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Human exposures to parabens in cosmetics-

a literature study

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HEL- 3950 Master's Thesis in Public Health
December 2013

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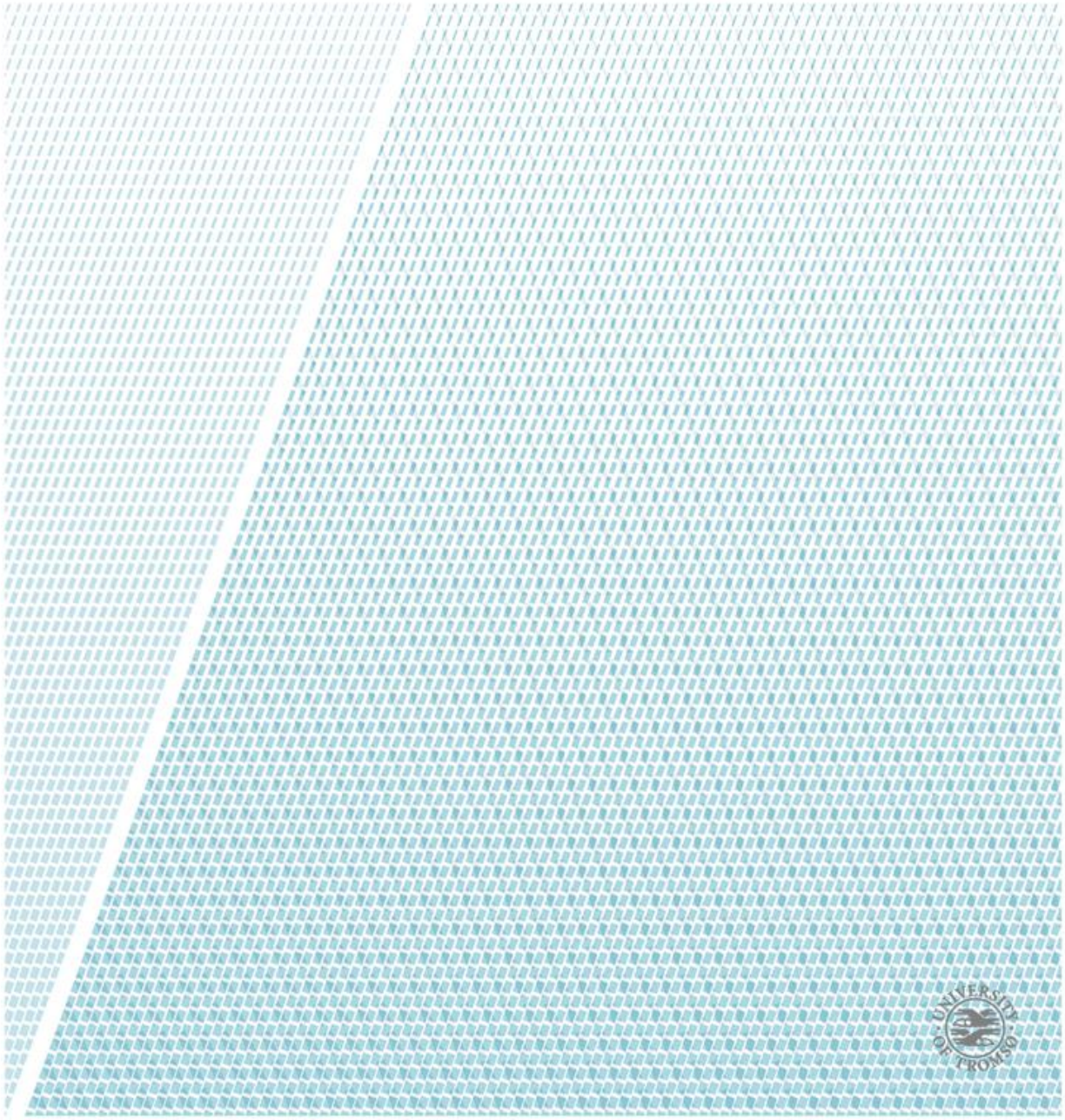

PREFACE

Writing about endocrine disrupting chemicals (EDCs) including parabens can basically be explained by a personal interest and a desire to get more in depth knowledge on the topic. The reason for this interest is especially related to the increasing concern of chemicals possession of endocrine disrupting effects in humans and animals, and the last 50 years increase in disease incidences and prevalences (1). This interest has gradually grown during the last years, as EDCs and parabens have received increasing attention in media, and as I have gained more knowledge on the topic during the course HEL- 3030 International and Environmental Health. The thesis focus on exposures through cosmetics is based on consumers' extensive and uncritical use of such EDC- containing products.

I find it important to increase knowledge on EDCs, both in terms of more scientific research, and informing consumers. Without knowledge it is not possible for consumers to take precautions. Precaution is important in terms of protecting the health of individuals and the public. And as stated in the precautionary principle: lack of significant data is not an excuse not to intervene (2).

ACKNOWLEDGEMENTS

First and foremost I would like to thank my supervisor Torkjel Manning Sandanger for dedicating his time to guide and share valuable knowledge during the educational and interesting period of thesis writing. Throughout the writing- period I have received all the guiding and advice I have needed, despite of his tight schedule.

I would like to thank other employed at ISM who has been advising in the process of accomplishing the thesis, and those who have performed the administrative tasks.

I will express gratitude to my family who has cared and supported me during this period.

And last but not least I will thank my fellow students and friends from the Master`s Program in Public Health. Good advice and constructive discussions always come in handy. A special thanks to Marko Lukic for proofreading on such a short notice.

Ragnhild L. Aarflot

Sunndalsøra 02.12.13

ABSTRACT

A literature study was performed in order to assess and compare evidence of human exposure to parabens in cosmetics. The focus of the thesis is on human concentrations, the rate of dermal absorption, metabolism and excretion; in order to increase our understanding of human exposures to endocrine disrupting chemicals in cosmetics. High detection rates of native and total parabens in blood and urine were identified. GMs of native parabens were lower than total paraben levels in urine as expected, because parabens need to be conjugated before excreted. More research is required to determine medians or means of native parabens in human plasma and serum, as disparity exist between median concentrations measured in the two existing studies. Based on available evidence it was not possible to conclude on the percentage of dermal absorption, but it is indicated that higher exposures to native parabens occur when dermally absorbed in contrary to orally. As paraben exposures are widely occurring and parabens have a half- life of less than 24 hours, regular or constant exposures are identified. Regular or constant exposures do most likely occur from the use of cosmetics, which is in conformity with evidence showing both higher cosmetic use and higher GMs of parabens among women than men. Elevated paraben exposures among women can also cause exposures to the most vulnerable groups; the fetus and breastfeeding infant. However, further research is required to investigate to what extent these EDCs with short half- lives reach the fetus and the infant through breast milk. Further research is also required to investigate effects of parabens in combination with other compounds, the so- called cocktail/ mixture effect, as this has been a neglected area in international studies. Based on available evidence it can be concluded that strong evidence exists on widely occurring paraben exposures among humans.

Keywords

Endocrine disrupting chemical, paraben, p- hydroxybenzoic acid, exposure, dermal absorption, metabolism, serum/ plasma concentration, urine excretion, vulnerable groups, mixture effects

LISTS OF FIGURES AND TABLES

CONTENTS

1. RATIONALE

Humans are daily exposed to a number of chemicals simultaneously (3). Some of these chemicals are endocrine disrupting chemicals (EDCs), which add to the already high body burden of persistent EDCs (4). To reduce human exposures to new EDCs and thereby decrease the potential to acquire adverse health effects (5), it is necessary to increase knowledge on chemical exposures.

Exposures happen through different routes (figure 1), but of increasing concern are exposures to EDCs in cosmetics. The concern is related to the extensive and increasing use of such products (6-8). To assess human exposures to parabens in cosmetics were decided because of their possession of estrogenic properties (9, 10), their extensive use in cosmetics (11, 12) and the increasing attention they have received in media. As paraben exposures and their potential to cause adverse health effects are still debated, it is important to increase knowledge and understanding.

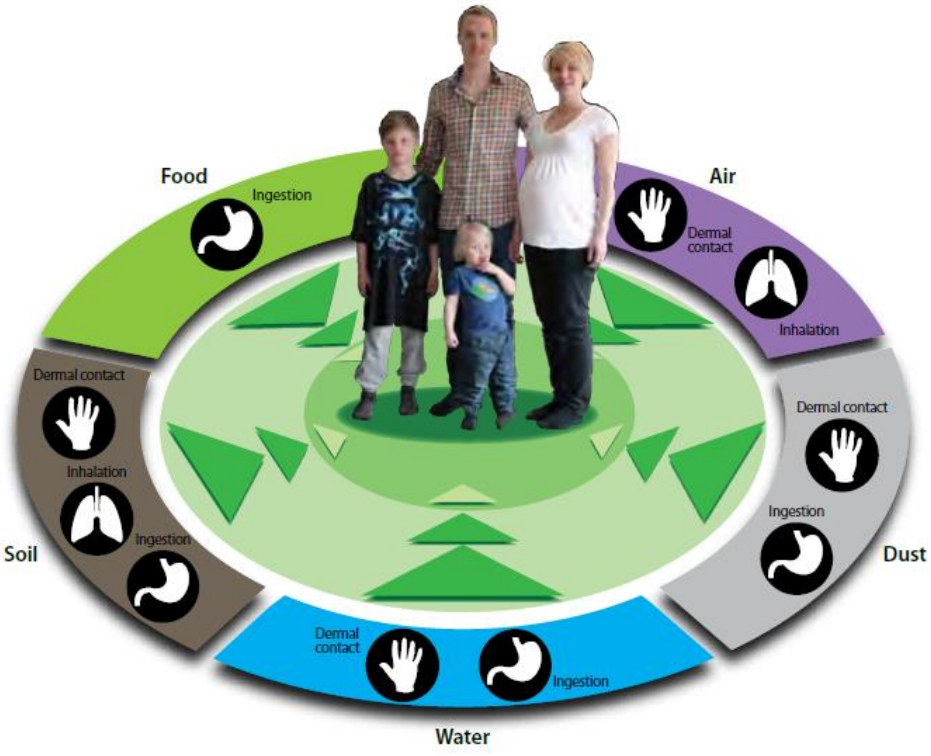

Figure 1: Exposure routes of EDCs. The sources (water, soil, food, air and dust) illustrate pathways for human absorption of EDCs. Figure reused with publishers permission (13).

2. OBJECTIVE

The objective of this literature study is to assess human exposure to parabens in cosmetic, with a particular focus on human concentrations, rate of dermal absorption, metabolism and excretion; and by this increasing our understanding of human exposure to EDCs in cosmetics.

