) up to 1000 mg/kg bw/day [70] Fisher et al. (1999): No effect on testicular excurrent ducts, single dose tested (NOEL 2 mg/kg bw/day) [67] Boberg et al. (2016) and Guerra et al. (2017a): Testicular toxicity effects, incl. sperm effects (LO(A)EL 10 mg/kg bw/day) [88, 95]
Reproductive Toxicity	Not determined (in OECD TG) Oishi (2004): Negative (NOAEL 1000 mg/kg bw/day) [97] Hoberman et al.	Not determined (in OECD TG) Oishi (2004): Negative (NOAEL 1000 mg/kg bw/day) [97] Vo et al. (2010): organ	Negative in OECD screening TG 422 up to ~1000-1500 mg/kg bw/day (highest dose) in 2012 Oishi (2002b): effects on sperm (LOAEL 12.4	Not determined (in OECD TG) Oishi (2001): effects on sperm count and testosterone concentrations (LOAEL 10.4 mg/kg bw/day) [68] Oishi (2002a): effects on sperm, serum testosterone concentrations and

Toxicological Endpoint	MeP	EtP	PrP	BtP
	(2008): effects on sperm (NOAEL 11.2 mg/kg bw/day) [71] Vo et al. (2010): delay vaginal opening , estrous cycle, organ weights (NOAEL 250 mg/kg bw/day) [98]	weights (NOAEL 250 mg/kg bw/day) [98]	mg/kg bw/day) [99] Vo et al. (2010): organ weights (NOAEL 250 mg/kg bw/day) [98] Gazin et al. (2013): Negative (NOAEL 1000 mg/kg bw/day) [100]	epididymal weights (LOAEL 14.4 mg/kg bw/day) [69] Hoberman et al. (2008): Effects on testosterone concentrations (NOAEL 10.9 mg/kg bw/day) [71]

3.10 Butylparaben and 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' [101]. 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) [102]. Although the intention is to use these criteria in other legal frameworks as well, it remains to be seen whether this will happen.

According to European criteria, a substance will be identified as an ED if it meets the following:

- It 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 its susceptibility to other influences;
- It has an endocrine mode of action, i.e. it alters the function(s) of the endocrine system;
- The adverse effect is a consequence of the endocrine mode of action, therefore a biologically plausible link between adverse effects and the endocrine mode of action has to be shown.

The European Food Safety Authority (EFSA) and European Chemicals Agency (ECHA) have recently developed a guidance document that describes how to implement the scientific criteria for the identification of the endocrine-disrupting properties of chemicals pursuant to the PPPR and BPR [103]. The guidance highlights how to gather, evaluate and consider all relevant information for the identification of EDs according to the scientific criteria in a weight of evidence analysis [102, 103].

The focus in the evaluation for butylparaben will only be related to estrogen, androgen, thyroid and steroidogenic (EATS) mediated parameters. Furthermore, since parabens are no pesticide or biocide tested in the PPPR and BPR, less official TG data about EDs are available than are for PPPs and BPs. Therefore, conclusions should be drawn based only on the available scientific data. These scientific data should be grouped and summarized as described in the guidance. Based on these available data, a 'weight of evidence' as to whether butylparaben fulfils the criteria will be performed, but no final conclusion can be drawn based on the lack of required data from TG studies and the limitations and differences in study design of the scientific data available.

During the development of criteria to identify EDs, various organizations published lists of (suspected) EDs, e.g. the European Commission (EU priority list), the International Chemical Secretariat (ChemSec) (SIN list), and The Endocrine Disruption eXchange (TEDX), which produced

easy-to-access, overall listings with substances associated with endocrine disruption:

- The EU Priority List of 2003 is a fixed list according to which butylparaben is a Category I (evidence of ED activity in at least one species using intact animals) endocrine disruptor [104].
- The SIN (Substitute It Now!) list contains substances identified as SVHCs according to the criteria in REACH by ChemSec, an NGO. According to this list, butylparaben is an endocrine disruptor with estrogenic and antiandrogen activity, affecting sperm function and reproductive organs, among other things. The substance has been detected in human urine and indoor air [105].
- The TEDX list is least selective and contains a broad list of (suspected) EDs. Substances are placed on this list when they are demonstrating effects on the endocrine system in at least one peer-reviewed literature study [106]. According to this list, butylparaben is an ED because of several peer-reviewed studies [107-111].

The ECHA – EFSA guidance was published in 2018 and compounds could be identified as an ED based on the available data described in this guidance. Although no sufficient guideline studies are available for the endocrine-disrupting effects of butylparaben, there are clear indications from peer-reviewed studies that measure relevant ED-related endpoints as described in the guidance that butylparaben acts via various ED modes of action. Based on the data in Appendix 10.1, the following indications exist in relation to butylparaben and endocrine disruption:

- There is sufficient evidence from different studies that exposure to butylparaben can affect hormone levels (testosterone, estrogen, luteinizing hormone (LH), follicle stimulating hormone (FSH), Appendix 10.1), which is evidence for the endocrine activity of butylparaben via the estrogen, androgen and steroid pathways [103].
- In the EFSA/ECHA guidance for PPPR and BPR, a list of parameters is described that could be used for a weight of evidence for endocrine disruption [103]. Butylparaben affects more of these ED-related endpoints (Appendix 10.1). In some studies, the weight of reproductive organs are affected (epididymis, uterine, prostate and ovary) or the histopathology of these organs (e.g. testes, epidydimus) is affected or hormone levels are affected. Many studies show an effect of butylparaben on sperm parameters (e.g. sperm count, production or morphology).

Altogether, several *in vitro* studies in particular support the ED MoA by butylparaben. But some *in vivo* studies, too, show ED effects, though they lack a clear dose-response relationship because of the dosing regime (limited doses). The available data give many indications that butylparaben has endocrine-disrupting effects via the estrogenic, androgenic and steroid pathways (Appendix 10.1). However, according to the ED criteria, it needs to be identified whether the adverse effects are a consequence of the endocrine mode of action and, therefore, a biologically plausible link between adverse effects and the endocrine mode of action has to be demonstrated. However, it should be discussed whether the available data presents a level of evidence that is high enough to properly identify whether butylparaben is an endocrine

disruptor based on the ED criteria and the EFSA-ECHA guidance, or whether additional functional assays are necessary. If additional *in vivo* experiments are to be conducted, these should be well-designed by taking into account the potentially much more effective metabolism in experimental animals compared to humans, at least with regard to the relevant route of human exposure (dermal).

4 Reviews and risk assessments

Reviews and risk assessments of butylparaben, have been conducted by several organizations. The reviews and risk assessments are often closely related to those conducted for other parabens, i.e. methyl-, ethyl- and propylparaben, and therefore these are briefly mentioned here as well. An overview is provided in the following sections.

4.1 SCCS opinions

History

The EU Scientific Committee on Consumer Products (SCCP) and its successor, the Scientific Committee on Consumer Safety (SCCS), published several opinions on parabens. In 2005, the “Extended opinion on the safety evaluation of parabens” [63], and the “Extended opinion on parabens, underarm cosmetics and breast cancer” [64] were published, focused on methyl-, ethyl-, propyl- and butylparaben. In 2006, the SCCP published a reaction to newly introduced data [65] and, in 2008, the description of the outcome of an industry hearing and some additional publications [66]. The SCCS updated the opinion in 2010 with regard to propyl- and butylparaben after a pharmacokinetic study and a survey conducted by the Danish authorities [59]. In 2011, it published a clarification of the 2005 opinion on parabens in light of the Danish ban of propyl- and butylparaben in cosmetic products for children up to three years of age [112]. In 2013, another update followed on propyl- and butylparaben based on new toxicity studies [51]. For an overview and understanding of the SCCP/SCCS opinion with time, the opinions are mentioned below chronologically, per year.

2005

In 2005, the SCCP concluded there was no concern for methyl- and ethylparaben [63]. 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%, a larger body surface per body mass of children versus that of adults with a factor of 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 p-hydroxybenzoic acid (PHBA) (liver, skin) was not accounted for and the contribution of dietary parabens was not considered (very small). They confirmed the ADI of 10 mg/kg bw/day based on the NOAEL of 1,000 mg/kg bw/day by the SCF in 1994 [113]. Endocrine-disrupting 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 have proven to be practically non-toxic, non-carcinogenic, non-genotoxic, non-co-carcinogenic, and non-teratogenic [113]. Parabens were not expected to accumulate in tissues and the ester linkage of the parabens was expected to be readily hydrolysed [113]. Therefore, the SCCP concluded that “methyl- and ethylparaben can be safely used up to the maximum authorized concentration as actually established (0.4%)” [63].

For butylparaben, a NOEL value of 2 mg/kg bw/day was proposed by Oishi (2002a) [69] based on Fisher et al. (1999) [67]. However, as recent studies were not taken sufficiently into account, the data available while making the opinion did not enable a decision to be taken on whether butylparaben (and propylparaben) can be safely used in cosmetic products at a concentrations of 0.4%.

For propylparaben, no NOAEL could be established, but as the potency of propylparaben is clearly lower than the potency of butylparaben [69], the SCCS suggested that the proposed NOEL value of 2 mg/kg bw/day for butylparaben [67] can be conservatively used for propylparaben [63]. More data with regard to the reproductive and developmental toxicity of propyl- and butylparaben with special focus on the male reproductive system was requested [63].

It was concluded that methyl-, ethyl-, propyl- and butylparaben can be safely used as a preservative up to a maximum concentration of 0.4% in the finished product for 1 ester and up to 0.8% for mixtures of esters.

2006

SCCP drafted an updated opinion because of a new oral, dietary reproduction toxicity study focused on methyl- and butylparaben in male rats, *in vitro* dermal penetration and metabolism studies with methyl- and butylparaben, and an *in vitro* kinetics and metabolism study using full-thickness human skin with butylparaben [65]. With regard to the new studies, it was concluded that they contain too many shortcomings in order to be considered as scientifically valid. Therefore, the conclusion of the opinion in the earlier 2005 opinion [63] remained unchanged.

2008

Upon industry's request in October 2007, a hearing took place at which the dossier was defended by SCCP. In 2008, the SCCP published the description of the outcome of an industry hearing and some additional publications. According to the SCCP, based upon the available data, the safety assessment of propyl- and butylparaben cannot be finalized yet.

2010

In November 2009, the Danish authorities submitted the report "*Survey and health assessment of the exposure of 2-year-olds to chemical substances in consumer products*" [114], 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. In February 2010, the Danish authorities submitted a report by the Danish National Food Institute (DTU) called "*Update on uptake, distribution, metabolism and excretion (ADME) and endocrine-disrupting activity of parabens*" [115]. In the meantime, this study was published as a scientific article by Boberg et al. (2010) [60].

The SCCS agreed that, based upon currently available *in vitro* data and *in vivo* rodent test results, the estrogenic properties displayed by parabens appear to increase with increasing chain length. Nevertheless, the SCCS stressed that the displayed potency levels remain about three

to six orders of magnitude lower than the potency of the positive controls.

For propyl- and butylparaben, the SCCS considered the use as preservatives in finished cosmetic products as safe for the consumer, as long as the sum of their individual concentrations does not exceed 0.19% (of esters), which is the concentration of butylparaben in the finished product in order to obtain a MOS ≥ 100 [59]. This conclusion is based on the lack of scientifically sound data on the pivotal link between dermal absorption in rats and humans, in particular with regard to the metabolism of the parent substance in the skin. The latter can only be addressed through additional human data.

With regard to methyl- and ethylparaben, the previous opinion, which stated that use at the maximum authorized concentrations can be considered safe, remained unchanged [59].

2011

On 21 March 2011, Denmark notified the European Commission that it had banned propyl- and butylparaben, the isoforms and salts in cosmetic products for children up to three years of age. On 10 October 2011, the SCCS adopted a clarification to its previous opinion of 2005, in light of the Danish clause of safeguard [112], and concluded that:

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

2013

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; therefore it did not confirm the conclusions of the previous studies that pointed to negative effects on reproduction. This new study, to be published as Gazin et al. (2013) [75], did not remove the previous concerns expressed by the SCCS with respect to the lack of sound scientific data. In the SCCS's 2013 opinion, the relevance of the animal studies after oral exposure for human risk assessment was discussed because of the rapid and effective metabolism of parabens in rats, which did not occur in humans [66]. For these reasons, the SCCS requested more data, particularly on the exposure of humans, including children, to propylparaben in cosmetic products and the toxicokinetics of propyl- and butylparaben in humans. For the methyl- and ethylparaben, conclusions were drawn conservatively and no argument is presented to change those based on these findings [66].

The SCCS reports that uncertainties exist which relate to data gaps and questionable data on:

- dermal uptake/absorption of parabens by human skin *in vivo* and *in vitro*,

- dermal and systemic metabolism of parabens in humans, in particular in neonates/newborns and early infants,
- systemic exposure to free parabens as seen in biomonitoring studies, in particular the contribution of carboxylesterases to the inactivation of parabens, and
- human exposure to parabens in cosmetic products.

4.2 EFSA opinion

In 1974, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an ADI of 0-10 mg/kg bw for the sum of methyl-, ethyl- and propylparaben and their sodium salts based on chronic toxicity studies from the 1950s and 1960s [116]. These studies showed a NOEL for all three parabens of 2% in the diet, equivalent to 900-1,200 mg/kg bw/day. The effect observed at the higher dose level of 8% in the diet was decreased weight gain accompanied by depression and death [117]. JECFA was unable to establish an ADI for butylparaben [117].

On the basis of the evaluation by JECFA, the EC Scientific Committee for Food (SCF) took over the results as a temporary ADI of 0-10 mg/kg bw for the sum of methyl-, ethyl- and propylparaben and their sodium salts in 1996 because the toxicological information available showed some inadequacies and uncertainties [113]. In 2004, the ADI was evaluated by EFSA [117]. The EFSA review panel used the NOAEL of 1,000 mg/kg bw/day level for methyl- and ethylparaben, but considered more data necessary to determine a NO(A)EL value for propylparaben [117]. Propylparaben is, therefore, not allowed to be used as a food additive in the EU anymore. Butylparaben, although not allowed as a food additive, was also taken into account for comparison with the other parabens.

With regard to exposure, the EFSA Panel noted that human exposure resulting from the use of parabens in food within 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 [118]. Total paraben exposure was estimated to be 77.5 mg/day (or 1.29 mg/kg bw/day for a 60 kg individual) [118]. Butylparaben is not allowed as a food additive in the EU. It is, however, used as a food additive in, for example, the USA and China.