3. METHOD AND MATERIALS

A literature study was performed with the purpose to describe paraben exposures from cosmetics in humans, by discussing and comparing evidence. As this is an exposure assessment and not a risk assessment, toxicities are not covered. Potential adverse health effects of EDCs and parabens are only shortly described in the introduction. As paraben exposures in humans are believed to be mainly caused by cosmetic use (9, 14), the discussion is focused on the human concentration, dermal absorption, metabolism and excretion of parabens.

3.1 Definitions and clarifications

Some of the frequently used terms in the thesis can be interpreted subjectively. Definitions and clarifications are therefore given to make sure readers interpret the thesis as similar as possible. Definitions are shown before potential clarifications:

"Exposure" is defined by the International Programme on Chemical Safety (IPCS) (15) as:

"a concentration or amount of a particular agent that reaches a target organism, system, or (sub) population in a specific frequency for a defined duration" (p. 12).

"Cosmetics" is defined by the European Commission (EC) (16) as:

"Any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours."

The Precautionary Principle or Precautionary Approach has several definitions. The European Unions (EU) (17) communication on the principle is as follows:

"*The Precautionary Principle applies where scientific evidence is insufficient, inconclusive or uncertain and preliminary scientific evaluation indicates that there are reasonable grounds for concern that the potentially dangerous effects on the environment, human, animal or plant health may be inconsistent with the high level of protection chosen by the EU*" (p. 13). The principle aims at pre- damage control. Interventions based on the precautionary principle can be initiated in the occurrence of morally unacceptable harms, to avoid or reduce them. Such harms are a risk to human life or health, severe and permanent, unjust to present or future generations, or imposed without concern of the human rights of the people affected (17). An example of a morally unacceptable harm can be the addition of an EDC in cosmetics. The precautionary principle is included in several international declarations and agreements, like the UN`s 1992 Rio Declaration on Environment and Development (17).

An "*exposure*" occurs, based on the IPCS (15) definition, when EDCs including parabens are absorbed and can be measured in a living organism. For instance, a human is exposed to parabens when measurable levels are detected in blood or urine, as native, metabolized or conjugated parabens. The definition is based on the fact that no compound can cause adverse health effects if not absorbed.

Based on the EC (16) definition, "*cosmetics*" in the thesis include hygiene products as well as make- up products, skin creams and lotions, deodorants and perfume, hairspray and shaving creams. The decision to use the term "cosmetic" instead of "personal care product" is based on the lack of an official definition of "personal care product", as well as the European Commission`s definition of "cosmetics" were satisfactory for use in the thesis.

There is no official definition of "*native paraben*", "*paraben metabolite*" and "*paraben conjugate*". In the thesis a native paraben is defined as a non- metabolized or non- conjugated paraben. Native parabens can be hydrolyzed into their main metabolite p-hydroxybenzoic acid (PHBA) by esterases in human skin (phase one metabolism), or into a glycine, sulfate or glucuronide conjugate (phase two metabolism) in the liver and intestines. Glycine, sulfate and glucuronide conjugates are conjugated PHBA (9, 18).

There is not an official definition of *limit of detection (LOD)* either. The LOD describe the lowest concentration of a component that can be detected by an analytical method, and is thereby used to distinguish whether a component is present or absent (19).

3.2 The thesis structure

The discussion of parabens is found in **Chapter 5** and titled "*human exposures to parabens"*. The thesis structure is gradually built- up, so even the unaware reader should be able to get an understanding of the thesis topic, and understand the content of the discussion and the conclusion.

A rationale is found in **Chapter 1** to give an overall explanation of the thesis topic and to explain why this topic is important. **Chapter 2** state the objective, while **Chapter 3** explains how the literature study was conducted and which materials were used. This chapter also includes definitions and clarifications of terms, and possible limitations in studies on parabens. **Chapter 4** covers the background theory. And it is here explained what EDCs and parabens are, and briefly where in the environment and in which products they can be found. It is also briefly explained what adverse health effects they can cause and their mechanisms of action in the human body. A subchapter on the use of cosmetics is also found here. **Chapter 5** gives the information about the human concentration of parabens in different matrices. The human concentration is discussed in two separate parts, after theme. The first discussion covers dermal absorption and metabolism of parabens. Because of overlapping's between dermal uptake and metabolism, these activities were discussed in the same chapter. The second discussion covers the excretion of parabens. A subchapter on paraben exposures in the vulnerable groups is located in chapter 5.2.1. **Chapter 6** is a methodological summary, explaining and studying common methodological challenges in studies on parabens. **Chapter 7** is reflecting upon effects of parabens in combination with other compounds, while the thesis conclusion is found in **Chapter 8.** The reference list and the appendix are found at the end.

In **Appendix A** is the example on how literature searches were performed, while literature matrices are shown in **Appendix B**. Matrices were used to make comparisons of paraben concentrations and detection rates easier. The studies presented in the matrices are the main

studies discussed in chapter 5. How these studies were found are explained in the succeeding chapter.

3.3 Literature search strategy

Systematic searches were performed to find all available relevant background theory, and through this identify the most relevant papers to discuss in chapter 5. Searches were performed on peer reviewed studies using the online search engines PubMed and Scopus. PICO (population, intervention, comparison and outcome) was used in the search strategy to find articles specific to the topics. Truncation was used to search for different variances of the same word, while boolean search was used to make the search more effective. Words where combined with "AND"; "OR" was used to find either one of the words searched for; and "NOT" was used to exclude papers not relevant to the topic. As a literature study is a dynamic process, searches were done several times during the writing process to find theory, and evidence showing an effect or no effect. Because of the large number of searches, it would not be possible to present them in the thesis. However, one search strategy is presented in appendix A (including included and excluded studies), to give an example on how systematic searches were conducted. This search was conducted to find studies on the excretion of parabens.

The search criteria's were as follows:

- articles had to be published between 1. January 2010 and 23. April 2013;
- only human *in vivo* studies;
- only single studies (no reviews);
- the primary objective had to concern measurements of parabens in urine.

Reviews were not included in this search to limit the number of studies, because of time and space limitations. Since the studies were intended for comparisons, only studies presenting urine concentrations as GMs were chosen.

In general for all searches, search criterias were decided to decrease the number of studies as a consequence of time and space limitations, but also to find articles specific to the topic. All studies discussed in chapter 5 were published before 23. April 2013. The most recent and relevant studies were always preferred, as long as they could be compared. To objectively evaluate the evidence, efforts were made to find and include studies that showed an effect and studies that showed no effect.

3.4 Materials

Theory and materials to support or disprove evidence were obtained from different sources. Scientific studies were obtained from Scopus, PubMed and Google Scholar. Background theory, laws and directives were obtained from reports (national, EU, WHO), web pages, and textbooks. EU- reports were used because they have been much debated, and there have been frequent meetings about parabens in scientific committees in the EU- system. No unpublished literature (grey literature) was used.

Human studies were preferred over animal and *in vitro* experiments. This preference was caused by the thesis objective of dermal exposures in humans, and because it is debated to what extent we can generalize evidence form animal and *in vitro* studies to humans. Where human studies were lacking, animal and *in vitro* studies were used to indicate evidence. However, the animal study by Aubert et al. (20) was also used in the discussion to show differences in animal and human evidence.

In the discussion in chapter 5, scientific studies were always used as primary literature sources. Policies, reports and literature reviews were used to support or disprove evidence if scientific

articles could not be obtained. Studies on parabens absorption and metabolism (chapter 5.1) are presented in literature matrix A and studies on the excretion of parabens (chapter 5.2) are presented in matrix B (appendix). Urine paraben concentrations shown in matrix B are all unadjusted values. The decision to compare only unadjusted values was made since a higher number of articles show unadjusted, than adjusted concentrations. The studies by Wang et al. (21) and Frederiksen et al. (22) on the other hand, do not mention or describe urine adjustments. It is therefore assumed that values from these studies are unadjusted.

Endnote was used for managing references, and the program was updated to Endnote X6 in March 2013. Because numbered references were used, year of publication was not applied, to keep in-text references as clear and reader friendly as possible.

3.5 Limitations in available studies

The limitations presented are general methodological limitations found within studies on parabens and other contaminant studies. Such limitations can decrease the reliability of the conclusion, and should therefore be introduced.

Firstly and maybe the most important, are the low number of randomized controlled trials (RCTs). Most of the studies used in the thesis have observational designs, and are thereby in the lower parts of the evidence pyramid. This is also the case for the animal studies, from which findings are difficult to extrapolate to humans. Secondly, many studies have small sample size, which reduce the confidence in study findings, and thereby decrease generalizability. Thirdly, several of the articles have different study populations (age, gender etc.). Such differences can make findings from articles less comparable and thereby less generalizable to other populations. Another limitation is the studies use of different limit of detection- levels (LODs). Different LOD- levels make findings less comparable, as concentrations also can be found below the LOD. However, this limitation can easily be observed by the reviewer, as the LOD usually is documented in the

research paper. Furthermore, more articles measure parabens in urine instead of blood. When studying dermal absorption of parabens, blood concentrations are preferred. The preference is caused by the fact that parabens to a larger extent are hydrolyzed and conjugated before excreted in urine, and that native parabens better reflect dermal exposures.

Some articles are unclear on how they define parabens, metabolites and conjugates, and whether the urine concentrations are specific- gravity or creatinine adjusted. Both these factors can affect the findings. However, uncertainty in whether it is native parabens or conjugates that are measured is likely to make larger differences in the findings, than unclearness in specific- gravity or creatinine adjustments. But at the same time, paraben detections in urine, independent of being conjugated or not, indicates exposure. Lastly, it cannot be completely excluded that small differences between the studies` results are caused by differences in the methods of detection. Evaluations and comparisons of methods have not been conducted as there are currently no quality assurance (QA)/ quality control (QC) programs for analyzing parabens in plasma/ urine, as there is for POPS (23), and as this is beyond the scope of the thesis. These limitations will be further studied in chapter 6, except from differences in methods of detection.

4. EDCs AND COSMETICS

More than 80 000 chemicals are manufactured and imported into the United States each year (24). The production of chemicals has recently reached 400 million tones worldwide, which as a consequence leads to increasing amounts of pollution (25). Some chemicals are widely used in for instance industry and agriculture, for medical purposes, and are found in consumer products such as cosmetics (5). It is estimated that 3000 - 5000 new chemicals are introduced each year, and most of the synthetic chemicals today were produced after World War II (24). Many of the chemicals have unknown properties, but the increase in hormonally related diseases (13) over the last 50 years (1), are suspected to be partly caused by an increasing amount of synthetic chemicals (25) (i.e. EDCs). Examples of such hormonally related diseases are breast- (figure 2), prostate- and testicular cancer (figure 3), diabetes, obesity and reproductive problems (1).[1]

An EDC is according to the European Commission (EC) (28):

"*an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function.*"

And a potential EDC is defined by the European Commission (EC) (28) as:

"*a substance that possesses properties that might be expected to lead to endocrine disruption in an intact organism.*"

[1] It is important to be cautious when interpreting statistics of disease prevalences and incidences. The last decades improve in diagnostics (26), increased reporting's and over- diagnosing (27) are examples of factors that can affect existing or new numbers of specific diseases.

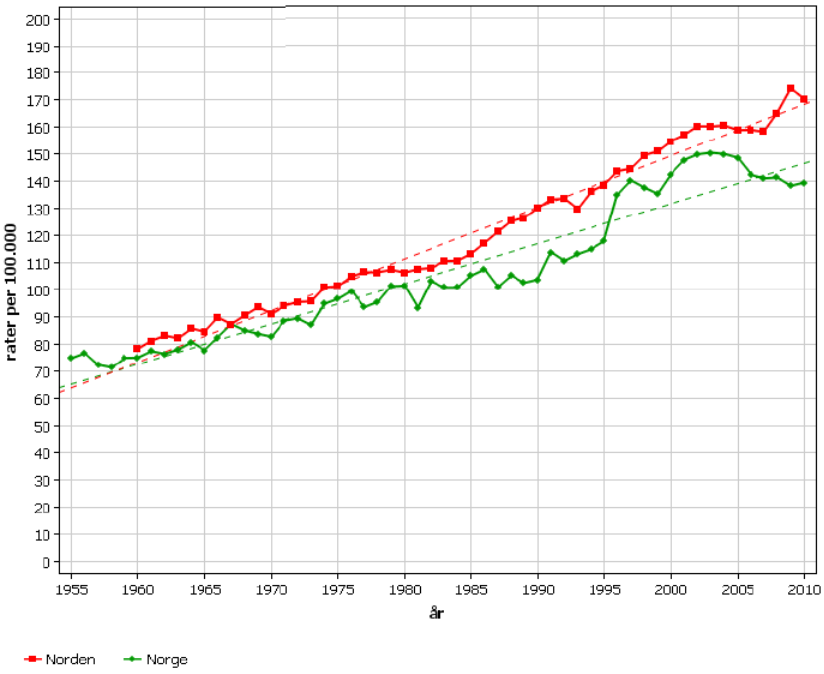

Figure 2: Mammary gland cancer. Age- adjusted incidence (N) in the Nordic countries 1955 - 2010 for women aged 20 - 79 (29).

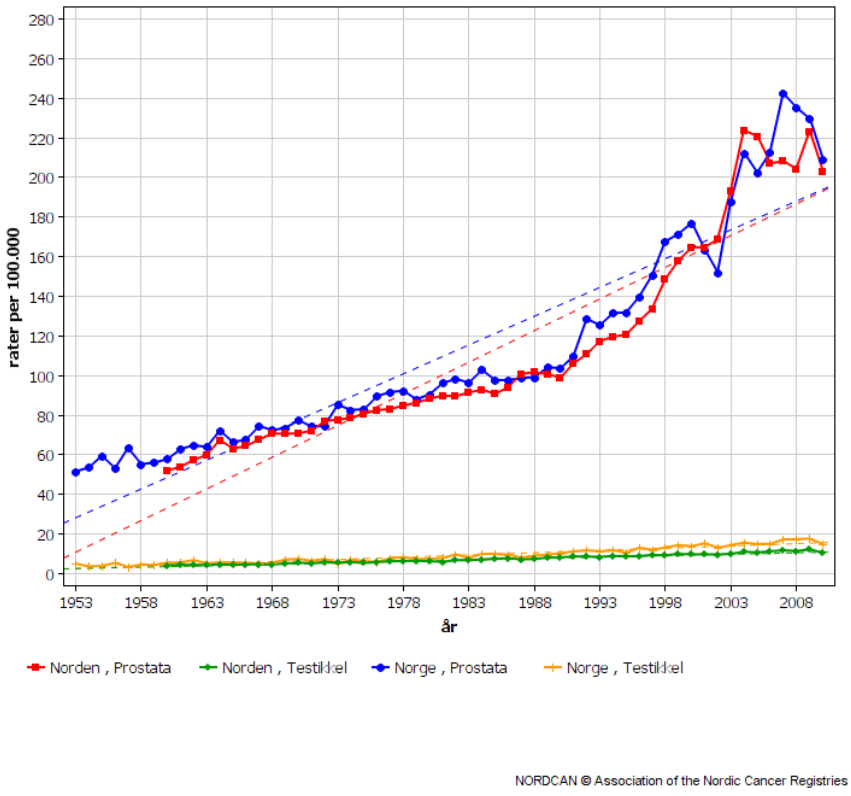

Figure 3: Prostate- and testicular cancer. Age- adjusted incidence (N) in the Nordic countries 1953 - 2008 in men aged 20 – 79 (29).

EDCs are a group of heterogeneous substances (1) in which most are synthetic (13). They are omnipresent in society and can be found:

- in industrial products (solvents/lubricants) (1, 4), e.g. polychlorinated biphenyls (PCBs) (4);
- as industrial byproducts, e.g. polybrominated biphenyls (PBBs) and dioxins (4);
- in plastics (1, 4), e.g. bisphenol A (BPA) (4);
- as plasticizers, e.g. phthalates (30);
- as pesticides (4, 31) (fungicides, insecticides, herbicides, rodenticides etc. (31)), e.g. methoxychlor, chlorpyrifos and dichlorodiphenyltrichloroethane (DDT) and vinclozolin (4);
- in/ as medications, e.g. DES (4) and parabens (18);
- in cosmetics, e.g. phthalates, parabens, UV- filters, synthetic polycyclic musks, antimicrobials such as triclosan (30), and siloxanes (32);
- as toxic metals like cadmium (Cd) and lead (Pb) (1).

According to Zeliger (24), both synthetic and natural hormones are considered endocrine disruptors, as they can cause alterations in the endocrine system. Hormones, both synthetic and natural, are used as medications, normally causing preferred effects. Looking back into history, it is also possible to find examples on endocrine disruptors causing unwanted effects. Diethylstilbestrol (DES) for example, was prescribed by physicians between 1948 and 1971 to prevent spontaneous abortions, but was found to cause negative health effects in girls and boys exposed prenatally. For instance, a reduction in fertility, abnormal pregnancies, immune system disorders, depression, early onset of vaginal clear- cell adenocarcinomas and reproductive tract cancers was detected in the girls at a higher rate than in the standard population (24). In boys a higher rate of testicular abnormalities was detected compared to non- exposed males (33).

Moreover, DES is suspected to cause hereditary diseases or health effects in third generations (34).

The concerns in relation to EDCs in general, are their possession of estrogenic, androgenic, antiestrogenic and antiandrogenic properties (1). Examples of EDCs with estrogenic properties are parabens (9, 35) and the UV- filters (35) benzophenone- 1 and phenyl salicylate. Large variations have been detected in the potencies of EDCs with estrogenic properties (36). EDCs with androgenic properties are less studied than estrogen disrupting chemicals (37). Examples of androgenic chemicals are the UV- filters benzophenone- 2 and octyl salicylate, while examples of chemicals with antiestrogenic properties are the UV- filters benzophenone- 3 (BP3) and homosalate. The UV- filters 4,4- dihydroxy- benzophenone and octocrylene are antiandrogenic (36). Some EDCs have a combination of properties, like for example the UV- filter 3- benzylidene camphor (3BC), which has shown estrogenic-, antiestrogenic- and antiandrogenic activity *in vitro* in human receptor systems (36). Animal studies have shown EDCs can cause alterations in thyroid- and corticoid function, and other metabolic functions (1). This means EDCs can affect normal development and reproduction, but also metabolism, growth, fluid balance, cardiovascular function and other biological functions involving hormones (30). Hormone- related cancers in breasts-, endometrium-, ovary-, testis-, prostate- and thyroid glands are more common in industrialized countries, and are still increasing. Hormone- related cancers has also been shown to increase in Asian countries, but data from especially Asia, Africa, Central- and South America are still in minority (13). Despite EDCs potential to cause adverse health effects, several factors must be considered to understand EDCs and their potential risks to human health:

- *Vulnerable groups:* humans have critical windows of developmental sensitivity to endocrine disruptors including natural- and synthetic hormones (4). The fetus and children are considered to be most vulnerable to EDC exposures (38), but also elderly are more sensitive to EDCs, as metabolism decrease with age (24).

- *Gender:* Some chemicals are shown to have greater impacts on men than females, and opposite. This can be related to testosterone- and estrogen levels. Few studies exist on this topic in humans (24).
- *Latency to health effects:* Diseases and symptoms can develop years after exposure. For example may exposures during development (pregnancy, infancy, childhood, puberty) be manifested first in middle- or old age (4). It can therefore be very difficult to connect the exposure with the symptoms.
- *Trans generational:* Diseases can occur first in the next generation and thereby cause diseases in subsequent generations (4).
- *Mixture effects:* Humans are exposed to a number of chemicals simultaneously, and combinations of EDCs are likely to increase the overall effect of hormonal influences (3).
- *Dose- response:* EDCs can act at very low concentrations, and they may cause stronger effects at low- compared to larger- doses (4). EDCs dose- response curves can be standard sigmoidal- shaped (monotonic) or for example U- or inverted U- shaped (non- monotonic). This means that maximum responses can happen at low- and high doses (U- shaped), or at intermediate doses (inverted U- shaped) (13).
- *Genetics:* Proneness to EDCs might vary because of genetic polymorphisms (4).
- *Half- life/ persistency:* Persistent chemicals (i.e. with long half- lives) can contribute to constant exposures over years (4), after one or more exposures, while chemicals with short half- lives can contribute to constant exposures when repeatedly exposed through cosmetics, food, medications etc.

As a means to better protect human health and the environment, the EU chemical regulation REACH (*the Registration, Evaluation, Authorization and Restriction of Chemical Substances*) (39) has listed 626 EDCs of very high concern (40). REACH was commenced in 2007 and work towards earlier and improved identification of chemical properties. This create more competitiveness within the EU chemical industry, and enhance innovation (39). According to the

2012 report by the WHO and United Nations Environmental Program (UNEP) on state of the science of EDCs (13), only a small part of the environmental EDCs are known and understood; simply the tip of the iceberg (13).