4.3 Other reviews

4.3.1 Soni et al. (2005)

Soni and co-workers at a consultancy bureau published several safety reviews on parabens: Soni et al. (2002) on methylparaben, Soni et al. (2001) on propylparaben, and Soni et al. (2005) on parabens in general [3, 118, 119]. According to these reviews, acute, subchronic, and chronic studies in rodents indicate that parabens are practically non-toxic and are rapidly absorbed, metabolized and excreted. In individuals with normal skin, parabens are, for the most part, non-irritating and non-sensitizing. However, the application of compounds containing parabens to damaged or broken skin has resulted in sensitization. Genotoxicity testing of parabens in a variety of *in vitro* and *in vivo* studies primarily produced negative results. The paraben structure is not indicative of carcinogenic potential and experimental studies support these observations. Some animal studies have reported adverse reproductive effects of parabens. In

an uterotrophic assay, methyl- and butylparaben administered orally to immature rats were inactive, while subcutaneous administration of butylparaben produced a weak positive response. The ability of parabens to transactivate the estrogen receptor *in vitro* increases with alkyl group size. The detection of parabens in a small number of breast-tumour tissue samples and the adverse reproductive effects of parabens in animals has provoked controversy over the continued use of these substances. However, the possible estrogenic hazard of parabens based on the available studies is equivocal and fails to consider the metabolism and elimination rates of parabens, which are dose-, route- and species-dependent. In light of the recent controversy over the estrogenic potential of parabens, conducting a reproductive toxicity study may be warranted, according to Soni et al. in 2005 [3].

4.3.2 Cosmetic Ingredient Review (CIR)

2008

The USA Cosmetic Ingredient Review (CIR), an industry-backed organization that reviews the safety of cosmetic ingredients in the USA, reported on the safety assessment of parabens as used in cosmetic products in 2008 in a scientific publication referred to as Andersen (2008) [120]. In it, an exposure estimate was performed based on the assumption that 0.4% of a single paraben was used in a cosmetic product (and 0.8% for multiple parabens), although the industry indicates a lower use concentrations. For an average daily personal care product use amount, 17.76 grams of products per day for adults and 378 mg of products for infants is assumed. This results 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 on exposure to parabens resulting from the use of cosmetic products to a NOAEL of 1,000 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 1,690 for single paraben products to 840 for multiple paraben products. The margin of safety for infants ranged from approximately 6,000 for single paraben products to approximately 3,000 for multiple paraben products. The Expert Panel considered these margin of safety determinations to be conservative and said they likely represent an overestimation of the possibility of an adverse effect (e.g. use concentrations may be lower, penetration may be less) and support the safety of cosmetic products in which parabens preservatives are used [120].

The Expert Panel did consider data in the category of endocrine disruption, including male reproductive toxicity and various estrogenic activity studies. Reiterating the absence of human data that can identify adverse effects associated with endocrine-active chemicals, it was stated that animal studies are necessary. It is critical that such studies, themselves, be designed to maximize the likelihood that adverse effects will be detected [120].

2012
In 2012, the CIR panel reconsidered the parabens. At its meeting, CIR carefully reviewed the EU SCCS opinions and concluded that there were little additional new data concerning parabens and reaffirmed the use of parabens in personal care products (i.e. cosmetics) to be safe in the USA [121].

2018
In August 2018, a draft safety assessment on parabens for their use in cosmetics was published [122]. CIR determined a NOAEL of 160 mg/kg bw/day for butylparaben based on the epididymal sperm concentration in the Zhang et al. (2014) study [87]. This was used for the margin of safety calculation for butylparaben (on the assumption that 0.4% butylparaben was used in a cosmetic product). Assuming an estrogenic mechanism, this NOEAL was considered to be adequately protective for methyl-, ethyl- and propylparaben for a cumulative MOS calculation (with 0.8% for multiple parabens in a cosmetic product). Using the same exposure calculation, this resulted in margin-of-safety values of 135 for adults exposed to multiple parabens, and 476 for infants exposed to multiple parabens, suggesting safe use [122].

However, the final assessment is awaited in 2019 and was delayed following pressure from the NGO Women's Voices for the Earth, raising comments concerning the calculated margin of safety, the use in vaginally applied cosmetics and sperm mortality, bioaccumulation, and the contribution made by personal care products to overall exposure [123]. CIR agreed to better justify the conclusions drawn in their final assessment with regard to these points [124].

4.3.3 Danish EPA (2013)

According to the Danish EPA, concerns have been raised about the endocrine-disrupting potential of parabens at high exposure levels [125]. Some studies in young male rats have shown adverse effects on sperm production and testosterone levels following oral exposure to parabens, i.e. propyl- and butylparaben, yet other studies with the same study design and of a more recent date did not confirm these findings even at a very high dose [125]. Both the studies, with positive and negative findings on reproductive toxicity, have shortcomings, which makes it difficult to assess and weigh the results [125]. According to the Danish EPA, parabens are known to be estrogenic *in vitro* and in uterotrophic assays *in vivo* and estrogenicity appears to increase with side chain length. Therefore, methyl-, ethyl-, propyl- and butylparaben are on the EU list of potential endocrine disruptors in category 1 (for human exposure). Isopropyl- and isobutylparaben are not on this EU list. Category 1 substances are substances for which endocrine-disrupting activity has been documented in at least one study of a living organism and are given the highest priority for further studies. The Danish EPA has concluded that the method for evaluating parabens for their endocrine-disrupting potential and their kinetics are still not agreed upon [125]. In addition, discussions on the most relevant NO(A)EL and the dermal absorption values have not yet come to a conclusion. So, considering the endocrine-disrupting effects, a final risk assessment still awaits a decision on which NO(A)EL to use and which dermal absorption fraction to use to further identify the overall exposure [125]. Currently a

new study concerning reproductive toxicity is being assessed by the SCCS. Only a few studies are available on the combined exposure to several parabens from several products [125].

4.3.4 NICNAS (2015)

The Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) performed a human health Tier II assessment for parabens in 2015 [126]. According to their recommendation, current risk management measures are considered to be sufficient to protect the public's and workers' health and safety, provided that all requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory [126]. The available data do not indicate any risks associated with exposure to the chemicals in this group [126]. The chemicals have been shown to have weak estrogenic activity, but there are no established adverse outcome pathways for this effect. Should further information on adverse outcome pathways in mammals associated with weak estrogenic activity become available, further assessment of these chemicals at Tier III could be required." [126].

4.3.5 National Toxicology Program (2005)

In 2005, the USA National Toxicology Program (NTP) published a review of toxicological literature on butylparaben [21]. Apart from being used in cosmetics and pharmaceutical products, in the USA butylparaben is also allowed to be used as a preservative in food "at very low levels" [21]. According to the NTP, human exposure to butylparaben may occur via inhalation, eye or skin contact, or ingestion. Inhalation exposure causes irritation to the respiratory tract. Contact with the eyes or skin can cause irritation, redness, pain, and/or itchiness, but patch test results show that the sensitization potential of parabens is low. Ingested butylparaben is rapidly absorbed from the gastrointestinal tract, metabolized and excreted in the urine. Large doses, however, may cause irritation to the gastrointestinal tract. In mice, rats, rabbits and dogs, butylparaben was reported to be practically non-toxic. Results from one chronic feeding study in mice showed that butylparaben caused a high incidence of amyloidosis, affecting the spleen, liver, kidney and/or adrenal gland. It was cytotoxic in isolated rat hepatocytes and mitochondria and in other animal cells *in vitro*.

Butylparaben was not mutagenic in several short-term bioassays and was reported to be non-carcinogenic in rats and mice [21]. Reproductive studies in mice and rats suggested that maternal exposure to butylparaben (100 or 200 mg/kg bw from GD6 to PND20) results in adverse effects on the reproductive system of F1 male offspring (According to Kang et al. (2002) [127] as cited by [21] – a study also taken into account by the SCCP/SCCS).

4.4 Summarizing reviews and risk assessments

When the group of parabens was considered together, an overall NOAEL of 1,000 mg/kg bw/day was established for methyl- and ethylparaben (by the SCF; resulting in an ADI of 0-10 mg/kg bw/day) or for all parabens (CIR). The SSCP concluded with a NOEL of 2 mg/kg bw/day for butylparaben based on a non-guideline study conducted by Fisher et al. (1999) in which juvenile rats were subcutaneously exposed for 17

days (only one dose group). Pragmatically and conservatively, according to the SCCP/SCCS, this NOEL of 2 mg/kg bw/day for butylparaben was also proposed for propylparaben, which has a lower toxicity than propylparaben [67].

In general, it has been concluded by most reviews that there is no risk attached to the use of methyl- and ethylparaben in food and no risk attached to the use of methyl-, ethyl-, propyl- and butylparaben in personal care products. However, uncertainty does exist and more information is needed on the (dermal) absorption and metabolism of parabens, as well as on the reproductive effects.

When comparing the exposure estimates, also taking into account that exposure estimations were mostly performed in a rough or worst-case manner, it was concluded that there was no risk attached to the use of methyl- and ethylparaben in personal care products (SCCS, CIR) [51, 59, 120] or food (EFSA) [117] or both (NICNAS, Soni et al.) [3, 128]. Because of the lack of a clear NOAEL for propylparaben, the substance cannot be used in food in the EU (EFSA) and butylparaben is not used as a food additive in the EU [106]. In the USA, both propyl- and butylparaben are allowed in food products [129]. SCCS regards propyl- and butylparaben safe for general cosmetics, excluding specific products for the nappy area of young children [51].

With respect to the endocrine-disrupting properties, ED properties were discussed and taken into account (as far as possible) by SCCS in setting the toxicological reference value for butylparaben [51]. Most risk assessments mention endocrine disruption as a point of discussion. In several risk assessments, it has been noted that more studies are needed and that further assessments are required when more information on these kinds of effects becomes available.

4.5 Discussion and uncertainties

Currently performed risk assessments do not result in any concern for health risks. However, with respect to dermal exposure, remarks are still being made to the effect that a final risk assessment is waiting on a proper definition of the dermal absorption fraction to be used. The uncertainty about interspecies difference (rat-human) with regard to toxicokinetics should also first be resolved and when missing studies on reproduction effects become available, the review should be updated accordingly.

Studies have reported that there is a difference in toxicokinetics between rats and humans in the metabolism and that 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 is relevant, especially with regard to the effects after dermal exposure from specific personal care products or medicinal products applied to the skin. When first-pass metabolism through oral uptake in rat experiments (from which the NO(A)ELs are derived) is very effective, but is not effective in humans via dermal exposure, this could lead to a relative higher internal exposure in humans and could influence the margin of exposure that is considered

sufficient between an aggregate exposure estimate (including dermal exposure) and the present NO(A)ELs.

The current toxicological reference value for butylparaben, the NOEL of 2 mg/kg bw/day from the subcutaneous exposure study by Fisher et al. (1999) [67], is regarded by the SCCS as conservative, but is not very conservative, as was shown by the several recent studies on reproductive toxicity from which a LO(A)EL of 10 mg/kg bw/day seems evident [87, 88, 92-94]. The NOEL of 2 mg/kg bw/day is the reference value taken from the most sensitive toxicological endpoint at present, but it is not possible to say with certainty that this reference value completely covers possible ED effects. The uncertainty with regard to differences in metabolism between rats and humans also introduces another uncertainty in the risk assessment [60].

When comparing the calculated aggregate exposure estimate of ~0.1-0.2 mg/kg bw/day for butylparaben (Tables 6 and 7) with the NOEL of 2 mg/kg bw/day, the margin of exposure may not be protective enough. However, the present calculated exposure values are very likely overly conservative (because of several factors (Table 8)), as is also indicated by back-calculated exposure values from biomonitoring studies (differing by 1 up to 2 orders of magnitude). Therefore, a refinement of the exposure assessment will very likely sufficiently increase this margin of safety to a level that is sufficiently protective. Overall, the extent to which people appear to be exposed to butylparaben and the current information on health effects do not seem to present a reason for concern.

5 Cumulative exposure to parabens, toxicity and risk assessment

As a group, parabens consist of similar chemical substances, which means that, with an apparent similar hazard profile, it might be relevant to add up the aggregated exposure to methyl-, ethyl-, propyl- and butylparaben. Such a cumulative risk assessment is justified when substances share a common mechanism of toxicity. In the present chapter, we initially describe approaches to a cumulative risk assessment. Thereafter, the discussion turns to whether such a cumulative exposure assessment of parabens is justified.

5.1 Definition, approaches and methodology

Traditionally, exposure and risk assessment has been single-substance-oriented, although exposure to mixtures and combinations of substances is the rule rather than the exception [130]. In recent years there has been a growing awareness of the importance of cumulative exposure and risk assessment, e.g. by the US-EPA, EC, WHO and OECD [101, 131-133], but at present there is still little space for cumulative exposure and risk assessment within regulatory practices [134]. In the EU, at least within the authorization of plant protection products, i.e. Reg. (EU) No. 1107/2009 on the placing on the market of plant protection products, and Reg. (EC) No. 396/2005 on maximum residue levels of pesticides in food or feed, the importance of taking into account cumulative effects is being addressed, though there is an admission that concrete methods need to be developed for this matter.
The issue and the development of methodology is being dealt with within several organizations, such as EFSA (methodology, work on cumulative assessment groups) [135], JRC [136], SCHER, SCCS, and SCENIHR [137], with specific task forces of the OECD (i.e. TFHA and TFEA), and in several EU projects (e.g. Acropolis, EUROMIX). The present chapter briefly describes the common mechanism of toxicity which justifies cumulative exposure assessment and different ways by which cumulative exposure and risk assessment can be performed.

A common mechanism of toxicity is needed, or the likelihood for the cumulation of a common toxic effect, in order to justify cumulative exposure and risk assessment. This requirement, however, can be approached by different levels of complexity, e.g. ranging from the same organ being affected in the critical endpoint used to set the toxicological reference value to the shared specific molecular mechanisms affected. To complicate the situation, mixture effects could potentially occur as well, i.e. interactions between the different compounds, such as antagonistic or synergistic effects. However, usually such interactions occur at medium or high dose levels (relative to the lowest effect levels). At low exposure levels, they are either unlikely to occur or are toxicologically insignificant [137].

There are several ways by which cumulative exposure assessment can be performed, e.g.:

Simple cumulative exposure and risk assessment
An initial approach is by simply adding up the exposure to the single substances considered. This could be done by summing the exposure concentrations, but with respect to further risk assessment this is only relevant when there is a common reference dose (i.e. a shared safety limit value, usually an acceptable exposure value, e.g. an ADI) to which this summed exposure can be compared. Often this is not the case, as the individual substances have their own reference doses. Because the substances concerned often differ in potency with regard to a certain toxicological mechanism or endpoint, as a worst case approach, the lowest limit value of the most potent substance could be used as a reference value (provided information on potency differences is known) [133, 138, 139].

Hazard Index (HI) approach
Dividing the exposure to a specific substance by its respective reference dose (resulting is a Hazard Quotient (HQ)) is an approach which enables the addition of relative risk ratios resulting from exposures to multiple substances with a common mechanism of toxicity, but having their own reference values. The summation of HQs results in a hazard index (HI). This way of cumulative exposure assessment already integrates risk assessment. However, one should take into account any differences in safety levels and the level of conservatism with regard to the reference doses concerned and to any differences with regard to the quality of the relative exposure assessments when drawing conclusions on a HI. The ratio of HQ and HI (as percentage) can be used to express the contribution of an individual substance to the risk of the mixture [5, 30, 140].

An example of the application of the HI approach is the REACH evaluation of phthalates. Four phthalates (DEHP, BBP, DBP and DIBP), known (suspected) EDCs, were placed on the Authorization List because of reproductive toxicity. In the risk assessment, cumulative exposure and risk assessment were taken into account, referred to as grouping, based on a similarity in chemical structure, exposure pattern, and specific anti-androgenic activities, effects on reproductive organs and fertility. Cumulative risk assessment was performed by applying the HI approach.

Toxicity Equivalency Factor (TEF) approach
Usually, through sharing a common mechanism of toxicity, one substance is more potent than the other with regard to a certain endpoint. To include a substance-specific difference in potency with regard to a toxicological mechanism of action in the calculation, a factor addressing this activity can be included in the summation. The activity is usually expressed as a Toxicity Equivalency Factor (TEF), an expression of relative potency equivalent to the activity of a model substance with the same effect. The resulting cumulative exposure value can be compared with a reference value belonging to the model substance [141-143].

5.2 Cumulative exposure, toxicity and risk assessment of parabens

With respect to parabens, there have been several peer-reviewed studies that performed cumulative exposure and risk assessments, often for a combination of different substances, including (specific) parabens, on the basis of specific (usually *in vitro*) effects. For example, Kortenkamp & Faust (2010) combined 15 anti-androgenic substances, including propyl- and butylparaben [144]. In their study, exposure estimates for the individual substances were divided by a reference dose for anti-androgenic effects and cumulated according to the HI approach [144]. Among these, propyl- and butylparaben were cumulated, although their evidence of anti-androgenicity was limited, and mainly observed *in vitro*, as was indicated by the authors [144]. The *in vivo* effects that were considered were the suppression of testosterone levels, decreased epididymis weights, and decreases in sperm production as reported after oral administration to post-weanling male Wistar rats by Oishi (2001, 2002b) [68, 99]. As a result, for propylparaben 100 mg/kg bw/day [99], and for butylparaben 10 mg/kg bw/day [68] were chosen as points of departure, both with an uncertainty factor of 100 and used in order to calculate a reference dose. As an illustration, this resulted in an HQ of 0.006 for propyl-, and an HQ of 0.06 for butylparaben, on an HI from all cumulated substances of 0.38 (resulting from median intakes of anti-androgens) [144]. The authors discussed, however, that propyl- and butylparaben are both *in vitro* AR antagonists, yet there was limited evidence of *in vivo* effects coming from scientific investigations not conformed to standards required for regulatory purposes [144].

A cumulative exposure and risk assessment requires a common mechanism of action. Though similarities in chemical structure and exposure pattern, such as with phthalates, trigger the performance of a cumulative exposure and risk assessment of parabens, the current knowledge on their specific toxicological effects at present does not allow a definite conclusion to be drawn on whether this is justified.

Methyl-, ethyl-, propyl- and butylparaben show at least estrogenic and anti-androgenic activities *in vitro*, as has been indicated by several studies that identify and share a potential of endocrine-modifying effects, with estrogenic activity as a function of chain length. However, different mechanistic effects have also been seen in different *in vitro* effects, as well as in *in vivo* studies. Moreover, many studies do not allow for a good comparison because of differences in methodology, endpoints studies or experimental conditions. In most studies, several different endpoints are affected for the parabens and no consistent effects on one or two specific endpoints are identified. At present, this is mainly due to the fact that no standard TG studies for developmental and reproductive toxicity have been performed and most peer-reviewed *in vitro* and *in vivo* studies have only tested one single paraben and do not compare the effects of the four parabens. Altogether, based on the current *in vivo* and *in vitro* studies available, there are indications that the parabens have a common mechanism of toxicity via the estrogenic, androgenic mechanism.

The maximum concentrations of parabens from the cosmetic regulation, based on the assessment by SCCS, give the impression that risk

assessment on parabens is already (partly) cumulative. For example, for propyl- and butylparaben, the sum of these individual parabens is restricted to 0.19%. The main reason for this, however, is that the NOEL of butylparaben is conservatively also used for propylparaben, for which no reference value could be derived. Of course, this is also based on the *in vitro* estrogenic effects of parabens with estrogenic activity as a function of chain length (butylparaben being more potent than propylparaben), and effects seen in the uterotrophic assay. This also accounts for methyl- and ethylparaben, sharing the same NOAEL, and estrogenic effects *in vitro*. As a result, the maximum concentration of all four parabens is restricted to 0.8% in Annex V of the cosmetic regulation.

In addition to the request for the performance of additional studies in order to identify a common mechanism of action for the separate parabens, combination studies have been suggested in order to investigate the cumulative exposure to several parabens or to parabens together with other ED substances, potentially leading to cumulative effects [60, 115].

In conclusion, there are indications that the four parabens share a common mechanism of toxicity, but the present information does not allow a conclusion to be drawn on this matter. More data are needed to truly identify a common mechanism of action and to come to a conclusion about the cumulative toxicity of parabens.

6 Legal frameworks

For butylparaben, some restrictions are laid down in different regulations, such as the Cosmetics Regulation. These are often closely related to those for other parabens, e.g. methyl-, ethyl- and propylparaben, and therefore these are mentioned here as well. An overview is provided in the following sections, as well as in Appendix 10.2.

6.1 Cosmetics Regulation

Based on SCCS opinions (see Section 4.2), the use of the different parabens in personal care products is regulated by the Cosmetics Regulation [145], which was adapted in 2014 [146].

With regard to butylparaben and propylparaben, SCCS concluded that their use as preservatives in finished cosmetic products is safe for the consumer, as long as the sum of their individual concentrations does not exceed 0.19% (as esters), which was also taken up into entry 12 of Annex V of 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 of Regulation (EC) No 1223/2009 should be maintained”. However, the SCCS maintained that, with respect to propyl- and butylparaben present in leave-on cosmetic products designed for application on the nappy area of children below the age of six months, a risk could not be excluded in 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. And therefore it says that 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 old, butylparaben and propylparaben should be prohibited in leave-on cosmetic products designed for application on the nappy area of children under 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 (see Appendix 10.2).

6.2 Food

Butylparaben is not allowed in food as a preservative in the EU according to Directive Regulation EC No 1333/2008, nor may it be used in the manufacture of plastic materials and articles intended to come into contact with food (Food Contact Materials (FCM), Commission Regulation (EU) No. 10/2011).

6.3 REACH

Butylparaben (CAS 94-26-8) was registered within the REACH legislation in June 2018. According to the ECHA website (consulted October 2018), butylparaben is manufactured and/or imported in the European Economic

Area in the amount of 10 - 100 tonnes per year. This tonnage is ten- or a hundred-fold less than is registered for propyl- and ethylparaben (100-1,000 tonnes per year), and methylparaben (1,000-10,000 tonnes per year), respectively. With regard to registered consumer uses, butylparaben is used in cosmetics and personal care products and pharmaceuticals.

6.4 CLP

According to the ECHA website (consulted October 2018), butylparaben has no harmonized hazard classification. Most notifiers self-classify butylparaben with Skin Irrit. 2 (H315: Causes skin irritation), Eye Irrit. 2 (H319: Causes serious eye irritation) and STOT SE 3 (H335: May cause respiratory irritation). But there are also many unclassified notifications. The REACH registration dossier of notifications consists of Skin Irrit. 2 (H315: Causes skin irritation) and Eye Dam. 1 (H318: Causes serious eye damage).

6.5 Specific legislation

On 15 March 2011, Denmark introduced a national ban on parabens in cosmetic products intended for children. It contains a ban on propyl- and butylparaben and their isoforms and salts in cosmetic products for children younger than 3 years.
(http://eng.mst.dk/media/mst/Attachments/Engelskparabenbekendtgrelse.pdf)

7 Conclusions and recommendations for further research

7.1 Conclusions

Exposure assessment

With regard to consumer exposure, we performed an inventory and discussion of estimates of exposure to butylparaben for consumers 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:

- Butylparaben can be present in personal care products, other consumer products and in medicinal products as a preservative. In China and the US, butylparaben has been detected in food. In the EU butylparaben is not allowed to be used as a food additive or to be used as a food contact material;
- Exposure estimations from the literature provided show a wide variety in the types of study with regard to methodology, level of detail and assumptions made, as well as in the resulting estimates (see Table 6 and Table 7). The main sources of uncertainty in the parabens exposure assessment are the product aggregation method, assumptions regarding the frequency of use and amount of product applied, the assumed concentration of butylparaben in products, the fraction of products in which butylparaben is used, the fraction of the product remaining on the skin, and the dermal absorption value (Table 8). Some of these factors have possibly been estimated unrealistically high;
- A worst-case aggregate, internal exposure estimate for butylparaben, taking into account personal care products and food, resulted in an exposure estimate of ~0.1 mg/kg bw/day for adults (Table 6) and ~0.2 mg/kg bw/day for children (Table 7). The estimated exposure via food is very limited (<1%) compared with personal care products. No estimation for the exposure via medicinal products could be made because of a lack of relevant data;
- It is unclear how well the estimates of exposure via food from China and the USA represent the situation in the Netherlands and/or Europe;
- There are indications that butylparaben is used as a preservative to a lesser extent than before and therefore exposure estimations based on old data easily overestimate the actual exposure. This impression is supported by some biomonitoring studies where 95th percentile values from urine metabolite concentrations were back-calculated to internal exposure or daily intake levels of butylparaben, which were one up to two orders of magnitude lower than the estimated exposure values;
- In addition to the lack of data with regard to medicinal products, information on actual levels in non-food consumer products in the Netherlands or Europe is currently missing.

Hazard assessment

With regard to the hazard profile of butylparaben, we aimed to describe the toxicity, including the possible endocrine-disrupting effects and the current toxicological reference values. The following can be concluded:

- Butylparaben has low acute toxicity and low repeated dose toxicity, it proved to be a moderate irritant in animal tests and it may cause eye and skin irritation in humans. Butylparaben has a low skin sensitization potential in humans. Butylparaben is not deemed genotoxic or carcinogenic in the available studies;
- Studies demonstrate that the metabolism of butylparaben in rats is more effective than it is in humans, especially during dermal uptake. Therefore, the relevance of rat studies are under debate, since they potentially underestimate the effects in humans. More data are needed on toxicokinetics to clarify interspecies differences in order to gain insight into the human relevance of animal studies for butylparaben;
- No effects were observed for butylparaben in a study similar to an OECD TG 414 developmental toxicity study dosed up to 1,000 mg/kg bw/day. A study by Fisher et al. (1999) assessing postnatal developmental toxicity resulted in a developmental NOEL of 2 mg/kg bw/day based on testicular toxicity, which was also taken into account. This value is used by the SCCS as a 'conservative' toxicological reference value;
- No official OECD TG studies have been performed focused on the reproductive toxicity of butylparaben. Several non-guideline studies were performed, including five recent studies not taken into account during earlier risk assessments (i.e. by the SCCS). Differences in methodologies and endpoints do not allow for a direct comparison, but all of these studies together show that there are many *in vivo* data available. A LO(A)EL of 10 mg/kg bw/day is evident. The NOEL of 2 mg/kg bw/day used by the SCCS is therefore not very conservative;
- Many *in vitro* and several *in vivo* studies indicate that butylparaben has ED properties via the estrogenic, androgenic and steroid pathways and support the ED MoA by butylparaben;
- It should be discussed whether the available data presents a level of evidence which is high enough to properly identify whether butylparaben is an endocrine disruptor based on the ED criteria and the EFSA-ECHA guidance, or whether additional functional assays are necessary;
- If additional *in vivo* experiments are to be conducted, these should be well-designed by taking into account the potentially much more effective metabolism in experimental animals compared with humans, at least with regard to the relevant route of human exposure (dermal);
- ED properties were discussed and taken into account (to the extent possible) by SCCS (2013) in setting the toxicological reference value for butylparaben. The NOEL of 2 mg/kg bw/day is the reference value taken from the most sensitive toxicological endpoint at present. This level is close to the level at which ED-related endpoints were identified (10 mg/kg bw day), but it is not possible to say with certainty that this reference value completely covers possible ED effects.

Risk assessment
With regard to the risk assessment of butylparaben (also related to other parabens), including a statement about the risk related to the exposure in the present study, the following can be concluded:

- In general, most risk assessments conclude there is no risk attached to the use of butylparaben in personal care products. The SCCS restricted the use of butylparaben in leave-on products designed for application in the nappy area of young children because of their immature metabolism and possibly damaged skin;
- The current toxicological reference value, the NOEL of 2 mg/kg bw/day, is regarded by the SCCS as being conservative, but it is not very conservative because more recent studies indicate that a LO(A)EL of 10 mg/kg bw/day for reproductive toxicity is evident;
- There are uncertainties about the risk assessment for butylparaben because it is not possible to say with certainty that this reference value completely covers possible ED effects and interspecies differences with regard to metabolism are possibly not taken sufficiently into account;
- The present calculated exposure value of ~0.1-0.2 mg/kg bw/day is very likely overly conservative;
- It is expected that a refinement of the exposure assessment would contribute to more realistic values and increase the margin of safety to a level that is sufficiently protective. Overall, the extent to which people appear to be exposed to butylparaben and the current information on health effects do not seem to present a reason for concern.

Cumulative exposure to parabens, toxicity and risk assessment
With regard to the potential cumulative exposure, toxicity and risk assessment of methyl-, ethyl-, propyl- and butylparaben, the following can be concluded:

- There are indications that methyl-, ethyl-, propyl- and butylparaben share a common mechanism of toxicity via an estrogenic and/or androgenic mechanism. However, more data are needed to properly identify a common mechanism of action;
- Therefore, a cumulative exposure and risk assessment of these four parabens can currently not be justified.