4.1 Persistent EDCs

Persistent chemicals are, as non- persistent chemicals, both synthetic and naturally occurring. Many of the persistent chemicals were and are intentionally produced to have a long half- life, like for example PCBs. Others are not intentionally produced, but are for example byproducts of different processes, like dioxins. Dioxins and PCBs have a half-life in the human body ranging from approximately 1 to 20 years (41). Dioxins and PCBs are persistent organic pollutants (POPs), and thereby on the Stockholm Conventions list of chemicals to be eliminated or regulated (42). The Stockholm Convention is a global treaty working on protecting human health and the ecosystem from POPs. It was adopted in 2001, commenced in 2004 (43), and has today listed 22 POPs for elimination or control (42). POPs affect humans and biota negatively because of several factors:

- they are toxic, even at small concentrations (44);
- they have high persistence (44) (i.e. persist in the environment for years);
- they bio- accumulates in the food chain (44) (i.e. the higher up in the food chain, the higher concentrations can be detected);
- they are lipophilic and accumulate in living organisms fatty tissues (44);
- they can be detected in remote areas where no industry exists (44), as in the Arctic, because of their ability to travel long distances by air and water (44) (long range transport).

Some persistent chemicals can cause detrimental effects in human- and animal health and in the environment (4). Already in the 1940`s, Rachel Carson raised concern regarding the use of pesticides. She manifested the detrimental effects pesticides, and especially DDT, had on animals and the environment in her book Silent Spring published in 1962 (45). Examples of other persistent EDCs of concern are chlordanes, which are an organochlorine pesticide like DDT, and furans that are byproducts of different chemical processes or waste incineration as dioxins (44).

Bans and regulations of persistent chemicals are shown to have a positive effect decreasing exposures. For example, after the banning of DDT in developed countries (44) from the 1970s (46), a decrease in the body burdens of DDT has been observed in exposed populations. However, due to DDTs persistency, it can still be detected in for instance human breast milk (46). Lipophilic chemicals like DDT (46) and toxic metals like lead (Pb), cadmium (Cd) and arsenic (As) can be transferred from the mother to the fetus (47) and infant. High positive correlations have been detected between organochlorine pollutants and maternal fat tissue, plasma, cord blood and breast milk (48, 49). During breastfeeding, the mother`s body burden of organochlorine pollutants decrease (50), and the body burden of these substances will be lower in women who have breastfed, compared to those who have not (51).

Even though many of the environmental chemicals are ubiquitous and cannot be avoided, it is possible to reduce exposures to certain EDCs (both persistent- and not- persistent chemicals). One example is to increase consumer awareness on EDCs in consumer products such as cosmetics.

4.2 EDCs in cosmetics

Despite of the European Union (EU) (52) law that states the use of cosmetics shall not pose a threat to human health, under normal or reasonably predictable conditions, a wide range of different EDCs and potential EDCs are used as ingredients in cosmetic products. Examples are:

- Parabens (30, 53);
- UV- filters, e.g benzophenone- 1, benzophenone- 3 and octinoxate (1, 30, 53, 54);
- Synthetic musks and other fragrance compounds (30, 53);
- Antimicrobials, e.g. triclosan (30, 53);
- Cyclosiloxanes: octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6) (53);
- Bisphenol- A (BPA) (1, 53);
- Alkylphenols, e.g. nonylphenols and octylphenol (53, 55);
- Glycol ethers (53).

Chemicals are used in cosmetics as preservatives or solubilizes, as fragrances, colorants and UV-protection. The substances are mostly non- persistent, but traces of for instance the persistent and toxic metal lead (Pb) have been detected at trace levels in at least lipstick. Despite the low concentrations identified (56, 57), lead may accumulate and add to the body burden of EDCs. Lead, BPA, musk xylene, the siloxsane D5 and triclosan are on the Norwegian Climate and Pollution Agency's (KLIF) "high concern- list", and are to be reduced or forbidden by 2020. The restriction or ban was decided because of the chemicals properties or potential to cause adverse effects in humans and/ or the environment (58). Other EDCs in cosmetics that are listed by KLIF as concerning, are diethyl phthalate, the siloxsane D4, the UV- filter isotiazoliner 4-Methylbenzylidene camphor, and lastly, toluene/ methylbenzene, which are found in nail polish (59). Although EDCs in cosmetics can cause adverse health effects, it will often be difficult to

connect these health effects with the chemicals, as humans are regularly exposed over years and it can take years before negative health effects occur (60).

The safety of cosmetics have for many years been tested by conducting animal studies, even though human studies produce more reliable results. But now, as a result of the European Commission`s (61) animal testing ban on cosmetics, the cosmetic industry must find equally good alternatives to be able to safety assure cosmetic ingredients and products. The directive came fully into force in March 2013, and prohibits animal testing of finished cosmetic products and cosmetic ingredients within the EU. The directive does also include a marketing ban of such products within the EU (61). A result of this directive can be a reduction in cosmetic innovations, where it can become harder for companies to find substitutes to EDCs in their products.

Consumers have a freedom to choose what products to buy. But not all product ingredients are always labeled. UV- filters, phthalates and parabens were for instance not labeled on some of the products tested by Dodson et al. (53). However, because of the EU- regulation (EC) no 1223/2009 (52), declaring that ingredients must be documented on the product, it is likely most chemicals are printed on the label. This also the case in Norway, because of the Norwegian Cosmetic Regulation`s implementation of the European Commission's regulation. The only difference in these regulations concern pharmacological active ingredients allowed in cosmetics (62). However, impurities from raw materials and lower concentrations of technical materials not present in the final product, are not required to be documented on the product (52). Neither is every single fragrance chemical, where it is permitted to only print "parfum" or "aroma" on the label (52). As a consequence it is more difficult for consumers to identify all of the products ingredients, and thus be able to avoid exposures.

In summary, many different EDCs and potential EDCs are used as ingredients in cosmetic products. Several of these chemicals are on KLIF`s "high concern- list", and are to be reduced or

forbidden by 2020. As cosmetics can contain EDCs and potential EDCs, public information and high consumer awareness are required.

4.3 EDCs mechanisms of action in the human body

The factors that cause diseases are many, but non- communicable diseases are usually caused by a combination of genetic- and environmental factors. Environmental chemicals can affect the hormone system (figure 4) through different mechanisms. Firstly, by affecting hormone receptors and receptor mechanisms directly, or by acting directly on specific proteins that regulate hormone delivery to the receptor. In the last mechanism mentioned can the protein be involved in hormone production (e.g. aromatase), it can be a transporter (e.g. sodium/ iodide symporter) or a carrier protein (e.g. cortisol binding protein). An EDC can also block the hormone- synthesis, making hormone concentrations rise or fall (13).

Hormones bind to and act on specific proteins, i.e. receptors, and cause actions (13) like alterations in developmental and reproductive mechanisms (25). Hormones can act by one specific- or several receptors. Estrogens for example act by estrogen receptor alpha (ERα), estrogen receptor beta (ERβ) and by distinct membrane receptors on certain cells. The numbers of receptors estrogens act by are unknown. Testosterone on the other hand, acts only by one receptor, the androgen receptor (AR) (13). Hormones act largely through membrane bound receptors that respond to peptide hormones (e.g. insulin), and through nuclear receptors (NRs) activated by interaction with lipophilic hormones (25) (e.g. steroid- and thyroid hormones) (13). The main endocrine systems that can be altered by EDCs are the estrogen, the androgen and the thyroid ones (49). Action through NRs is one of the EDCs main ways of action (25). When hormones act by NRs, new proteins are developed as a result of the hormone and receptor binding to particular areas of the DNA, regulating the development of gene transcription. Steroids like estrogens and progestin's though, act by both membrane receptors and NRs (13). Lipophilic chemicals, such as

many xenobiotics, can act through specific NRs, which are likely to disturb or modulate downstream gene expression. One example is EDCs causing alterations in the mechanisms of the ER and/or AR, altering the normal action of estrogens- and androgens ligands. Reproductive and developmental alterations caused by EDCs, are suspected to stem from this mechanism (25). EDCs can also act through for example non- steroid receptors like neurotransmitter receptors (serotonin-, dopamine- and norepinephrine receptors), but also through orphan receptors like aryl hydrocarbon receptors, and enzymatic routes affecting steroid biosynthesis and/or metabolism (1).

EDCs can act as both agonists and antagonists, and may impact hormone secretion, biological half- life or alter the feedback relationships that exist in the hypothalamic- pituitary target organ systems like the gonadal, thyroid or adrenal ones (30). Thyroid disrupting chemicals, like PCBs (4), can especially cause adverse health effects in humans exposed in sensitive periods of life. This is because these hormones are important in brain maturation, cognitive growth and behavior, and development (5). Pregnant women, fetuses, premature children, infants and toddlers are more vulnerable to permanent effects on neurodevelopment, while exposures to thyroid disruptors in older children and adolescents primarily cause negative effects associated with growth and reproductive development (63). Adverse effects related to thyroid hormones can be caused by EDCs binding to thyroid hormone receptors (TR) on target cells, decreasing bioavailability of thyroid hormones to the NRs. Certain EDCs can act by the TR, as agonist or antagonist, or indirectly by regulating expression of the TR- genes. Normal development of the central nervous system can be disturbed by alterations in TR- expression or TR- binding (64).

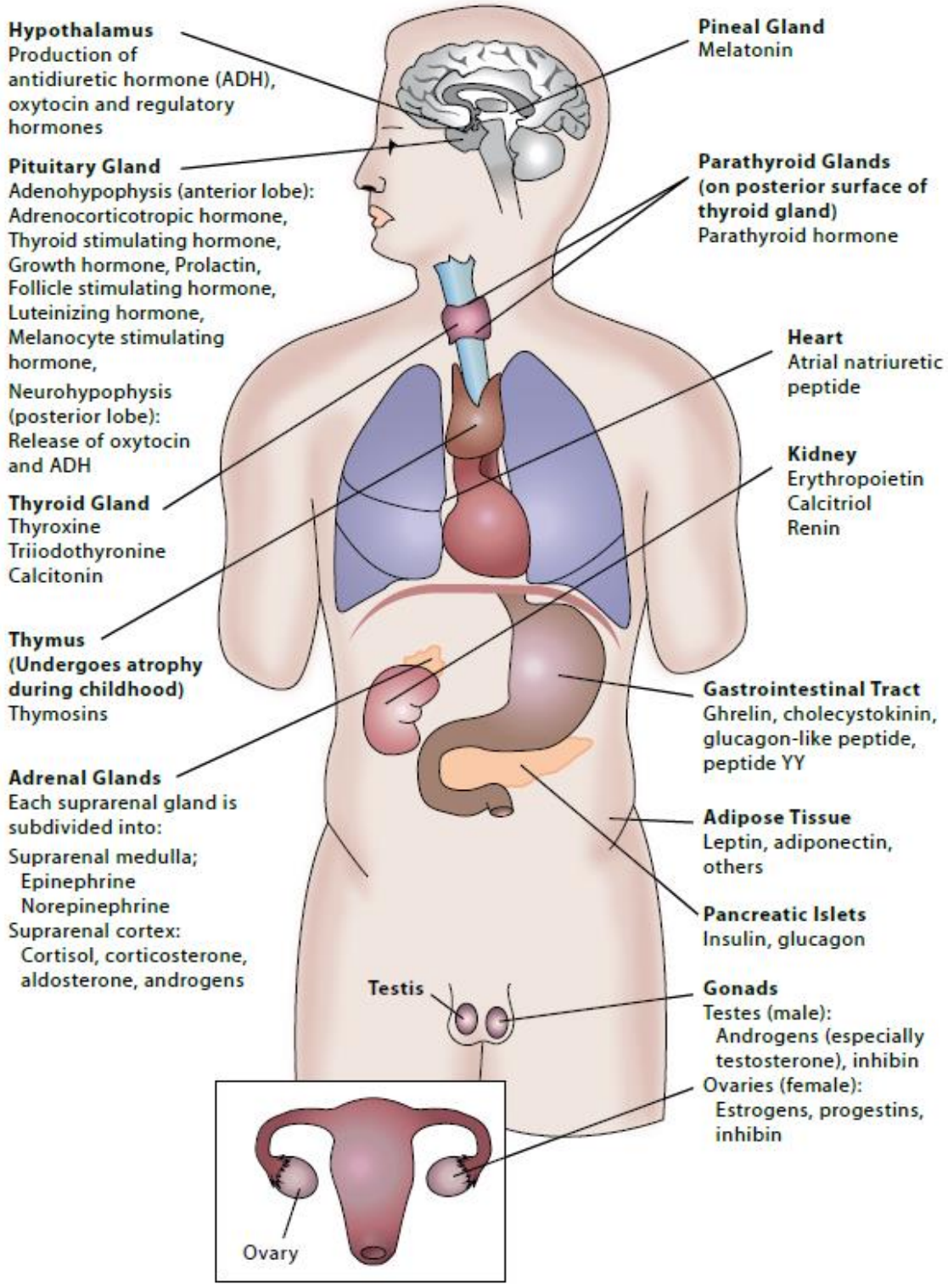

Figure 4: Human hormones. After being created in specific cells in different glands and tissues, hormone molecules are transported through the blood, and acts on target tissues. Figure reused with publishers permission (13).

Development of different cancers has also been linked to EDCs. Alterations in ERs caused by EDCs have been associated with breast- , lung- , kidney- , brain- , pancreas- and reproductive system cancers (50), while chemicals with estrogenic or antiandrogenic properties may cause

alterations in the masculinization process of the male fetus, triggering development of low sperm count, testis cancer, hypospadias and cryptorchidism (15, 51). It is an assumption that breast cancer can be triggered by EDCs, following alterations in ERs, as estrogens can stimulate growth, progression and metastasis of breast cancer (13). Parabens are an example of a group chemicals that possess both estrogenic (9) and antiandrogenic effects (65, 66), acting as ER agonists and AR antagonists (10). However, research also shows EDCs may have an impact on metabolic alterations, for example in the development of obesity. Obesity is on the other hand linked with metabolic syndrome, diabetes type 2, liver disease, cardiovascular- and pulmonary diseases, psychological- and social problems, reproductive defects and some types of cancer (25). After all, there is still a lack of knowledge on EDCs mechanisms of action in the body and their long- term effects.

4.4 The use of cosmetics

Cosmetics are not a new invention. Already 10 000 BC Egyptians used fragranced oils and ointments to clean themselves, soften their skin and cover body odors. Essential oils were vital in their belief: "cleanliness is godliness". Hygiene was important (67). Trends have changed through time, and certain cosmetic trends have not always been so fortunate. From the middle ages, lead and arsenic were used for skin- whitening, as it was more status to be pale as the aristocrats than sun- tanned like the lower class workers working outdoors (67). Cosmetics are today used for different reasons, and even though fragrances and skin paling ointments still are widely used, ingredients have changed; for good and for bad.

Today cosmetics are used for hygiene, as colorants, fragrance, protection and appearance. Products are applied random or regularly every day, to smaller or larger areas of the body and are often used over years. Exposed parts of the body are the skin, hair and scalp, lips, oral-, ocular- and vaginal mucosae, axillae, nails (68) and lungs (60). Exposures happen orally, dermally and by inhalation. Biesterbos et al. (7) identified over 50 % of their 516 study participants to use

cosmetics like deodorant, facial cream and mascara every day, while 54.7 % of the 332 women in the study by Sandanger et al. (69) was shown to use facial cream once every day. Sandanger et al. (69) also detected 45 % of the population to apply cream on the whole body once or more every day. Even though the cosmetic demand is already high, it is still increasing (70). The total cosmetic market value increased in Europe (27 EU countries + Norway and Switzerland) with 2.2 % in 2010 compared to 2009. In Norway only, it increased with 1.8 % in the same time period (values based on retail sales prices) (8).

The use of cosmetics is very individual and large- scale population- based studies, like the Norwegian Women and Cancer study (NOWAC) (71), are therefore needed to increase knowledge on exposures to chemicals in such products. Yet, not much research exists on the usage patterns of cosmetics. Two studies did however study gender- and age- differences (6, 7), and both identified a higher prevalence and frequency of cosmetic use, for most cosmetic products, among women compared to men. Still, according to one of the studies, men had a higher use frequency for at least shampoo and bath gel (6). According to Elsner (2012) (72), the number of men using cosmetic products is increasing. However, cosmetics are also used by groups considered more sensitive to EDCs. For instance, a frequent use of cosmetics has been reported in infants and children, where girls were shown to have a higher use frequency than boys (6). The same study did also investigate correlations of cosmetic use between parents and children (6). When interpreting the spearman correlation coefficients (r_s) (non- parametric test) as suggested by Pallant et al. (2007) (73), moderate correlations ($p = < 0.05$) were detected for different cosmetic products (6). Another study showed mostly low and moderate correlations ($p = < 0.01$) when investigating Spearman correlations between mothers and children's urine concentrations of phthalates, phenols and parabens. High correlations where only detected for benzophenone- 3 and triclosan ($p = < 0.01$) (74). The results indicate differences between parents and children`s use of cosmetics.

Humans are exposed to EDCs through different types of cosmetics, but some cosmetics cause a higher exposure to EDCs than others. Shower gel, sunscreen, body lotion etc. gives a higher exposure than for example mascara and eyeliner, as they are applicated to larger areas of the body (75). The daily exposure (mg/kg bw/day) of individual products have been calculated to be 1.12 mg and 0.57 mg for liquid body soap and cleansing products, respectively; and 268.33 mg and 17.43 mg for body lotion and roll- on antiperspirant, respectively (76). This shows that leave- on products contributes to a larger relative daily exposure than wash- off products. And, the larger the area applicated the larger is the exposure. To be able to assess risks of EDC- exposure through cosmetics adequately, it is necessary to map usage patterns and to use biomonitoring. Usage patterns are mapped by identifying exposure history and by for instance using questionnaires, while biomonitoring can be conducted by measuring chemical concentrations in blood and urine.

In summary, usage patterns of cosmetics are very individual, but some population groups have a higher use than others. Studies indicate a higher use among women than men; and a higher use among girls than boys (6). Correlations do however indicate differences between chemical exposures in children and their parents (74). As some populations use more cosmetics than others, they are likely more exposed to EDCs. The use of cosmetics in more sensitive groups is however most concerning.

4.5 Parabens

Parabens, the alkyl esters of p- hydroxybenzoic acids (PHBA) (68), are a group of non- persistent chemicals (74) used individually or in mixtures to reach preferred antimicrobial- and preservative effects. They are especially effective against molds and yeasts, and are widely (18) used because of their antimicrobial-, and relatively non- irritating- and non- sensitizing properties. Parabens have low acute toxicity (9, 68, 77), but have been associated with allergy. They also have low cost, (9) and are pH-stable (i.e. they help in preventing too rapid product degradation) (68, 77). Short chained parabens are more hydrophilic and the long chained are more lipophilic (figure 5). When the chain length of the paraben increases, the resistance to hydrolysis and antimicrobial activity increase (9), but water solubility decrease. As a consequence, methylparaben (MP) and propylparaben (PP), which have shorter chains, are the ones most used in cosmetics (9). MP and PP are however also preferred for use in foods (68, 77). MP, PP, butylparaben (BP), ethylparaben (EP), heptyl- (HP) and benzylparaben (BzP), isopropyl- and isobutylparaben are homologous (30, 68, 77).