7.2 Recommendations for further research

Taking into account the uncertainties present in the available data and methodology with regard to the exposure, hazard and risk assessment of butylparaben (but also with regard to other parabens), the following issues should be addressed in (future) studies or discussions:

- Further discussion among an expert group about whether butylparaben is an endocrine disruptor based on the ED criteria and the EFSA-ECHA guidance or whether additional functional assays are necessary;
- Better information with regard to (toxico)kinetics and metabolic interspecies differences is required in order to assist with setting more realistic toxicological reference values, as metabolic inactivation in rats is likely more effective than it is in humans, which could affect the relevance of animal studies (this also accounts for other parabens);

- To obtain improved information about dermal absorption, including metabolism, to facilitate more realistic exposure estimates (this also accounts for other parabens);
- As there are indications that butylparaben is used to a lesser extent than before, a market surveillance to study whether butylparaben is still being used as a preservative in personal care products, as well as in other non-food consumer products, will identify whether further exposure studies are still relevant to conduct (this also accounts for other parabens);
- In order to derive an actual level of exposure to compare with the calculated aggregate exposure estimate, more realistic biomonitoring data are needed that are representative of the current situation in the Netherlands or, as an alternative, the conformation of the (possibly limited) current exposure to butylparaben (this also accounts for other parabens);
- A new (probabilistic) exposure assessment for butylparaben via personal care products using more recent products in use, concentration and presence data that better represent the current situation in the Netherlands in order to adjust the margin of safety to a more realistic level, which will very likely be larger than the current margin and possibly help to confirm that there is no reason for concern (this also accounts for other parabens);
- When relevant (i.e. still used in the market), the performance of an additional exposure study with regard to the exposure via non-food consumer products other than personal care products, and especially medicinal products, might be necessary in order to establish a more realistic aggregate exposure estimate (this also accounts for other parabens);
- The performance of more mechanistic studies, potentially addressing several parabens simultaneously, in order to determine whether cumulative exposure and risk assessment of parabens is justified.

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9 References

1. Brand, W., et al., *Exposure to and toxicity of methyl-, ethyl- and propylparaben - A literature review with a focus on endocrine-disrupting properties. RIVM Report 2017-0028.* 2018, RIVM: Bilthoven.
2. Delmaar, J.E. and J.G.M. Van Engelen, *Aggregating human exposure to chemicals. RIVM Report 630700001/2006.* 2006, RIVM: Bilthoven.
3. Soni, M.G., I.G. Carabin, and G.A. Burdock, *Safety assessment of esters of p-hydroxybenzoic acid (parabens).* Food and Chemical Toxicology, 2005. **43**(7): p. 985-1015.
4. 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): p. 14442-9.
5. 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): p. 208-14.
6. Gosens, I., et al., *Aggregate exposure assessment of chemicals in consumer products. Exposure to parabens in cosmetics in children as a case study. RIVM Letter Report 320015005/2011.* 2011, RIVM: Bilthoven.
7. Cowan-Ellsberry, C.E. and S.H. Robison, *Refining aggregate exposure: example using parabens.* Regulatory Toxicology and Pharmacology, 2009. **55**(3): p. 321-9.
8. 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): p. 113-9.
9. 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**(2): p. 152–159.
10. 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**(12): p. 2008–2018.
11. Loretz, L.J., et al., *Exposure data for cosmetic products: Facial cleanser, hair conditioner, and eye shadow.* Food and Chemical Toxicology, 2008. **46**(5): p. 1516–1524.
12. Loretz, L.J., et al., *Exposure data for cosmetic products: lipstick, body lotion, and face cream.* Food and Chemical Toxicology, 2005. **43**(2): p. 279-291.
13. US-EPA, *Exposure Factors Handbook*. 2011, U.S. Environmental Protection Agency: Washington, DC.
14. Rastogi, S.C., et al., *Contents of methyl-, ethyl-, propyl-,butyl-, and benzylparaben in cosmetic products.* Contact Dermatitis, 1995. **32**(1): p. 28-30.
15. Wormuth, M., et al., *What are the sources of exposure to eight frequently used phthalic acid esters in Europeans?* Risk Analysis, 2006. **26**(3): p. 803-24.

16. 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**: p. 68-74.
17. 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): p. 3918-25.
18. EMA, *Reflection paper on the use of methyl- and propylparaben as excipients in human medicinal products for oral use*. 2015.
19. Rowe, R.C., P.J. Sheskey, and M.E. Quinn, *Handbook of pharmaceutical excipients. Sixth edition.* 2009: Pharmaceutical press and American Pharmacists Association.
20. Moreta, C., M.T. Tena, and K. Kannan, *Analytical method for the determination and a survey of parabens and their derivatives in pharmaceuticals.* Environmental Research, 2015. **142**: p. 452-60.
21. NTP, N.T.P., *Butylparaben [CAS No. 94-26-8] Review of Toxicological Literature*. 2005.
22. 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.* Environmental Health Perspectives, 2016. **124**(8): p. 1155-65.
23. 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.* Environmental Science & Technology, 2016. **50**(24): p. 13539-13547.
24. 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**: p. 8-15.
25. Pastor-Nieto, M.A., et al., *Preservatives in personal hygiene and cosmetic products, topical medications and household cleaners in Spain.* Actas Dermosifiliograficas, 2017. **108**(8): p. 758-770.
26. 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.* Environmental Science & Technology, 2012. **46**(21): p. 11584-93.
27. Mitro, S.D., et al., *Consumer product chemicals in indoor dust: a quantitative meta-analysis of U.S. studies.* Environmental Science & Technology, 2016. **50**(19): p. 10661-10672.
28. Ye, X., et al., *Parabens as urinary biomarkers of exposure in humans.* Environmental Health Perspectives, 2006. **114**(12): p. 1843-6.
29. Guo, J., et al., *Urinary paraben concentrations and their associations with anthropometric measures of children aged 3 years.* Environmental Pollution, 2017. **222**: p. 307-314.
30. 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.* Journal of Exposure Science & Environmental Epidemiology, 2017. **27**(6): p. 591-600.

31. Moos, R.K., et al., *Parabens in 24 h urine samples of the German Environmental Specimen Bank from 1995 to 2012.* International Journal of Hygiene & Environmental Health, 2015. **218**(7): p. 666-74.
32. VWA, *Cosmetische producten voor kinderen: Inventarisatie van de markt en de veiligheidsborging door producenten en importeurs.* 2007.
33. Biesterbos, J.W., et al., *Usage patterns of personal care products: important factors for exposure assessment.* Food and Chemical Toxicology, 2013. **55**: p. 8-17.
34. Ficheux, A.S., et al., *Consumption of cosmetic products by the French population second part: Amount data.* Food and Chemical Toxicology, 2016. **90**: p. 130-41.
35. Ficheux, A.S., et al., *Consumption of cosmetic products by the French population. First part: frequency data.* Food and Chemical Toxicology, 2015. **78**: p. 159-69.
36. Garcia-Hidalgo, E., et al., *Use-patterns of personal care and household cleaning products in Switzerland.* Food and Chemical Toxicology, 2017. **99**: p. 24-39.
37. Ma, W.L., et al., *A survey of parabens in commercial pharmaceuticals from China and its implications for human exposure.* Environment International, 2016. **95**: p. 30-5.
38. 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): p. 293-306.
39. Nowak, K., et al., *Parabens and their effects on the endocrine system.* Molecular and Cellular Endocrinology, 2018. **474**: p. 2238-251.
40. 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.* Journal of Exposure Science & Environmental Epidemiology, 2015. **25**(3): p. 317-23.
41. Dudzina, T., et al., *The probabilistic aggregate consumer exposure model (PACEM): validation and comparison to a lower-tier assessment for the cyclic siloxane D5.* Environment International, 2015. **79**: p. 8-16.
42. ECHA-EFSA, et al., *European Chemicals Agency European Food Safety Authority) with the technical support of the Joint Research Centre (JRC). Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009.* EFSA Journal, 2018. **16**(6): p. 5311, 135.
43. OECD, *Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption*. 2018.
44. Ozaki, H., et al., *Comparative study of the hydrolytic metabolism of methyl-, ethyl-, propyl-, butyl-, heptyl- and dodecylparaben by microsomes of various rat and human tissues.* Xenobiotica, 2013. **43**(12): p. 1064-72.

45. Aubert, N., T. Ameller, and J.J. Legrand, *Systemic exposure to parabens: pharmacokinetics, tissue distribution, excretion balance and plasma metabolites of [14C]-methyl-, propyl- and butylparaben in rats after oral, topical or subcutaneous administration.* Food and Chemical Toxicology, 2012. **50**(3-4): p. 445-54.
46. Moos, R.K., et al., *Metabolism and elimination of methyl, iso- and n-butyl paraben in human urine after single oral dosage.* Archives of Toxicology, 2016. **90**(11): p. 2699-2709.
47. Abbas, S., et al., *Metabolism of parabens (4-hydroxybenzoic acid esters) by hepatic esterases and UDP-glucuronosyltransferases in man.* Drug Metabolism and Pharmacokinetics, 2010. **25**(6): p. 568-77.
48. Harville, H.M., R. Voorman, and J.J. Prusakiewicz, *Comparison of paraben stability in human and rat skin.* Drug Metabolism Letters, 2007. **1**(1): p. 17-21.
49. Janjua, N.R., et al., *Urinary excretion of phthalates and paraben after repeated whole-body topical application in humans.* International Journal of Andrology, 2008. **31**(2): p. 118-30.
50. 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.* Environmental Science & Technology, 2007. **41**(15): p. 5564-70.
51. SCCS, *Scientific Committee on Consumer Safety. Opinion on parabens. Updated request for a scientific opinion on propyl- and butylparaben. SCCS/1514/13*. 2013.
52. Seo, J.E., S. Kim, and B.H. Kim, *In vitro skin absorption tests of three types of parabens using a Franz diffusion cell.* Journal of Exposure Science & Environmental Epidemiology, 2017. **27**(3): p. 320-325.
53. Calafat, A.M., et al., *Urinary concentrations of four parabens in the U.S. population: NHANES 2005-2006.* Environmental Health Perspectives, 2010. **118**(5): p. 679-85.
54. Frederiksen, H., N. Jorgensen, and A.M. Andersson, *Parabens in urine, serum and seminal plasma from healthy Danish men determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS).* Journal of Exposure Science & Environmental Epidemiology, 2011. **21**(3): p. 262-71.
55. Meeker, J.D., et al., *Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage.* Environmental Health Perspectives, 2011. **119**(2): p. 252-7.
56. Fasano, W., J., *Butylparaben: In Vitro Dermal Penetration and Metabolism Using Full Thickness Human Skin*. 2004, E.I. du Pont de Nemours and Company, Haskell Laboratory for Health and Environmental Sciences, Report November 17.
57. Fasano, W., J., *Methylparaben and Butylparaben: In Vitro Dermal Penetration and Metabolism in Rat and Human Skin*. 2004, E.I. du Pont de Nemours and Company, Haskell Laboratory for Health and Environmental Sciences, Report November 22.

58. Fasano, W., J., *Butylparaben: In Vitro Kinetics and Metabolism Using Full Thickness Human Skin*. 2005, E.I. du Pont de Nemours and Company, HaskellSM Laboratory for Health and Environmental Sciences, Report August 29.
59. SCCS, *Scientific Committee on Consumer Products. Opinion on parabens. SCCS/1348/10. Revision 22 March 2011.* 2010.
60. Boberg, J., et al., *Possible endocrine disrupting effects of parabens and their metabolites.* Reproductive Toxicology, 2010. **30**(2): p. 301-12.
61. NICNAS, N.I.C.N.a.A.S. *Parabens: Human Health Tier II Assessment, Accessed August 2018*. 2018; Available from: https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-group-assessment-report?assessment_id=1714.
62. 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 para hydroxybenzoates (E 214-219). Question number EFSA-Q-2004-063.* EFSA Journal, 2004. **83**: p. 1-26.
63. SCCP, *Scientific Committee on Consumer Products. Extended Opinion on the Safety Evaluation of Parabens. SCCP/0873/05*. 2005.
64. SCCP, *Scientific Committee on Consumer Products. Extended Opinion on parabens, underarm cosmetics and breast cancer. SCCP/0874/05*. 2005.
65. SCCP, *Scientific Committee on Consumer Products. Opinion on parabens. SCCP/1017/06*. 2006.
66. SCCP, *Scientific Committee on Consumer Products. Opinion on parabens. SCCP/1183/08*. 2008.
67. 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.* Environmental Health Perspectives, 1999. **107**(5): p. 397-405.
68. Oishi, S., *Effects of butylparaben on the male reproductive system in rats.* Toxicology and Industrial Health, 2001. **17**(1): p. 31-9.
69. Oishi, S., *Effects of butyl paraben on the male reproductive system in mice.* Archives of Toxicology, 2002. **76**(7): p. 423-9.
70. Daston, G.P., *Developmental toxicity evaluation of butylparaben in Sprague-Dawley rats.* Birth Defects Research. Part B, Developmental and Reproductive Toxicology, 2004. **71**(4): p. 296-302.
71. Hoberman, A.M., et al., *Lack of effect of butylparaben and methylparaben on the reproductive system in male rats.* Birth Defects Research. Part B, Developmental and Reproductive Toxicology 2008. **83**(2): p. 123-33.
72. Zhang, Z., et al., *Inverse antagonist activities of parabens on human oestrogen-related receptor gamma (ERRgamma): in vitro and in silico studies.* Toxicology and Applied Pharmacology, 2013. **270**(1): p. 16-22.

73. Pop, A., et al., *Estrogenic and anti-estrogenic activity of butylparaben, butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate and their binary mixtures on two estrogen responsive cell lines (T47D-Kbluc, MCF-7).* Journal of Applied Toxicology, 2018.
74. Khanna, S. and P.D. Darbre, *Parabens enable suspension growth of MCF-10A immortalized, non-transformed human breast epithelial cells.* Journal of Applied Toxicology, 2013. **33**(5): p. 378-82.
75. Roszak, J., et al., *Inhibitory effect of silver nanoparticles on proliferation of estrogen-dependent MCF-7/BUS human breast cancer cells induced by butyl paraben or di-n-butyl phthalate.* Toxicology and Applied Pharmacology, 2017. **337**: p. 12-21.
76. 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, 2013. **65**(2): p. 484-93.
77. Gonzalez, T.L., et al., *Metabolites of n-Butylparaben and iso-Butylparaben Exhibit Estrogenic Properties in MCF-7 and T47D Human Breast Cancer Cell Lines.* Toxicological Sciences, 2018. **164**(1): p. 50-59.
78. 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): p. 1041-50.
79. Alam, M.S. and M. Kurohmaru, *Disruption of Sertoli cell vimentin filaments in prepubertal rats: an acute effect of butylparaben in vivo and in vitro.* Acta Histochemica, 2014. **116**(5): p. 682-7.
80. Brown, S.E., et al., *Pancreatic beta cells are a sensitive target of embryonic exposure to butylparaben in zebrafish (Danio rerio).* Birth Defects Research, 2018. **110**(11): p. 933-948.
81. Kjaerstad, M.B., et al., *Mixture effects of endocrine-disrupting compounds in vitro.* International Journal of Andrology, 2010. **33**(2): p. 425-33.
82. Kassotis, C.D., K. Hoffman, and H.M. Stapleton, *Characterization of Adipogenic Activity of House Dust Extracts and Semi-Volatile Indoor Contaminants in 3T3-L1 Cells.* Environmental Science & Technology, 2017. **51**(15): p. 8735-8745.
83. Hu, P., et al., *Methylparaben and butylparaben alter multipotent mesenchymal stem cell fates towards adipocyte lineage.* Toxicology and Applied Pharmacology, 2017. **329**: p. 48-57.
84. Hu, P., et al., *Effects of parabens on adipocyte differentiation.* Toxicological Sciences, 2013. **131**(1): p. 56-70.
85. Klopcic, I., K. Kolsek, and M.S. Dolenc, *Glucocorticoid-like activity of propylparaben, butylparaben, diethylhexyl phthalate and tetramethrin mixtures studied in the MDA-kb2 cell line.* Toxicology Letters, 2015. **232**(2): p. 376-83.
86. Ali, E.H. and A.H. Elgoly, *Combined prenatal and postnatal butyl paraben exposure produces autism-like symptoms in offspring: comparison with valproic acid autistic model.* Pharmacology Biochemistry and Behavior, 2013. **111**: p. 102-10.

87. Zhang, L., et al., *Effects of n-butylparaben on steroidogenesis and spermatogenesis through changed E2 levels in male rat offspring.* Environmental Toxicology & Pharmacology, 2014. **37**(2): p. 705-17.
88. Boberg, J., et al., *Multiple Endocrine Disrupting Effects in Rats Perinatally Exposed to Butylparaben.* Toxicological Sciences, 2016. **152**(1): p. 244-56.
89. Goswami, P. and J.C. Kalita, *Alteration in uterine histoarchitecture and uterine protein following butylparaben exposure in adult mice.* International Research Journal of Pharmacy, 2016. **7**(2): p. 25-30.
90. Hu, P., et al., *Differential effects on adiposity and serum marker of bone formation by post-weaning exposure to methylparaben and butylparaben.* Environmental Science Pollution Research International, 2016. **23**(21): p. 21957-21968.
91. Roberts, G.K., et al., *Exposure to butyl paraben during gestation and lactation in Hsd:Sprague dawley SD rats via dosed feed.* Toxicology Reports, 2016. **3**: p. 774-783.
92. Zhang, L., et al., *n-butylparaben induces male reproductive disorders via regulation of estradiol and estrogen receptors.* Journal of Applied Toxicology, 2016. **36**(9): p. 1223-1234.
93. Garcia, T., et al., *Effects on the reproductive system of young male rats of subcutaneous exposure to n-butylparaben.* Food and Chemical Toxicology, 2017. **106**: p. 47-57.
94. Guerra, M.T., et al., *Maternal exposure to butyl paraben impairs testicular structure and sperm quality on male rats.* Environmental Toxicology, 2017. **32**(4): p. 1273-1289.
95. Guerra, M.T., et al., *Long-term effects of in utero and lactational exposure to butyl paraben in female rats.* Environmental Toxicology, 2017. **32**(3): p. 776-788.
96. Lee, J.H., et al., *Parabens Accelerate Ovarian Dysfunction in a 4-Vinylcyclohexene Diepoxide-Induced Ovarian Failure Model.* International Journal of Environmental Research and Public Health, 2017. **14**(2): p. 161.
97. Oishi, S., *Lack of spermatotoxic effects of methyl and ethyl esters of p-hydroxybenzoic acid in rats.* Food and Chemical Toxicology, 2004. **42**(11): p. 1845-9.
98. Vo, T.T., et al., *Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model.* Reproductive Toxicology, 2010. **29**(3): p. 306-16.
99. Oishi, S., *Effects of propyl paraben on the male reproductive system.* Food and Chemical Toxicology, 2002. **40**(12): p. 1807-13.
100. 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): p. 392-401.
101. WHO, *Global assessment of the state-of-the-science of endocrine disruptors. WHO/PCS/EDC/02.2. Geneva: World Health Organization.* 2002.

102. 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.
103. ECHA, et al., *Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009.* EFSA Journal, 2018. **16**(6): p. 5311.
104. EC. *Priority list.* *http://ec.europa.eu/environment/chemicals/endocrine/strategy/substances_en.htm#priority_list* *[Accessed April 2017]*.
105. SIN List. *http://sinlist.chemsec.org/* *[Accessed August 2018]*.
106. TEDX list. *https://endocrinedisruption.org/interactive-tools/tedx-list-ofpotential-endocrine-disruptors/search-the-tedxlist#sname=&searchfor=any&sortby=chemname&action=search&searchcats=all&sortby=chemname* *[Accessed August 2018]*.
107. Boberg, J., et al., *Impact of diisobutyl phthalate and other PPAR agonists on steroidogenesis and plasma insulin and leptin levels in fetal rats.* Toxicology, 2008. **250**(2-3): p. 75-81.
108. Chen, J., et al., *Antiandrogenic properties of parabens and other phenolic containing small molecules in personal care products.* Toxicol Appl Pharmacol, 2007. **221**(3): p. 278-84.
109. Gomez, E., et al., *Estrogenic activity of cosmetic components in reporter cell lines: parabens, UV screens, and musks.* Journal of Toxicology and Environmental Health, Part A, 2005. **68**(4): p. 239-51.
110. Song, B.L., et al., *Evaluation of the effect of butyl p-hydroxybenzoate on the proteolytic activity and membrane function of human spermatozoa.* Journal of Reproduction and Fertility, 1991. **91**(2): p. 435-40.
111. Taxvig, C., et al., *Do parabens have the ability to interfere with steroidogenesis?* Toxicological Science, 2008. **106**(1): p. 206-13.
112. SCCS, *Scientific Committee on Consumer Products. Clarification on Opinion SCCS/1348/10 in the light of the Danish clause of safeguard banning the use of parabens in cosmetic products intended for children under three years of age. SCCS/1446/11.* 2011.
113. 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. p. 9-12.
114. 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.
115. Boberg, J., et al., *Update on uptake, distribution, metabolism and excretion (ADME) and endocrine disrupting activity of parabens 2009.* . 2009, Department of Toxicology and Risk Assessment, National Food Institute, DTU.
116. JECFA, *Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents.*, in *WHO Food Additives Series*. 1974, WHO: Geneva.

117. 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.* EFSA Journal, 2004. **83**: p. 1-26.
118. Soni, M.G., et al., *Evaluation of the health aspects of methyl paraben: a review of the published literature.* Food and Chemical Toxicology, 2002. **40**(10): p. 1335-73.
119. Soni, M.G., et al., *Safety assessment of propyl paraben: a review of the published literature.* Food and Chemical Toxicology, 2001. **39**(6): p. 513-32.
120. 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.* International Journal of Toxicology, 2008. **27**: p. 1-82.
121. CIR, *Parabens. New data. CIR expert panel meeting March 5-6, 2012. https://www.cir-safety.org/sites/default/files/paraben_build.pdf.* 2012.
122. CIR, *Amended safety assessment of parabens as used in cosmetics. Draft tentative amended report for panel review. August 29, 2018. https://www.cir-safety.org/sites/default/files/Parabens.pdf.* 2018.
123. WVE, *Womens's voices for the earth. Comments on parabens. Letter to CIR, September 12, 2018. https://www.womensvoices.org/wp-content/uploads/2018/09/WVE-comments-on-Parabens-Sept-2018-lh.pdf.* 2018.
124. CIR, *letter to Women's voices for the earth. https://www.womensvoices.org/wp-content/uploads/2018/11/Letter-to-Alexandra-Scranton-November-9-2018.pdf.* 2018.
125. Danish EPA, *Survey of parabens. Part of the LOUS-review.* 2013.
126. NICNAS, *Inventory multi-tiered assessment and prioritisation (IMPA). Human Health Tier II assessment for parabens.* 2015.
127. Kang, K.S., et al., *Decreased sperm number and motile activity on the F1 offspring maternally exposed to butyl p-hydroxybenzoic acid (butylparaben).* Journal of Veterinary Medical Science, 2002. **64**(3): p. 227-35.
128. NICNAS. *Parabens: Human Health Tier II assessment, Accessed August 2018*. 2018.
129. NTP, *Butylparaben [CAS No. 94-26-8] Review of Toxicological Literature. National Toxicology Program.* 2005.
130. Staal, Y. and L. van der Ven, *Risk assessment of subtsances in combined exposures (mixtures). RIVM Letter report 2015-0189.* 2015, RIVM: Bilthoven.
131. EC, *Commission Communication on the combination effects of chemicals/chemical mixtures (COM(2012) 252 final).* 2012.
132. US-EPA, *Guidance on cumulative risk assessment of pesticide chemicals that have common mechanisms of toxicity. Washington DC: USEPA; 2002.* 2002.

133. Meek, M.E., et al., *Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework.* Regulatory Toxicology and Pharmacology, 2011. **60**(2): p. S1-S14.
134. Kortenkamp, A. and M. Faust, *Regulate to reduce chemical mixture risk.* Science, 2018. **361**(6399): p. 224-226.
135. EFSA, *Panel on Plant Protection Products and their Residues. Scientific opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile. EFSA Journal. 2013; 11(7):3293.* 2013.
136. JRC, *Scientific methodologies for the combined effects of chemicals – a survey and literature review; EUR 27471 EN.* 2015.
137. SCCS, SCHER, and SCENHIR, *Toxicity and Assessment of Chemical Mixtures (Preliminary Opinion approved for Public Consultation).* 2011.
138. Moretto, A., et al., *A framework for cumulative risk assessment in the 21st century.* Critical Reviews in Toxicology, 2017. **47**(2): p. 85-97.
139. Sexton, K., *Cumulative risk assessment: an overview of methodological approaches for evaluating combined health effects from exposure to multiple environmental stressors.* International Journal of Environmental Research and Public Health, 2012. **9**(2): p. 370-90.
140. Pelletier, M., N. Bonvallot, and P. Glorennec, *Aggregating exposures & cumulating risk for semivolatile organic compounds: A review.* Environmental Research, 2017. **158**: p. 649-659.
141. Borgert, C.J., S.P. Baker, and J.C. Matthews, *Potency matters: thresholds govern endocrine activity.* Regulatory Toxicology and Pharmacology, 2013. **67**(1): p. 83-8.
142. Schoeny, R.S. and E. Margosches, *Evaluating comparative potencies: developing approaches to risk assessment of chemical mixtures.* Toxicology and Industrial Health, 1989. **5**(5): p. 825-37.
143. van Meeuwen, J.A., et al., *Aromatase inhibiting and combined estrogenic effects of parabens and estrogenic effects of other additives in cosmetics.* Toxicology and Applied Pharmacology, 2008. **230**(3): p. 372-82.
144. Kortenkamp, A. and M. Faust, *Combined exposures to anti-androgenic chemicals: steps towards cumulative risk assessment.* International Journal of Andrology, 2010. **33**(2): p. 463-74.
145. 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.
146. 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.
147. 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): p. 1051-9.
148. Charles, A.K. and P.D. Darbre, *Combinations of parabens at concentrations measured in human breast tissue can increase proliferation of MCF-7 human breast cancer cells.* Journal of Applied Toxicology, 2013. **33**(5): p. 390-8.

149. Zhang, Z., et al., *Inverse antagonist activities of parabens on human oestrogen-related receptor gamma (ERRgamma): in vitro and in silico studies.* Toxicology and Applied Pharmacology, 2013. **270**(1): p. 16-22.
150. 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, 2013. **65**(2): p. 484-93.
151. Alam, M.S. and M. Kurohmaru, *Disruption of Sertoli cell vimentin filaments in prepubertal rats: an acute effect of butylparaben in vivo and in vitro.* Acta Histochemica, 2014. **116**(5): p. 682-7.
152. 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): p. 1041-50.
153. Pan, S., et al., *Parabens and Human Epidermal Growth Factor Receptor Ligand Cross-Talk in Breast Cancer Cells.* Environmental Health Perspectives, 2016. **124**(5): p. 563-9.
154. Guerra, M.T., et al., *Effects of in vitro exposure to butylparaben and di-(2 ethylhexyl) phthalate, alone or in combination, on ovarian function.* Journal of Applied Toxicology, 2016. **36**(9): p. 1235-45.
155. Yang, C., et al., *Butylparaben promotes apoptosis in human trophoblast cells through increased oxidative stress-induced endoplasmic reticulum stress.* Environmental Toxicology, 2018. **33**(4): p. 436-445.
156. Haggard, D.E., et al., *Transcriptomic and phenotypic profiling in developing zebrafish exposed to thyroid hormone receptor agonists.* Reproductive Toxicology, 2018. **77**: p. 80-93.
157. Klopcic, I., K. Kolsek, and M.S. Dolenc, *Glucocorticoid-like activity of propylparaben, butylparaben, diethylhexyl phthalate and tetramethrin mixtures studied in the MDA-kb2 cell line.* Toxicology Letters, 2015. **232**(2): p. 376-83.
158. Ali, E.H. and A.H. Elgoly, *Combined prenatal and postnatal butylparaben exposure produces autism-like symptoms in offspring: comparison with valproic acid autistic model.* Pharmacology, Biochemistry & Behavior, 2013. **111**: p. 102-10.
159. Pollock, T., et al., *Butylparaben and propylparaben modulate bisphenol A and estradiol concentrations in female and male mice.* Toxicology and Applied Pharmacology, 2017. **325**: p. 18-24.
160. Riad, M.A., et al., *Reproductive toxic impact of subchronic treatment with combined butylparaben and triclosan in weanling male rats.* Journal of Biochemical and Molecular Toxicology, 2018. **32**(3): p. e22037.

10 Appendices

10.1 Overview *in vitro* and *in vivo* data of endocrine parameters and toxicity of butylparaben

10.1.1 In vitro data of endocrine parameters and toxicity of butylparaben

Table A1. In vitro data of endocrine parameters and toxicity of butylparaben (BtP) published after 2010.