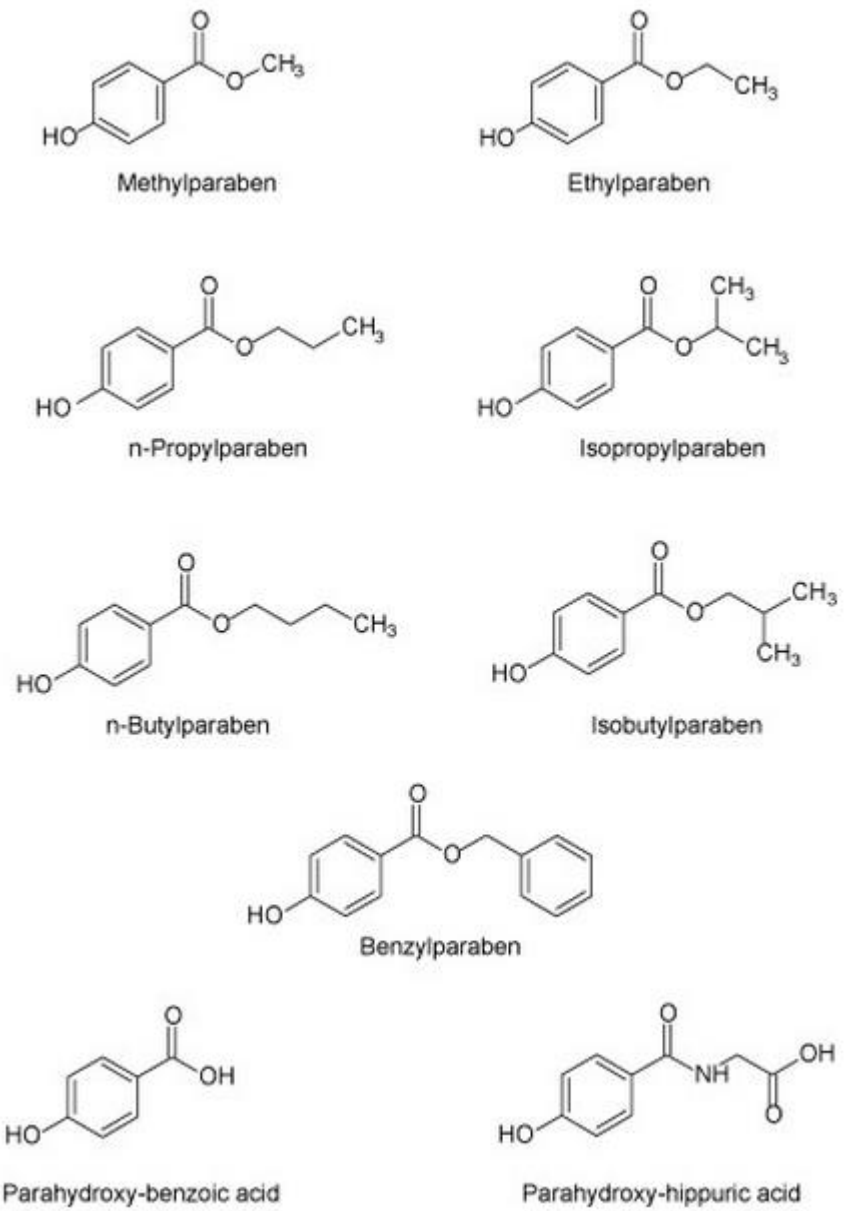

Figure 5: The chemical structures of parabens. The paraben metabolite p- hydroxybenzoic acid (PHBA) develops after hydrolysis of the ester linkage. PHBA can conjugate into p- hydroxyhippuric acid (PHHA). Figure reused with publishers permission (18).

The use of parabens have caused concern due to their possession of estrogenic- (9) and antiandrogenic properties (65, 66) (i.e. they can act as ER agonists and AR antagonists). Parabens may affect health at lower concentrations and more precise than non- receptor mediated mechanisms, because of their capability binding to ERs (69). Several studies, both *in vitro* and *in vivo*, have demonstrated parabens disruptive effects in physiologically important mechanisms. The disruptive effect most described in research, is the parabens ability to bind to human ERs, and

thereafter regulate gene expression and cell growth in estrogen- responsive cells through ER mediated mechanisms (figure 6). But parabens do also have the potential to antagonize AR–mediated effects in androgen- responsive cells, and to behave as sulfotransferase enzyme (SULTs) inhibitors (10) and act on the regulation of steroids (61). In fact, both *in vitro* and *in vivo* assays show all common native parabens possess estrogenic effects (10). And the longer paraben chain, from MP to n- BP, the larger are these effects (9, 78). A higher estrogenic effect is also associated with branching in the alkyl chain, from n- PP to isopropylparaben (79), and n- BP to isobutylparaben (80). However, the estrogenic effects have been detected to be 10 000 to 100 000-fold weaker than natural 17β- estradiol, after subcutaneous administration to rats (78). According to Darbre et al. (80), isobutylparaben has the strongest estrogenic effect. PHBA, parabens main metabolite, have a weaker estrogenic effect than native parabens (10). Despite unclear estrogenicity of glycine-, sulfate- and glucuronide conjugates, the Scientific Committee on Consumer Safety (SCCS) (81) has concluded that they are most likely not estrogenic. This conclusion was mainly based on the fact that steroid conjugates themselves cause no effect at the receptor (81). In theory, as a consequence of parabens estrogenic- and antiandrogenic effects, parabens may cause diseases related to the endocrine system. Reproductive diseases and endocrine cancers have been of special concern. For instance, parabens have been detected in larger concentrations in the axilla area compared to the lateral, mid and medial side of the breast, and a link between parabens in underarm cosmetics and breast cancer has been suggested (82). No association, however, has been made between single parabens and breast cancer. A recent study by Charles and Darbre (83) on the other hand, showed that combinations of parabens in human breast tissues are large enough to stimulate proliferation of MCF- 7 breast cancer cells. In some tissue samples, all single parabens measured were detected at concentrations below "no- observed-effect- concentration" (NOEC) (83). This shows the importance to also consider mixtures of EDCs.

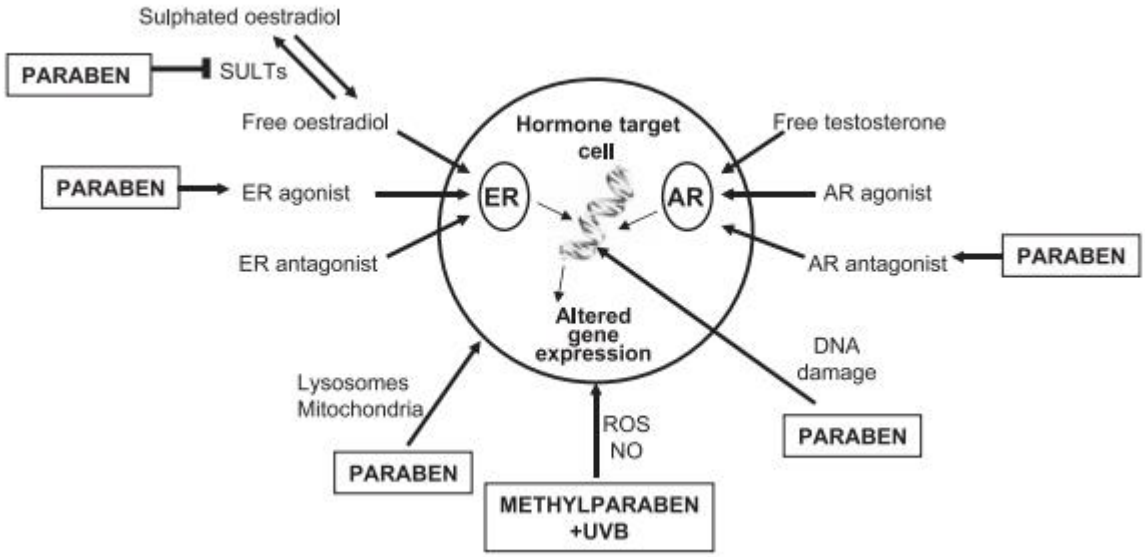

Figure 6: Parabens molecular pathways in cells. As parabens are EDCs, they can act as ER- agonists, as AR-antagonists or as inhibitors of SULTs. But they can also disturb lysosomal and mitochondrial mechanisms, cause DNA damage, and amplify UVB- induced damage through production of reactive oxygen species (ROS) and nitric oxide (NO). Figure reused with publishers permission (10).

When considering reproductive diseases, Oishi (65, 66) reported decreased sperm production and adverse effects on testosterone- concentrations after oral exposures to PP and BP in young male rats. These adverse effects are assumed to be caused by parabens estrogenic activity. It is however also likely the effects were caused by anti- androgenic mechanisms (10), as several parabens are shown to bind to human ARs and antagonize the effects of testosterone on reporter gene expression (63). A similar study by Oishi (84) on the other hand, did not observe any adverse effects after oral exposure of MP and EP, and neither did the study by Hoberman et al. (85) where young rats where fed with MP and BP. It is however likely that the differences in observed effects were caused by exposures to parabens with different estrogenic properties. PP and BP are as a matter of fact more estrogenic than MP and EP (9, 78).

Recent studies have also suggested parabens to be genotoxic, which indicate parabens may influence the development of malignant melanoma through both genotoxic- and estrogenic activity (10). However, very little is known about this topic. And yet, there is still a lack of knowledge on parabens possibility to cause negative health effects in general.

The main source of parabens is expected to be through cosmetics (14), and based on this the SCCS (86) has estimated the systemic exposure dose (SED) of native BP in cosmetics to be 0.043 mg/kg bw/day. Calculations shown below:

Maximum concentration of single parabens: 0.4 %

Cumulative exposure of cosmetics added parabens/ day: 17.4 g[2]

Maximum dermal absorption: 3.7 %

Body weight (bw): 60 kg

$$\text{SED} = \frac{17400\ \text{mg/day} * \frac{0.4}{100} * \frac{3.7}{100}}{60\ \text{kg}} = 0.04292\ \approx 0.043\ \text{mg/kg bw/day}$$

The margin of safety (MoS), which is the threshold between safety and risk, has been estimated by using a "no– observed– effect- level" (NOEL) of 2.0 mg/kg bw/day. The NOEL is based on subcutaneous injections of BP to neonatal rats (87). The calculation of MoS:

MoS = NOEL / SED = 2.0 / 0.04292 = 46.59 ≈ <u>46.6</u>

As a result of this estimate, the SCCS has recommended to reduce the maximum concentration of BP in cosmetics to 0.19 %. Only then will the MoS be kept over 100 (86), which is the WHO`s limit to conclude with the safety of a chemical (88).

The calculation has however been adjusted for children. For a 3 months old child weighing 5.3 kg and with a body surface of 0.31m^2, cumulative exposure to leave- on products a day would be 17.4 g * 0.31 m^2 / 1.75 m^2 = 3.08 g/day.

$$\text{SED} = \frac{3080\ \text{mg/day} * \frac{0.19}{100} * \frac{3.7}{100}}{5.3\ \text{kg}} = 0.0408\ \text{mg/kg bw/day}$$

[2] Based on an adults body surface of 1.75 m^2 (86).

NOEL = 2.0 mg/kg bw/day

MoS = NOEL / SED = 2.0 / 0.0408 = 49

However, data on the amount of cosmetics children are exposed to must be obtained, as it is not realistic that children are exposed to as many products as adults.

4.5.1 Paraben use and regulations

Parabens were first used in pharmaceuticals in the 1920s (89). Today parabens are used in pharmaceuticals, food- (9), and consumer products such as detergents (12) and cosmetics (9). Humans are therefore exposed through inhalation, ingestion and dermal absorption (60). Even though exposures happen through different products and routes, the main expected source of exposure is through cosmetics (14). The total aggregate exposure from food is assumed to be less than 4 - 5 % (9, 14). Inhalation and digestion are therefore considered less important sources. In 1981, producer reporting's to the U.S. FDA identified parabens in 13 282 cosmetic formulations (11). This number is large, but it is likely to be underestimated as the reporting's where voluntarily. The number may however not be generalizable to the European population as the number is based on U.S. producers. A study from 2008 on the other hand, investigated chemical concentrations in 204 cosmetic products bought from stores in Stockholm (12). 44 % of the products tested contained parabens. MP was detected in 41 % of the products and was thereby most commonly observed. PP was detected in 25 % of the products, EP in 22 %, BP in 14 % and isobutylparaben in 13 % of the products (12). Since the Norwegian Cosmetic Regulation implements the European Cosmetic Regulation (EC) no 1223/2009, and Sweden is an EU member state, these results can most likely be generalized to Norway (62, 90).