Test system	Test principle(s)	Results	Reference
In vitro assays – Estrogenic activity: carcinogenesis			
MCF-10A immortalised, non-transformed human breast epithelial cells	Aim: To investigate the potential of parabens (including BtP) to enable suspension growth of MCF-10A cells Compounds and concentrations: 70 nM 17β-estradiol (positive control); 0.1, 1, 4, 10 and 100 µM BtP Exposure: Up to 17 days Endpoints tested: Number of colonies and average colony size under non-adherent conditions. Number of cell doublings under adherent conditions	BtP increased the number of colonies grown in suspension at all doses, with a maximal colony formation at 1 µM BtP. 10 µM BtP increased the average colony size. No effect of BtP on cell doublings was observed in monolayer culture of MCF-10A cells. Anchorage-independent growth of MCF-10A cells is closely related to transformation and is suggested as a good predictor of carcinogenicity *in vivo*.	Khanna & Darbre (2013) [74]
MCF-7 human breast cancer cells; T-47-D and ZR-75-1 human breast cancer cells	Aim: To investigate the effects of parabens (including BtP) on motility, migration and invasion of human breast cancer cell lines Compounds and concentrations: 10 nM 17β-estradiol (positive control); 10 µM BtP Exposure: 1 week (short term) or 20±2 weeks (long term) Endpoints tested: Motility in MCF-7, T-47-D and ZR-75-1 cells, migration and invasion and proliferative response of MCF-7 cells, protein levels in MCF-7 cells (E-cadherin, β-catenin, ERα)	Long-term and short-term treatment with BtP increased motility of MCF-7 cells. Long-term treatment increased migratory and invasive properties of MCF-7 cells and motility in T-47-D and ZR-75-1 cells. Addition of BtP to MCF-7 cells prevented the loss of a proliferative response to estrogen. ERα levels were downregulated. Expression of E-cadherin and β-catenin was downregulated, which might be suggestive for the process of epithelial to mesenchymal transition.	Khanna et al. (2014) [147]
MCF-7 human breast cancer cells	Aim: To investigate the potential of (combinations of) parabens (including BtP) to stimulate proliferation of	The maximal number of doublings was reached at 7 and 5 µM, after respectively 7	Charles & Dabre (2013) [148]

Test system	Test principle(s)	Results	Reference
	MCF-7 cells at concentrations as measured in human breast tissues. Compounds and concentrations: 10 nM 17β-estradiol (positive control); a range of concentrations of BtP Exposure: 7 and 14 days Endpoints tested: Proliferation	and 14 days exposure. The NOEC and LOEC values were respectively 500 and 700 nM after 7 days, and 500 and 200 nM after 14 days. At concentrations measured in human breast tissue, BtP did not induce proliferation, but proliferation was stimulated when combined with other parabens (methyl-, ethyl-, propyl- and isobutylparaben). The concentration of BtP measured in the region of the ER+ and PR+ tumour of one specific patient were sufficient to induce proliferation of MCF-7 cells.	
Human ERRγ coactivator recruiting assay	Aim: To investigate the binding activities of parabens (including BtP) on the human estrogen-related receptor γ (ERRγ) Compounds and concentrations: 0.1 nM - 100 μM bisphenol A (positive control) or BtP Exposure: 7 and 14 days Endpoints tested: *via* antagonist (4-hydroxy-tamoxifen) competitive binding	BtP displayed inverse antagonist activity on ERRγ, with a REC50 (50% relative effective concentration that offset 50% of the antagonist activity) of 309 nM. Binding to the active site of ERRγ was supported by molecular docking analysis.	Zhang et al. (2013) [149]
MCF-7 human breast cancer cells and MCF-10A immortalised, non-transformed human breast epithelial cells	Aim: To investigate the potential of parabens (including BtP) to induce proliferation and the effect on estradiol secretion and expression of aromatase (*CYP19A1*) Compounds and concentrations: 0.2 nM – 2 μM BtP; 100 nM 17β-estradiol (positive control) Exposure: 24 hours (expression of aromatase), 72 hours (estradiol secretion, proliferation), 6 days (proliferation, repeated exposure) Endpoints tested: gene and protein expression of aromatase, proliferation, estradiol secretion	At 20 nM BtP, *CYP19A1* expression was not affected in MCF-7 cells and decreased in MCF10-A cells. Estradiol secretion was increased at 0.2 nM only in MCF-7 cells, and decreased (not dose dependently) at all doses in MCF-10A cells. Proliferation was increased following single exposure to 0.2, 2, 20 and 200 nM (MCF-7 cells) and to 2, 20 and 200 nM (MCF-10A cells). Following repeated exposure, proliferation was only increased following 96 hours, not after 144	Wróbel & Gregoraszczuk (2013) [150]

Test system	Test principle(s)	Results	Reference
		and 196 hours. The positive control gave expected results.	
Mixed cultures of primary rat Sertoli and spermatogenic cell	Aim: To determine the direct effects of BtP exposure on Sertoli cells Compounds and concentrations: 1, 100 and 1000 µM BtP; Exposure: 6 and 24 hours Endpoints tested: Light microscopy observations and vimentin immunohistochemical analysis	Treatment induced and increased number and size of vacuoles in Sertoli cells. Vimentin filaments were disrupted.	Alam & Kurohmaru (2014) [151]
MCF-7 human breast cancer cells and MCF-10A immortalised, non-transformed human breast epithelial cells	Aim: To investigate whether paraben-induced (including BtP) proliferation is the result of a direct effect on cell cycle and apoptotic gene expression Compounds and concentrations: 20 nM BtP; 10 nM 17β-estradiol (positive control) Exposure: 48 hours Endpoints tested: Apoptosis and cell cycle regulatory gene and protein expression	Butylparaben affected gene expression of G1/S phase genes and of cell cycle progression inhibitors in MCF-7 cells. No effects were found in protein expression of cyclins and p21. Apoptosis gene expression analysis in MCF-7 cells showed only downregulated TNF receptor superfamily member 21. In MCF-10A cells BtP treatment affected expression of G1/S phase and G2/M phase genes, and of cell cycle progression inhibitors. On protein level, cyclin expression was increased and p21 protein expression was decreased. Few genes involved in apoptosis were affected. On protein level, only expression of Bcl-xL was increased. Alterations in expression were comparable to 17β-estradiol in MCF-10A cells, differences were observed in MCF-7 cells.	Wróbel & Gregoraszczuk (2014) [152]
Human BT-474 (HER2 and ERα positive), MCF-7 (ERα positive) and	Aim: To investigate the estrogenic effects of BtP and other parabens in presence of activators of the HER2 pathway (recombinant human heregulin-β1, HRG) Compounds and concentrations: 10 µM	Of all parabens, butylparaben co-treatment with HRG led to the highest increase *of c-Myc* mRNA levels (maximum increase at 10 µM). The combination with HRG had a	Pan et al. (2016) [153]

Test system	Test principle(s)	Results	Reference
SKBR3 (HER2 positive) breast cancer cell lines	metylparaben, ethylparaben and propylparaben (real-time-PCR only); and; 0.01, 1, 100 µM butylparaben (concentration dependent on endpoint); 0.01 µM 17β-estradiol (positive control) Exposure: 2 hours (real-time RT-PCR, western blot); 1-3 hours (chromatin immunoprecipitation); 24 hours (cell cycle analysis); 1-5 days (cell proliferation) Endpoints tested: *c-Myc* transcript levels (BT-474 cells), c-Myc protein levels; proliferation (BT-474 cells), ERα phosphorylation and recruitment	synergistic effect. 1 and 10 µM butylparaben increased expression of c-Myc protein in BT-474 cells to levels comparable to that induced by 17β-estradiol. No effects on protein expression were found in SKBR3 cells. Butylparaben increased the number of BT-474 cells entering S-phase in absence and presence of HRG with an EC50 of 0.551 and 0.024 µM, respectively. The addition of HRG lowered the concentration of butylparaben required to increase cell proliferation from 1 to 0.01 µM. Co-treatment of butylparaben and HRG had a synergistic effect on ERα recruitment to the *c-Myc* enhancer.	
MCF-7/BUS human breast cancer cells	Aim: To investigate the potential of BtP to stimulate proliferation of MCF-7 cells Compounds and concentrations: 4-500 µM BtP (alone or together with silver nanoparticles) Exposure: 6 days Endpoints tested: Proliferation, mRNA expression of ER genes and ER-dependent genes	BtP induced cell proliferation at 4-31 µM, with a maximal increase at 16 µM. BtP increased expression of pS2 and PGR.	Roszak et al. (2017) [75]
MCF-7 human breast cancer cells and T47D human mammary ductal carcinoma cells	Aim: To investigate the potential of a BtP metabolite to promote estrogen signaling by interacting with the ER Compounds and concentrations: 100 nM – 32 µM 3-hydroxy-*n*-butyl 4-hydroxybenzoate (3OH-BtP) and BtP; 100 pM 17β-estradiol (positive control) Exposure: 2 hours - 6 days Endpoints tested: Proliferation, mRNA expression of the estrogen responsive gene *GREB1*, luciferase AR reporter-gene activation	3OH-BtP promoted proliferation of MCF-7 cells with and EC_{50} of 8.2 µM. For BtP, the EC_{50} was 1.2 µM. 3OH-BtP induced proliferation in T47D cells, an EC_{50} was not reached. 10 µM 3OH-BtP and BtP induced GREB1 expression, which could be blocked by the anti-estrogen ICI 182, 780. 3OH-BtP activated the AR reporter. Interaction of 3OH-BtP to human ERα was supported with computational docking studies.	Gonzalez et al. (2018) [77]

Test system	Test principle(s)	Results	Reference
MCF-7 human breast cancer cells and T47D-Kbluc human mammary ductal carcinoma (containing an estrogen-responsive reporter gene construct) cells	Aim: To investigate estrogenic and anti-estrogenic effects of BtP, alone or in mixture with other compounds Compounds and concentrations: 0.3-60 (MCF-7 cells) or 0.3-100 (T47D-Kbluc cells) µM BtP; 0.005-5000 nM 17β-estradiol (positive control) Exposure: 72 (MCF-7) or 24 (T47D-Kbluc) hours Endpoints tested: Cell viability, luciferase reporter-gene activation, proliferation	A non-monotonic inverted U-shaped dose response was observed in BtP-mediated activation of the reporter and BtP induced proliferation of MCF-7 cells, resulting in EC_{50} values of respectively 4.05 and 60.33 µM, and 1.38 and 25.73 µM. Anti-estrogenic activity was observed in both the reporter gene and proliferation assay, with IC_{50} values of respectively 60.55 µM and 47.76 µM.	Pop et al. (2018) [73]
In vitro assays – Estrogenic activity: reproductive and developmental toxicity			
Follicles isolated from immature F_1 hybrid (C57B/6j x CBA/Caj) mice and human granulosa cells (hGCs)	Aim: To investigate the direct effects of BtP (alone or in combination with di-(2-ethylhexyl)phthalate, DEHP) on follicle growth and ovarian steroidogenesis Compounds and concentrations: 10 nM – 10 µM BtP; 1-100 nM DEHP Exposure: 12 days (follicles) or 96 hours (hGCs) Endpoints tested: Growth, survival and 17β-estradiol output (follicles); progesterone output (hGCs)	BtP treatment alone did not affect any of the evaluated parameters. Together with DEHP estradiol output was attenuated, BtP attenuated DEHP-induced decreases in progesterone concentrations.	Guerra et al. (2016) [154]
Human trophoblast (HTR8/SVneo) cells	Aim: To investigate the effects of BtP on placental development Compounds and concentrations: 50, 100 and 200 µM BtP Exposure: 1-48 hours Endpoints tested: 48 hours: proliferation, apoptosis, intracellular Ca^{2+} concentrations, mitochondrial potential; 24 hours: proliferating cell nuclear antigen (PCNA) expression, invasion and migration; 6 hours: expression of proteins related to ER stress; 2 hours: phosphorylation of proteins involved in the P13K/AKT pathway; 1 hour: ROS production	BtP inhibited proliferation, induced apopotosis, increased the expression of proteins related to ER stress, increased intracellular ROS production and Ca^{2+} concentrations, and induced a loss of mitochondrial potential in a dose-dependent manner. 200 µM BtP reduced PCNA expression and inhibited invasive properties. BtP treatment inhibited the activation of P13K/AKT pathways. The results additionally suggested the involvement of ERK1/2 pathways.	Yang et al. (2018) [155]
Wildtype (AB)	Aim: To investigate BtP-induced oxidative stress in	The NOEC for islet variant morphology in	Brown et al. (2018)

Test system	Test principle(s)	Results	Reference
zebrafish embryos and *Tg(ins:GFP)* embryos	the developing embryo and pancreatic beta cells as a sensitive target for embryotoxicity Compounds and concentrations: 1, 2, 5 µM BtP (screening test 1); 0.25, 0.5, 1, 3 µM BtP (screening test 2); 62.5, 125, 250 nM BtP (main test 1); 0.5, 1 µM BtP (main test 2 and 3) Exposure: 72 hours and 7 days (screening test 1 and 2); 4 days, 24 hours and 3 days (main test 1, 2 and 3) Endpoints tested: toxicity (screening test 1); microscopy and image analysis (screening test 1, main test 1), total glutathione and cysteine concentrations (main test 2), expression of genes related to pancreatic endocrine hormone axis and glutathione	*Tg(ins:GFP)* embryos was 125 nM. BtP increased total developmental deformities (pericardial edema, yolk sac utilisation, intestinal effusion, craniofacial malformations, spinal malformations), with no affects at 250 nM and all observation of all deformities at 3 µM BtP. Swim bladder inflation was impaired. No clear relationship was found between the occurrence of deformities and islet variant morphology. BtP increased GSH concentrations. Transcription factor *pdx1* and genes involved in GSH synthesis were downregulated, *gsr* was upregulated.	[80]
Wild-type Tropical 5D zebrafish	Aim: To identify and classify endocrine bioactivity using phenotypically-anchored transcriptomics Compounds and concentrations: 0.25-64 µM BtP; 24 other known EDC Exposure: 114 hours or 42 hours Endpoints tested: developmental toxicity across 22 endpoints, transcriptome profiling.	The EC80 value for BtP was around 10 µM. Clustered correlation analysis following transcriptome profiling indicated the involvement of multiple MOAs for the BtP developmental toxicity profile.	Haggard et al. (2018) [156]
In vitro assays – Androgenic activity			
CHO cells containing an AR reporter gene construct	Aim: To investigate AR antagonistic effects of BtP in alone and in mixture with other parabens Compounds and concentrations: BtP, methylparaben, ethylparaben, propylparaben; Concentrations between 0.01 and 100 µM Exposure: 24 hours Endpoints tested: Cytotoxicity and luciferase reporter-gene activation	BtP antagonised the AR only at cytotoxic concentrations (> 10 µM). In mixture, antagonistic effects were evident from 2 µM. Mixture effects were not additive.	Kjaerstad et al. (2010) [81]
In vitro assays – Adipocyte differentiation			