The EU (52) has set the maximum total concentration of parabens permitted in cosmetics to 0.8 % for a combination of native paraben, and the maximum total concentration permitted of a single

paraben to 0.4 % (regulation (EC) no 1223/2009). The safety of parabens has been evaluated several times since 2005. And because of parabens potential risks to cause adverse health effects in infants and children (60, 91), the Danish Government banned PP, isopropyl-, BP and isobutyl-parabens in cosmetics for children up to three years of age in March 2011 (81). The banning was meant as a precautionary measure, and was implemented by Denmark as the only country within the EU (91). After the Danish Governments banning, the SCCS published a clarification of the previous published opinion from 2010 (86). The clarification stated that the use of cosmetics containing parabens in general is safe, as long as the concentrations added are no more than the maximum permitted levels. An exception, however, was made for certain products used in the nappy area. This result was based on their calculation of MoS, which they consider conservative (81). In May this year (2013) a new opinion was published, stating the concentrations of parabens allowed in cosmetics are still considered safe for humans. But, as a result of the MoS, the SCCS has recommended to reduce the maximum concentration of PP and BP in cosmetics from 0.4 % to 0.19 %. The recommendation was grounded on the absence of adequate knowledge on dermally absorbed parabens in rats compared to humans. But still, the risks of isopropyl-, isobutyl-, benzyl-, pentyl- and phenyl parabens are unidentified (86).

In summary, parabens are widely used in cosmetics, and the main expected source of exposure is through cosmetics (14). Maximum permitted level of parabens in cosmetics are 0.8 % for a combination of native parabens, and 0.4 % for single native parabens (52). The SCCS considers their maximum permitted level of concentration to be safe, but recommend reducing the maximum total concentration of PP and BP to 0.19 % (86). The safety of paraben exposures to the vulnerable group of infants and children is unknown (60, 91).

5. HUMAN EXPOSURES TO PARABENS

5.1 Blood metabolism

Human and animal *in vivo* and *in vitro* studies have been performed to investigate dermal absorption and metabolism of parabens. In this chapter, state of the art knowledge on dermal absorption and metabolism of parabens in plasma and serum is compared and discussed. Despite urine as such can be used as matrix, blood is preferred. The reason is because native parabens, which reflect dermal compared to oral exposures most (22), are found to be less conjugated in blood than in urine, and that parabens in blood are more directly linked to potential effects. So far, it has been debate on how high percentage of native parabens is absorbed. But what is known is that parabens are dermally absorbed and metabolized by esterases in the skin. When orally absorbed they are mainly metabolized by esterases in liver and intestine (18). And as parabens are more hydrolyzed in human liver than in human skin (92), more systemic available native parabens are observed when dermally absorbed compared to when orally absorbed (22).

Mostly animal and *in vitro* studies have been conducted to identify maximum dermal absorption rates of parabens in humans. According to a study by Cowan- Ellsberry et al. (14), 80 % was chosen as a conservative measure of the maximum amount of native parabens and their metabolites to be dermally absorbed. The estimate was based on mostly *in vitro* studies, where dermal uptake ranged from 15 % - 75 % (14). Also another *in vitro* study on native parabens and their metabolites found the minimum concentration dermally absorbed to be 15 % (18). Their maximum value of 57 % on the other hand (18), was 18 % lower than what observed by Cowan-Ellsberry et al. (14). Conversely did a study on rats (20) observe 0.5 % - 9 % native MP, PP and BP and their metabolites to be systemically available. This is even lower values than observed in the two previous studies (14, 18). The latter study`s estimates of systemic availability was considered worst possible case of the applied dose (20), and the higher permeability of topical applied substances in rat skin compared to human skin (93) where accounted for. The paraben

concentrations were however measured in urine and faeces (20), in contrary to the two other studies measurements in blood and skin (14, 18). One reason for the much lower concentrations observed in the animal study (20), could be differences in the metabolism of rats and humans or the *in vitro* models. Data for such conversions is still lacking. As there is a shortage of proper human studies on the rate of dermal absorption, the SCCS (86) decided to use 3.7 % as a measure of maximum dermal absorption of native BP. The conclusion was based on three *in vitro* studies (94-96), where the estimate was calculated from the average dermal absorption of 37 %, measured in split- thickness skin (95). A correction factor of 10 was used to correct for skin metabolism as detected in full thickness skin experiments (94, 96). However, because *in vitro* and animal studies cannot be directly generalized to humans, they can only be used as indications on exposure.

RCT is the best study design when studying exposures, but only two RCTs, conducted by Janjua et al. (97, 98), exist on parabens in humans. Janjua et al. (97) investigated 24 hour urine excretion level of total BP after dermal application, and observed a mean recovery of only 0.32 %. This is a small percentage considering the large dose of BP applied (2 %) (97). However, as sulphated parabens have been shown to be the main conjugate of at least MP and PP (99), and PHBA have been shown to be the main metabolite of parabens in general (9, 18), it is likely the result of 0.32 % is an underestimation as neither glucuronidated conjugates nor PHBA were measured. Since lipid solubilizes in creams can decrease skin absorption (100), this could be another reason for the low percentage of recovery. The amount of BP applicated on the subjects is nevertheless not realistic compared to a real life context (97). However, as native parabens are less conjugated in blood than urine, more native BP would have been detected if blood was used as matrix.

When studying dermal absorption of parabens, it is important to consider differences in paraben uptake in whole and damaged skin. As damaged skin is more common in the nappy area of infants compared to adult skin, it is especially important that exposures are known in order to conduct risk assessments. The SCCS (101) concluded, it cannot be ruled out that children less than 6 months of

age are in risk when leave- on products containing parabens are applied to the nappy area. The assumption of risk is based on infant's immature metabolism and the likelihood of damaged skin in this area (101). Research on paraben exposures through damaged skin is in general lacking, but one study observed an increase in systemic availability of MP and PHBA in damaged skin, using a pig ear model (102). Despite the percentage of parabens dermally absorbed in general is unknown, measurements of native and total parabens can be used to show exposures.

Three studies have measured native paraben concentrations in human plasma or serum (69, 98, 103), but there is only one RCT (98). Janjua et al. (98) conducted a crossover experimental study on 26 Danish men aged 21 – 36, and proved that native BP is systemically absorbed after dermal application. Before whole- body application of a cream containing 2 % BP, 2 % diethyl phthalate (DEP) and 2 % dibutyl phthalate (DBP), untraceable or a maximum of 1.0 µg/L BP could be detected in serum. Three hours after the first application, BP reached an average (± standard error of the mean, SEM) concentration of 135 (± 11) µg/L. At peak concentration, 0.81 mg of native BP was calculated to be present in the circulatory system. 0.81 mg was distinguished by multiplying the mean peak BP concentration with the estimated average blood volume of an adult man: 0.135 µg/L x 6 L = 0.81 mg. Mean (± SEM) serum levels of BP decreased to 18 (± 3) µg/L after 24 hours, before the second application (98). The observed concentration of native parabens in this study can however be overestimated. High concentrations of all three chemicals applied, can cause saturation of skin esterases, and thereby cause higher concentrations of native parabens (86). Because 0.4 % is the maximum permitted concentration of single parabens in one product within the EU (52), 2 % BP in one product is unrealistically high. Because cosmetics usually do not contain that much paraben and 2 % phthalates, saturation of skin esterases cannot happen to the same extent with a normal use of creams.

Sandanger et al. (69) measured native MP, EP and PP in plasma from 332 postmenopausal women with an average age of 55 years. Native MP was detected with a median concentration of 9.4 ng/ml, EP < 3 ng/ml and PP < 2 ng/ml (p = < 0.001). 95 % of the subjects reported applying lotion to 50 – 100 % of the body each day[3]. Median (range) plasma concentration of MP, EP and PP were in this group 12.8 ng/ml (3.5 – 129.3), 1.5 ng/ml (1.5 – 45.9) and 1.0 ng/ml (1.0 – 43.9), respectively (p = < 0.001). For EP and PP this were the highest concentrations detected, compared to any of the other groups. Maximum concentration of MP (142.7 ng/ml) was on the other hand detected in the group of those applying cream to "150 – 200 %" of their body each day (equivalent to covering their whole body 1.5 – 2 times per day). A significant association was observed between the use of cream and paraben plasma concentrations (69). Ye et al. (103) on the other hand, observed lower concentrations of native MP, PP and EP than Sandanger et al. (69). The respective median (range) values of MP and PP were as follows: 0.2 (< 0.1 - 9.8) ng/ml and < 0.2 (< 0.2 - 2.3) ng/ml. EP was not detected (103), which may indicate a lower use of EP in cosmetics. The detection rates of MP and PP were 60 % and 47 %, respectively (103). In comparison, Sandanger et al. (69) detected MP, PP and EP in 63 %, 29 % and 22 % of the samples.

On the contrary, not all *in vivo* experimental animal studies have detected native parabens after dermal application (18). Aubert et al. (20) for instance, did not detect native parabens in male or female rats after oral or dermal administration of 100 mg/kg MP, PP and BP, or after subcutaneous administration of 100 mg/kg BP (20). This was however expected as no *in vivo* study yet has detected native parabens in rat serum. As also suggested in other studies (18), that may be related to an easier hydrolyzation of parabens in rats than in humans. Because of differences in the absorption and metabolism of humans and rats, results from rat studies cannot be directly extrapolated to humans. Methods of converting results from rat studies to humans must first be produced and validated. According to the SCCS (86) it is a shortage of data for the conversion from rat to human absorption (86). As long as such data do not exist, results from rat

[3] 100 % application is equivalent to the whole body creamed once daily (69).

studies can only be used as indications before further studies on humans, if ethically and medically correct. To summarize, even though native parabens have not been detected in rat serum in *in vivo* studies, Janjua et al. (98), Sandanger et al. (69) and Ye et al. (103) proved humans are widely exposed to native parabens.

Dermal exposures of parabens can also be investigated by measuring total concentrations of parabens in blood, as total levels include both native parabens and conjugates. But as long as native and conjugate levels not are presented separately, paraben metabolism cannot be studied. Total concentrations of parabens in blood were measured in two studies (22, 103). EP and PP were detected at very low concentrations in both (22, 103), but median concentration MP was identified by Ye et al. (103) to be over seven times higher than the one observed by Frederiksen et al. (22). Ye et al. (103) did also detect higher percentages of MP and PP than EP, which draw parallels to the detections of conjugates. Mean percentages of conjugated MP, EP and PP were 90 %, 100 % and 87 %, respectively (103). The high levels of conjugates are similar to another study (18), where over 90 % of the parabens administered to animals were found to be present as conjugates.