Test system	Test principle(s)	Results	Reference
Murine 3T3-LI preadipocytes without and with PPARγ transactivation reporters or GR responsive reporters and human adipose-derivedmu multipotent stromal cells (hADSC)	Aim: To investigate adipogenic activity of parabens (methylparaben, ethylparaben, propylparaben, BtP) Compounds and concentrations: 1, 10, 100 µM paraben (3T3-LI cells); 1, 10, 100 µM 4-hydroxybenzoic acid (3T3-LI cells); 50 µM paraben (hADSC) Exposure: During adipogenic differentiation (7 days, 3T3-LI cells) and maintenance (14 days, hADSC) Endpoints tested: Effects on adipocyte differentiation (morphology, lipid accumulation, mRNA expression of specific adipocyte marker genes), reporter activation, effects of PPARγ or GR antagonism on adipogenic potential, adipose conversion of hADSC	Of the tested parabens, BtP was the most potent promoter of adipogenesis om 3T3-LI cells and hADSC. The common paraben metabolite 4-hydroxybenzoic acid did not display adipogenic activity in 3T3-LI cells. All parabens activated both PPARγ and GR (GR activation was not mediated by direct binding or modulating ligand binding of GR). The adipogenic activity of BtP was attenuated by antagonism of PPARγ or GR. During differentiation of 3T3-LI cells, BtP can substitute dexamethasone when it is combined with methylisobutylxanthine and insulin.	Hu et al. (2013) [84]
MDA-kb2 human breast cancer cells containing an androgen and glucocorticoid responsive reporter	Aim: To investigate the glucocorticoid-like activity of BtP alone and in mixture with other glucocorticoid-like compounds Compounds and concentrations: 20 nM and 1 µM BtP, propylparaben, diethylhexyl phthalate and tetrametrihrin Exposure: 24 hours Endpoints tested: Cytotoxicity and luciferase reporter-gene activation	BtP was cytotoxic at concentrations higher than 75 µM. The EC_{50} was 1.75 µM. At 10 nM BtP was the only compound displaying glucocorticoid-like activity (1.44 fold over control). In mixtures, activation was apparent as well, although this was not synergistic or additive.	Klopcic et al. (2015) [157]
Multipotent stem cells C3H10T1/2 without and with PPARγ transactivation reporters or GR responsive reporters	Aim: To investigate the potential of parabens (including BtP) to modulate adipogenic, osteogenic or chondrogenic differentiation of C3H10T1/2 cells Compounds and concentrations: 100 µM BtP; 100 methylparabeen Exposure: During differentiation (8, 12 or 6 days for adipogenic, osteogenic or chondrogenic differentiation respectively); 18 hours for reporter assays	BtP promoted adipogenic differentiation, which was attenuated by PPARγ knockdown. BtP suppressed osteogenic differentiation, which was attenuated by both PPARγ and GR knockdowns. BtP also suppressed chondrogenic differentiation. Treatment with BtP resulted in activation of PPARγ, but not of GR. Similar results were found for methylparaben.	Hu et al. (2017) [83]

Test system	Test principle(s)	Results	Reference
	Endpoints tested: Effects on differentiation, reporter activation, effects of PPARγ or GR knockdown on adipogenic potential		
Murine 3T3-LI preadipocytes	Aim: To investigate adipogenic activity of semivolatile organic chemicals (including BtP) often found in indoor environments Compounds and concentrations: 0.1 nM – 10 µM BtP Exposure: During adipogenic differentiation (10 days) Endpoints tested: Promotion of triglyceride accumulation, preadipocyte proliferation	BtP treatment did not lead to cell proliferation. Over the tested dose-range, maximal 0.9% triglyceride accumulation was found.	Kassotis et al. (2017) [82]

10.1.2 In vivo data of endocrine parameters and toxicity of butylparaben

Table A2. In vivo data of endocrine parameters and toxicity of butylparaben (BtP) published after 2010, plus the key studies taken into account by the SCCS.

Test system	Test principle(s)	Results	Reference
Wistar **rats**	Neonatal repeated dose study on potential effects of BtP on the development of the testis. *Dose:* 2 mg/kg bw/day (n=6) *Route:* s.c. injection *Duration:* postnatal days (PNDs) 2-18 *Examinations:* Following sacrifice, the testes and epididymides were removed, weighted, and prepared for histopathology and immunochemistry. The potent estrogenic compounds diethylstilbestrol (DES) and ethinyl estradiol (EE) and the less potent estrogenic compounds bisphenol A, genistein and octylphenol were included as well.	No detectable effects on the assessed parameters (testis weight, distension of the rete testis and efferent ducts, epithelial cell eights in the efferent ducts, immunoexpression of aquaporin-1). All parameters were affected with the potent estrogenic compounds, minor observations were made in histopathology only with the less potent estrogenic compounds **NOEL = 2 mg/kg bw/day**	Fisher et al. (1999)[1] [67]
Wistar **rats**	Repeated dose study in immature rats (19-21 days old) on potential reprotoxic effects of BtP. *Doses:* 0.01%, 0.10% and 1.00% (n=8) *Route:* oral (in diet, *ad libitum*) *Duration:* 8 weeks *Examinations:* Following sacrifice, reproductive organs (i.e. the testes, epididymides, ventral prostates, preputial glands and seminal vesicles with coagulation glands) were weighted. Sperm counts were determined in the testes and epididymides and testosterone levels were assessed in serum.	*Average BtP intake:* 10.4, 103 and 1026 mg/kg bw/day, respectively The relative weight of the epididymides decreased at 0.10 and 1.00%. The cauda epididymal sperm reserves, daily sperm production and the efficiency of sperm production decreased dose dependently. Testosterone concentrations also decreased at all doses (significant from 0.10%). LOAEL = 10.4 mg/kg bw/day	Oishi (2001)[1] [68]
Sprague-Dawley Crl:CD®BR VAF/Plus® **rats**	Developmental toxicity study *Doses:* 10, 100 and 1000 mg/kg bw/day (n=25) *Route:* oral (gavage) *Duration:* GD 6-19, inclusive *Examinations:* Dams were monitored for body weights, feed consumption and clinical signs. Dams	Maternal body weights and feed consumption were decreased in the high dose group. No treatment-related developmental toxicity or foetal alterations were found. NOAEL (maternal toxicity) = 100 mg/kg	Daston (2004)[1] [70]

Test system	Test principle(s)	Results	Reference
	were sacrificed on GD 20 and were Caesarian-sectioned. Further examinations included gravid uterus weight, number and distribution of corpora lutea, implantation sites, live and dead foetuses, and early and late resorptions. Examinations on foetuses included sex determination, gross external alterations, body weights, soft tissue alterations and skeletal alterations.	bw/day NOAEL (developmental toxicity) = 1000 mg/kg bw/day	
Wistar (Crl:(WI) BR) **rats**	Repeated dose study in immature rats (22 days old) on potential reprotoxic effects of BtP. *Doses:* 100, 1000 and 10000 ppm (n=8) *Route:* oral (in diet, *ad libitum*) *Duration:* 8 weeks *Examinations:* From week 3, blood was collected bi-weekly (every other week) for assessment of hormones. Following sacrifice, gross necropsy was performed. Selected organs (including the reproductive organs) were weighted and/or subjected to histopathology. Sperm counts were determined in the testes.	*Average BtP intake:* 10.9, 109.3 and 1087.6 mg/kg bw/day, respectively In week 3, testosterone concentrations were decreased in the mid- and high dose group. At later timepoints, testosterone, follicle stimulating hormone and luteinizing hormone concentrations were increased in the high dose group. These effects were dismissed by the authors. NOAEL = 1087.6 mg/kg bw/day	Hoberman et al. (2008)[1] [71]
Albino **rats**	Developmental neurotoxicity study in male offspring rats (n=12) from female albino rats exposed to BtP. *Dose:* 200 mg /kg bw/day *Route:* oral or subcutaneous *Duration:* gestation day (GD) 1 to lactation day 21 *Examinations:* Treatment with valproic acid was included as positive control. Rats were subjected to the three-chamber sociability test and the morris water maze task. Following sacrifice, brains were dissected out and subjected to biochemical studies.	Treatment resulted in reduced social approach to foreigner rats and disturbances in learning and memory abilities. Monoamine content (in different sections of the brain), free amino acids contents (in the frontal cortex) and brain derived neurotrophic factor (BDNF) content were altered. Similar effects were noted in offspring from valproic acid-treated dams. LOAEL = 200 mg/kg bw/day	Ali et al. (2013) [158]
Sprague-Dawley	Reproductive toxicity study in 3-week old male rats	Treatment led to histopathological changes	Alam & Kurohmaru

Test system	Test principle(s)	Results	Reference
rats	(n=8) to investigate acute effects of BtP on testicular tissues *Dose:* 1000 mg /kg bw *Route:* oral *Duration:* single administration *Examinations:* Rats were sacrificed 3, 6 and 24 hours after administration, after which their testes were collected and prepared for histopathology. The incidence of apoptotic spermatogenic cells was quantified and spermatogenic cell types that underwent apoptosis were evaluated.	in the seminiferous tubules (i.e. reduction and/or disappearance of tubular lumen at 3 and 6 hours, thin seminiferous epithelia and wide tubular lumen at 24 hours) and an increased incidence of spermatogenic cells. LOAEL = 1000 mg/kg bw/day	(2014) [151]
Sprague-Dawley **rats**	Reproductive toxicity study in 3-week old male rats (n=8) to investigate acute effects of BtP on Sertoli cell skeleton. *Dose:* 1000 mg /kg bw *Route:* oral *Duration:* single administration *Examinations:* Rats were sacrificed 3, 6 and 24 hours after administration, after which their testes were collected and prepared for histopathological and immunohistochemical examinations.	BtP treatment induced a collapse of Sertoli cell vimentin filaments and disrupted microfilaments. No changes were observed in the pattern of microtubule network. Spermatogenic cells became separated from the basement membrane and sloughed into the lumen. LOAEL = 1000 mg/kg bw/day	Alam & Kurohmaru (2014) [151]
Wistar **rats**	The development of the reproductive system of male offspring was investigated by treatment of pregnant dams (n=7-8) with BtP *Doses:* 64, 160, 400 or 1000 mg/kg bw/day *Route:* oral (gavage) *Duration:* GD 7 to PND 21 *Examinations:* On PND 4, litters were culled to 8 pups (preferably all male) per litter. The anogenital distance (AGD) (PND 1 and 21), time of testicular descent (TD) (PND 15) and preputial separation (PPS) (from PND 33) were determined. Following	At 400 and 1000 mg/kg bw/day, AGD and PPS were reduced, hormone levels were altered at selected ages, epididymal sperm counts and daily sperm production were decreased and histopathological observations were made. The weight of the testis was affected from 160 mg/kg bw/day. NOAEL = 64 mg/kg bw/day	Zhang et al. (2014) [87]

Test system	Test principle(s)	Results	Reference
	sacrifice at PND 21, 35, 49, 90 or 180, blood was collected for hormone assays (testosterone, 17β-estradiol, progesterone, luteinizing hormone, follicle-stimulating hormone), selected organs (including androgen-sensitive organs) were weighted and the tests was subjected to histopathology. Epididymal sperm counts and daily sperm production were determined.		
HanTac:WH **rats**	The development of the reproductive system of male and female offspring was investigated by treatment of pregnant dams (n=18) with BtP. *Doses:* 10, 100 or 500 mg/kg bw/day *Route:* oral (gavage) *Duration:* GD 7 to GD21 and from PD 1 to 22 *Examinations:* Pups were checked for anomalies. The AGD, number of areolas/nipples (NR) and pubertal onset were determined. Following sacrifice, the number of implantation sites in dams were recorded. At PD 16 (males) and 17 (females), reproductive organs were collected from 1 male and female pup/litter to determine weights and histological and gene expression changes. Blood was collected for hormone analysis. At PD 22, 1 male and female pup/litter were sacrificed for hormone analysis in blood and determination of reproductive organ weight. Mammary glands of female pups were subjected to whole mounting. At PD80-90, 1 male and female pup/litter was sacrificed to determine (reproductive) organ weights. Selected organs were prepared for histopathology. Gene expression in testis was analysed and epididymal sperm counts were determined.	Effects at 10, 100 and 500 mg/kg bw/day: Sperm count reduced, testicular *CYP19a1* (aromatase) expression reduced in prepubertal males. Effects at 100 and 500 mg/kg bw/day: AGD reduced, ovary weights reduced and mammary gland outgrowth increased in prepubertal females. Effects only at 500 mg/kg bw/day: Adult prostate weights decreased. Prostate histology altered. LOAEL = 10 mg/kg bw/day	Boberg et al. (2016) [88]

Test system	Test principle(s)	Results	Reference
Swiss albino **mice**	Effects on the uterus were investigated in mice. *Doses:* 10, 50 or 100 mg/kg bw/day (n≥5) *Route:* subcutaneous *Duration:* 7 days *Examinations:* Following sacrifice, the uterus was weighted (also done for 21-day exposure) and prepared for histological and morphological analysis. Uterine endometrial glands and total uterine tissue protein were quantified.	Uterine weight was increased and histological observations were made at the mid and high dose. The thickness of endometrium and mycometrium and the total uterine tissue protein content were increased at the high dose. The number of uterine glands were increased at all doses. LOAEL = 10 mg/kg bw/day	Goswami & Kalita (2016) [89]
C57BL/6J **mice**	Effects on adipogenesis were investigated in 4-week old female obesity prone mice. *Dose:* 100 mg/kg bw/day (n=7 to 8) *Route:* oral (gavage) *Duration:* 12 weeks *Examinations:* Mice were fed either a chow diet or high-fat diet (ad libitum). Mice were subjected to glucose and insulin tolerance tests after respectively 10 and 11 weeks of treatment. After 12 weeks, whole blood was collected for measurement of plasma insulin, triglycerides, serum adiponectin, leptin and serum procollagen type 1 N-terminal propeptide and collagen type I C-terminal telopeptide. Mice were euthanised and major fat pads and liver were removed, weighted and processed for RNA isolation for quantitative real-time PCR analysis.	No effects on adiposity and serum leptin levels were found. In both diet groups, treatment induced changes in gene expression in the white adipose tissue and the liver, that are related to adipocyte differentiation and lipogenesis. In the chow diet group a decrease in serum procollagen type 1 N-terminal propeptide was observed, which can be linked to bone formation. LOAEL = 100 mg/kg bw/day	Hu et al. (2016) [90]
Hsd:Sprague Dawley SD **rats**	Internal exposure of BtP was investigated in pregnant rats. *Doses:* 1500, 5000 or 15000 ppm (n=35) *Route:* oral (diet, *ad libitum*) *Duration:* GD 6 to PND 28 *Examinations:* Free (unconjugated) and total (unconjugated and conjugated) BtP concentrations	Throughout the dosing period, average BtP exposure was 106.6-339.2, 360.3-1224.5 and 1217.8-3493.8 mg/kg bw/day for respectively 1500, 5000 and 15000 ppm. No effects were observed on littering rate, litter size, live litter size and sex ratio. Pup body weights were lower in the high dose	Roberts et al. (2016) [91]

Test system	Test principle(s)	Results	Reference
	were quantified in dam plasma, amniotic fluid and foetuses on GD 18 and in dam and pup plasma on PNDs 4, 10, 14, 21, and 28.	group. Results suggested a limited placental transfer and low lactational transfer, as analyte levels in amniotic fluid were < 1% compared to maternal plasma, and total BtP in PND4 pup plasma was <5% compared to dam plasma. The data suggested limited BtP conjugation in pups (26 to 53% conjugation at PNDs 4 and 10), only reaching similar levels as dams (>99% conjugation) at PND 21 to 28. NOAEL = 360.3-1224.5 mg/kg bw/day	
Wistar **rats**	Possible mechanisms of endocrine and reproductive disorders were investigated by treatment of pregnant rats. *Doses:* 64, 160, 400 or 1000 mg/kg bw/day (n=7 or 8) *Route: (*oral gavage) *Duration:* from GD 7 to PND 21 *Examinations:* On PND 4, litters were culled to 8 pups (preferably all male) per litter. On PNDs 21 and 90 one (random) male per litter was sacrificed, and blood was collected to determine steroid hormone concentrations in serum. Reproductive organs were collected and weighted. Testes of the three highest dose groups were prepared for histopathology, immunohistochemical analysis (protein expression of AR, ERα and ERβ), RNA isolation for quantitative PCR analysis (RNA expression of *StAR, P40SCC, Cyp19, Sult1E1, ERα, ERβ, AR, Dnmt1, Dnmt3a and Dnmt3b*), DNA extraction for bisulfite sequencing PCR (DNA methylation in the promotor region of the ERα	Treatment with 400 and 1000 mg/kg bw/day resulted in increased epididymis indices, testosterone and estradiol serum levels and CYP19 and mRNA and protein expression. Histopathological observations were made it the 400 and 1000 mg/kg bw/day dose groups and included reduced and loosely arranged germ cells, and decreased layers of germinal epithelium. ERα expression was increased at 400 and 1000 mg/kg bw/day or at all doses, at respectively transcript or protein level. Dnmt3b mRNA expression was increased at high dose, while DNA methylation of the estrogen receptor was decreased. 400 and 1000 mg/kg bw/day exposure decreased P450cc (at protein level also at 160 mg/kg bw/day), AR (at protein level also at 160 mg/kg bw/day) StAR (at transcript level only at 1000	Zhang et al. (2016) [92]

Test system	Test principle(s)	Results	Reference
	gene) and protein extraction for western blotting (protein expression of StAR, P450scc, CYP19, ERα ERβ, AR and SULT1E1.	mg/kg bw/day) and SULT1E1 expression levels. Higher levels of CYP19 and lower levels of SULT1E1 may increase estradiol concentrations, which promotes ERα expression. Epigenetic hypomethylation of ERα may also promote expression of ERα. NOAEL = 160 mg/kg bw/day	
Sprague-Dawley **rats**	Reproductive toxicity of BtP exposure during a complete spermatogenic cycle was investigated by treating 6-week old male rats. *Doses:* 150, 300 or 600 mg/kg bw/day (n=10) *Route:* subcutaneous *Duration:* 57 days *Examinations:* On day 30, urine samples were collected for toxicokinetic studies. After treatment animals were sacrificed and blood was collected for haematological, biochemical and hormonal analyses. Liver and reproductive organs were weighted and prepared for histopathological exam. Sperm motility, maturity, viability and morphology were determined. Sperm counts were determined in testis and epididymis.	BtP and its metabolites (*p*-hydroxybenzoic acid and *p*-hydroxyhippuric acid) were excreted in urine in a dose-dependent manner. Spermatozoa counts in epididymis were decreased and abnormal spermatozoa in the testis were increased at all doses. Histopathological observations (contraction and decreased secretory content) were made in seminal vesicles in all dose groups. At the high dose, prostate weight was increased. LOAEL = 150 mg/kg bw/day	Garcia et al. (2017) [93]
Wistar **rats**	Developmental toxicity in male offspring (foetuses) was investigated treatment of pregnant rats. *Doses:* 10, 100 or 200 mg/kg bw/day (n=8) *Route:* subcutaneous *Duration:* from GD 12 until GD 20 *Examinations:* After treatment, rats were sacrificed and uterus and ovaries were investigated for number of corpora lutea, implantation sites, resorptions and live and dead foetuses. One testis from one male	No treatment-related effects were found. NOAEL = 200 mg/kg bw/day	Guerra et al. (2017a) [94]

Test system	Test principle(s)	Results	Reference
	foetus per litter was prepared for histological analysis (seminiferous cord diameter, number of gonocytes per cord, number of foetal Leydig cells).		
Wistar **rats**	Developmental toxicity in male offspring (pups) was investigated by treatment of pregnant rats. *Doses:* 10, 100 or 200 mg/kg bw/day (n=9) *Route:* subcutaneous *Duration:* GD 12 until PND 22 *Examinations:* Pups were sexed and culled to 8 pups (similar numbers of females/males) on PND 1. Further examinations were performed on male pups. The AGD was determined on PND 1. The number of nipple/areolas was recorded on PND 13. Pups were weaned at PND 22 and examined daily for complete preputial separation from PND 30. At 110 days of age, one male per litter was sacrificed and reproductive and detoxifying (liver and kidney) organs were weighed. The left testis and epididymis were prepared for histopathological evaluation. Testis transverse sections were prepared for immunohistochemistry. The right testis and epididymis were prepared to determine daily sperm production per testis, sperm number and transit time in the epididymis. Blood was collected for hormone analysis (testosterone, FSH, LH). 1-2 rats per litter were paired to a sexually receptive adult female for examinations on sexual behaviour and mating. Sperm quality was further assessed by isolation of proximal cauda sperm from male rats and subsequent insemination of receptive females. Naturally and artificially inseminated females were sacrificed on GD 20 and uterus and ovaries were	Treatment resulted in an increased number of Adult Leydig Cells at the mid and high dose and altered spermatogenesis kinetics at low and high dose. At the high dose immunostaining of EsR1 and AR was impaired, testosterone concentrations were increased and LH and FSH concentrations were depressed. Sperm motility was impaired at the low dose. Sperm head abnormalities were increased at all doses. LOAEL = 10 mg/kg bw/day	Guerra et al. (2017a) [94]

Test system	Test principle(s)	Results	Reference
	collected to investigate fertility of the male rats (fertility potential and rate of postimplantation loss). Sperm motility and morphology was assessed using the isolated proximal cauda sperm.		
Wistar **rats**	Estrogenicity was investigated by treatment of weaned immature female rats. *Doses:* 10, 100 or 200 mg/kg bw/day *Route:* subcutaneous *Duration:* PND 20 to PND 22 *Examinations:* Pups were weighed and sacrificed on PND 23, after which uterus wet weight was determined.	No treatment-related effects were found. NOAEL = 200 mg/kg bw/day	Guerra et al. (2017b) [95]
Wistar **rats**	Developmental toxicity in female offspring (foetuses) was investigated by treatment of pregnant rats. *Doses:* 10, 100 or 200 mg/kg bw/day (n=8) *Route:* subcutaneous *Duration:* from GD 12 until GD 20 *Examinations:* After treatment, rats were sacrificed and uterus and ovaries were investigated for number of corpora lutea, implantation sites, resorptions and live and dead foetuses. The number of germ cells was determined in one ovary from one female foetus per litter by histological analysis.	No treatment-related effects were found. NOAEL = 200 mg/kg bw/day	Guerra et al. (2017b) [95]
Wistar **rats**	Developmental toxicity in female offspring (pups) was investigated by treatment of pregnant. *Doses:* 10, 100 or 200 mg/kg bw/day (n=9) *Route:* subcutaneous *Duration:* from GD 12 until PND 21 *Examinations:* Pups were sexed and culled to 8 pups (similar numbers of females/males) on PND 1. Further examinations were performed on female pups. The AGD was determined on PND 1. The	No treatment-related effects were found. A non-significant impairment of sexual behaviour was found at the high dose, which may suggest central nervous effects. NOAEL = 200 mg/kg bw/day	Guerra et al. (2017b) [95]

Test system	Test principle(s)	Results	Reference
	number of nipple/areolas was recorded on PND 13. Pups were weaned at PND 22 and examined daily for complete puberty onset (vaginal opening followed by the day of the first estrous) from PND 30. On PND 60 to PND 75 the estrous cyclicity was evaluated, after which one female per litter was sacrificed and reproductive and detoxifying (liver and kidney) organs were weighed. The uterus and ovary were prepared for histopathological evaluation. Blood was collected for hormone analysis (estrogen, progesterone, FSH, LH). During the first proestrus after PND 100, another set of females were paired to a sexually experienced male rat for examinations on sexual behaviour. Thereafter fertility performance was assessed by pairing the females for an additional 4 hours with a sexually experienced male. Naturally inseminated females were sacrificed on GD 20 and uterus and ovaries were collected for histopathological examination (gestation rate, preimplantation loss, postimplantation loss).		
Sprague-Dawley **rats**	Effects on ovarian folliculogenesis and steroidogenesis were investigated in female rats. *Dose:* 100 mg/kg bw/day (n=6) *Route:* oral *Duration:* 5 weeks *Examinations:* The estrous cycle was monitored during treatment. Following sacrifice, distributions of ovarian follicles were determined by histopathology, total RNA was extracted from ovaries to determine mRNA expressions of genes associated with ovarian steroidogenesis, and blood was collected to determine FSH concentrations.	Treatment with BtP shortened the interval of the estrous cycle, and decreased the number of total and preovulatory follicles. mRNA expression of steroidogenic enzymes (i.e. *Cyp19a1* and *Hsd3b1*) and hormone receptors (i.e. *Lhr*) in ovaries was decreased. FSH concentrations were increased. LOAEL = 100 mg/kg bw/day	Lee et al. (2017) [96]

Test system	Test principle(s)	Results	Reference
CF1 **mice**	The potential of BtP to modulate concentrations of 17β-estradiol was investigated in mice *Dose:* 91.5 mg /kg bw or 89.8 mg /kg bw for respectively females and males (n=10) *Route:* subcutaneous *Duration:* single injection *Examinations:* 2, 4, 6, 8, 10 and 12 hours following injection urine was collected non-invasively.	Concentrations of urinary 17β-estradiol were elevated following treatment in both females and males. LOAEL = 89.8 mg/kg bw	Pollock et al. (2017) [159]
Wistar albino **rats**	Subchronic reproductive study on the effects on male gonadal toxicity *Dose:* 50 mg/kg bw/day (n=6) *Route:* oral *Duration:* 8 weeks *Examinations:* Following treatment, blood was collected to determine serum concentrations of hormones (testosterone, follicle stimulating hormone, luteinizing hormone, estradiol). Animals were sacrificed and the testes and epididymides, the seminal vesicles (full of secretion) and ventral prostate gland were removed. Organ to body weight ratios were calculated. Sperm count and motility were determined in the epididymides. The testes were prepared for oxidant/antioxidant determination and for the comet and histopathological examination.	Following treatment, superoxide dismutase enzyme activity was reduced, catalase activity was inactivated. Malondialdehyde content was increased. Treatment reduced sperm integrity and motility and decreased serum levels of testosterone and follicle stimulating hormone. Estradiol levels were elevated. BtP induced testicular DNA damage. Histopathology revealed a reduction in Leydig cells population with spermatogenic arrest. LOAEL = 50 mg/kg bw/day	Riad et al. (2018) [160]

[1] Data already available and taken into account by the SCCS

10.2 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 1223/20093.

From Annex V "List of preservatives allowed in cosmetic products"								
	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-	Not to be used in leave-on products designed for application on the	For leave-on products designed for children under three years of age: 'Do not use on

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

From Annex V "List of preservatives allowed in cosmetic products"								
	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
						and propylparaben and their salts does not exceed 0.14%	nappy area of children under three years of age.	the nappy area'

Table A4. Entries 1374 – 1378 in Annex II "List of substances prohibited in cosmetic products" in the Cosmetics Regulation (EC) No 1223/20094.

	From Annex II "List of substances prohibited in cosmetic products"		
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

10.3 List of abbreviations

ADI	Acceptable Daily Intake
AR	Androgen Receptor
BIT	Benzisothiazolinone
BPR	Biocidal Products Regulations
CBG-MEB	Medicines Evaluation Board
CIR	Cosmetic Ingredient Review
CMIT	Chloromethylisothiazolinone
COLIPA	Cosmetic Toiletry and Perfumery Association
DTU	Danish National Food Institute
DNEL	Derived No Effect Level
ECHA	European Chemicals Agency
EATS	Estrogen, Androgen, Thyroid and Steroidogenic
EC	European Commission
ED	Endocrine-Disrupting
EDC	Endocrine Disrupting Chemical
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EPA	Environmental Protection Agency
ER	Estrogen Receptor
ERRγ	Estrogen-Related Receptor gamma
FAO	Food and Agricultural Organisation
FCM	Food Contact Material
GD	Gestation Day
GR	Glucocorticoid Receptor
HI	Hazard Index
HQ	Hazard Quotient
JECFA	Joint FAO/WHO Expert committee on Food Additives
JRC	Joint Research Centre
LH	Luteinizing Hormone
LOAEL	Lowest Observed Adverse Effect Level
LOQ	Limit of Quantification
MI	Methylisothiazolinone
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
NTP	National Toxicology Program
NVWA	Netherlands Food and Consumer Product Safety Authority
OECD	Organisation for Economic Co-operation and Development
OIT	Octylisothiazolinone
PACEM	Probabilistic Aggregate Consumer Exposure Model
PCP	Personal Care Product
PHBA	p-hydroxybenzoic acid
PHHH	p-hydroxyhippuric acid
PPPR	Plant Protection Products Regulation
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety

SCENHIR	Scientific Committee on Emerging and Newly Identified Health Risks
SCF	Scientific Committee for Food
SCHER	Scientific Committee on Health and Environmental Risks
TEF	Toxicity Equivalence Factor
TFEA	Task Force on Exposure Assessment
TFHA	Task Force on Hazard Assessment
TG	(OECD) Test Guidance
TSH	Thyroid-Stimulating Hormone
UDP	Uridine diphosphate
WHO	World Health Organization

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