Even less studies exist on human concentrations of PHBA compared to paraben conjugates. The few studies can be related to PHBAs reduced usefulness as a biomarker of exposure. PHBA are mainly produced in the body after metabolism of native parabens, and is non- specific (21). As with conjugates, if higher concentrations of native parabens than PHBA are detected, it is more likely the uptake occurred from dermal application. Findings from Wang et al. (21) for instance, indicate a dermal exposure route in contrast to an oral.

What determines the percentage of dermal absorption, and the level of paraben exposure, are most likely to be the use of cosmetics, the paraben content in the cosmetics applied and biological mechanisms. As mentioned in the background theory, there are large individual variations in the frequency of cosmetic use, while paraben content in cosmetics is determined by country and union directives. For instance, maximum permitted levels of parabens in cosmetics are decided by the

EU Cosmetic Regulation (52), while the U.S. FDA (104) do not regulate the use. Based on available evidence it is more likely that the differences in paraben exposures are caused by variations in the use of cosmetics and by products paraben content compared to differences in biological mechanisms regulating paraben absorption and metabolism.

In conclusion, findings from the available evidence show native parabens were detected in approximately 60 % of the samples, while the one study showing detection rates of total parabens observed MP in 100 % of the samples. More research are required to determine median rates of native parabens in human plasma and serum, as there is a lack of studies, and disparity exist between median concentrations measured in the two existing studies. Based on available evidence it is not possible to conclude with the rate of dermal absorption. More and improved RCTs are required. More studies are especially required on native parabens in plasma and serum, as they are more directly linked to potential effects. And as there is also a lack of studies on differences in paraben uptake in whole and damaged skin, further research is needed to determine the risks of applying parabens to especially the nappy area of infants. Evidence show animal studies should only be used as indications on human paraben exposures.

5.2 Excretion of parabens

This chapter investigates exposures of native and total parabens, by comparing and discussing state of the art knowledge on paraben concentrations in urine. It is expected to find lower concentrations of native parabens in urine compared to blood.

The measure of native parabens in urine shows the extent of paraben metabolism and excretion. As expected, few studies have used urine as matrix when measuring native parabens, but among the few that have are Shirai et al. (64) and Wang et al. (21). They both measured urine concentrations of native MP, EP and PP. The highest GMs of all three parabens were observed by Shirai et al. (64). But, even though MP, EP and PP were detected with GMs (range) of only 6.99 ng/ml (< 0.57 – 544 ng/ml), 0.62 ng/ml (< 0.47 - 63.2 ng/ml) and 0.60 ng/ml (< 0.48 – 14.3 ng/ml), respectively, maximum values were considerably higher. The maximum values are higher than the 95th percentiles in the populations investigated by Wang et al. (21), except from PP in Chinese adults (21). Variations in ranges and percentiles reflect individual differences in exposures. Despite of the high concentrations detected by Shirai et al. (64), the detection rates are all below 100 %. Less than 50 % were exposed to EP and PP, while 88 % were exposed to MP (64). The highest detection rates were detected in Wang et al. (21) population of Chinese children. Native MP, EP and PP were here detected in all samples (100 %), while BP was discovered in as much as 98 % of the samples. Despite low concentrations of parabens in Chinese children, the detection rates show almost all of the children were exposed (21). Exposures to also the most estrogenic parabens are especially concerning, since children are more sensitive to EDCs than adults. However, it is necessary to be aware that unadjusted paraben concentrations in general can be affected by differences in each spot sample`s urine volume. Such differences can be related to age, pregnancy, medications, kidney disease etc. (105). Differences in unadjusted urine samples can make results less comparable, and make it harder to link back to human concentrations and effects as compared to blood. Furthermore, in all populations studied, MP was the paraben most detected (21, 64), which indicates a higher use of this paraben in cosmetics.

High concentrations and detection rates of total parabens in urine have been detected in several studies (21, 22, 60, 64, 106, 107), and show widely, but individual human exposures to parabens. For instance, the highest GM of MP was observed at a concentration of 140 ng/ml (95 % confidence interval (CI) 117, 167) (106), while the lowest GM was 1.53 (range < 0.36 – 59.6) ng/ml (22). The highest GM (106) is more than 90 times higher than the lowest value (22). The detection rates of MP and PP are over 60 % in all studies assessed. However, when excluding Calafat et al. study (107), that only use "over 60 %" and "under 60 %" as a measure for percentages of detection, the lowest detection rate of MP and PP are 94 % (64) and 80 % (22), respectively. The detection rates of BP and EP differ more than the detection rates of MP and PP. The lowest and highest detection rate of BP (10 % and 99 %) was observed in different populations in the same study (21). In this study, EP was also detected in all samples (21), while two studies did not detect EP at all (60, 106). Less than 100 % detection rate shows that some individuals are not exposed. Overall, the observed paraben concentrations differ, but detection rates are high.

When studying exposures to parabens it is also necessary to observe half- life and whether parabens accumulate. Several studies have observed a rapid decrease in paraben concentrations after administration (20, 97, 98), but Janjua et al. (98) is the only study to investigate paraben half-life in humans. Native BP was here shown to reach a mean (SEM) serum concentration of 135 (± 11) µg/L three hours after the first application. The concentration had decreased to 18 (± 3) µg/L 24 hours after application. Aubert et al. (20) on the other hand, detected dermally absorbed MP and BP to reach maximum concentration after only one hour in male rats. Parabens in the other groups reached maximum concentrations after eight hours. Therefore, it is also likely the BP concentrations observed by Janjua et al. (98) would have been detected at a much lower level if measured earlier than first after 24. However, according to Aubert et al. (20) it is likely the maximum concentration reached already after one hour, was caused by a small oral paraben uptake. A rapid decrease in paraben concentration has also been identified in urine. While

parabens measured by Janjua et al. (97) were excreted 8 - 12 hours after exposure, Aubert et al. (20) identified paraben excretion 12 - 22 hours after exposure. These results indicate parabens have a short half- life in humans as well as in animals, and do not accumulate. Frederiksen et al. (108) on the other hand, indicated a possible accumulation of EP and BP in rat amniotic fluid after subcutaneous administration of 100, 200 and 400 mg/kg bw/day (108). The measurement was however only conducted once after administration, but should have been conducted several times to provide evidence of accumulation. More studies have however disproved parabens likelihood to accumulate (9, 18, 20, 97, 98).

In conclusion, evidence show paraben concentrations in general differ among individuals, but GMs of native parabens are lower than for total concentrations. High detection rates are observed of both native and total parabens. Total levels of MP and PP are detected with both highest GMs and detection rates. Literature shows, parabens have a half- life of less than 24 hours and do not accumulate, but more human RCTs are warranted. Further research is also needed especially on native paraben levels in urine, to further study paraben metabolism and the extent of dermal exposures.

5.2.1 Vulnerable groups and paraben exposure

As explained in the background theory, humans have critical windows of developmental sensitivity to EDCs (4). Fetuses and children are considered to be the most vulnerable to EDC exposures (38), and that is why it is important to study exposures to especially these groups. In this chapter, paraben exposures to fetuses and newborns are investigated, by comparing and discussing state of the art knowledge.

The fact that parabens have been detected in human cord blood (109), urine samples from South-Korean newborns (110) and in rat placenta, amniotic fluid and whole- body fetuses (108), show fetuses can be exposed to parabens. No study, however, exists on paraben exposures to human fetuses. As pregnant women have to be exposed to parabens if fetuses are exposed, measurements of paraben concentrations and detection rates should be identified to determine the degree of exposure to women during pregnancy. Shirai et al. (64) and Meeker et al. (106) are two of the few biomonitoring studies primarily investigating parabens in pregnant women. And compared to the other studies discussed in chapter five, highest urine concentrations of total MP, PP and EP were observed in these studies populations of pregnant women (64, 106). Detection rates of MP, PP and BP were observed to be higher by Meeker et al. (106). However, as explained earlier, it is important to be aware of possible differences in spot urine volume when comparing unadjusted measures. And such differences are likely to be more prevalent when comparing studies of pregnant populations, because of increased urine production during pregnancy (105).

Even though these studies indicate that fetuses can be exposed to parabens through their mothers, it has been discussed whether fetuses are better protected from parabens than infants and children exposed dermally. This has for instance been suggested by the SCCS (101). The suggestion arises from parabens rapid metabolism, and the likelihood of parabens to be metabolized in the mothers' body before reaching the fetus. As one study identified higher concentrations of MP, EP and PP in the mother's urine than in the newborns (110), this assumption may be true. But as there is to my

knowledge, no other study comparing levels of dermally applied parabens in newborns or infants with their mother`s, only assumptions can be made. The same study also observed MP and PP in 100 % of the newborns urine samples, EP in 98 % and BP in 41 %. Significant correlations of MP, EP and PP were observed between mothers and newborns (110). But as only medians and adjusted measures were shown, estimates cannot be compared with the other studies discussed.

Even though it is likely that fetuses also get exposed to parabens, one study observed a decrease in total paraben concentrations during pregnancy compared to before. GMs was not calculated, but medians showed lower paraben concentrations in the second and the third trimester than in the first. Within- person GMs, comparing pre- pregnancy measures with pregnancy measures, showed higher concentrations of MP, EP and PP in urine before pregnancy than in pregnancy. Spearman's correlations of MP, EP and PP were high ($r_s = 0.55$, $r_s = 0.56$ and $r_s = 0.55$, respectively) (60), when interpreted as suggested by Pallant et al. (73). The reductions in paraben concentrations during pregnancy can be a result of changes in cosmetic use, but also changes in physiological factors has been assumed as a reason. Such physiological factors can be an increased BMI, plasma volume expansion and bone mobilization (111). Changes in cosmetic usage patterns can for example be related to the use of products especially meant for pregnancy or maternity (creams against stretch marks etc.), or it may be caused by a general reduction in the use of cosmetics.

As both Calafat et al. (107) and Smith et al. (60) observed a higher total level of parabens in women's urine than men`s, potentially unnecessary exposures of the fetus and breastfeeding infant are indicated. This can be suggested as women use more cosmetics than men, both in terms of frequency and number of products (6, 7). Calafat et al. (107) detected the GM of MP in women to be over three times higher than in men ($p = < 0.01$). But, despite of a wide CI (95 % CI: 80.8, 135) in women, the results indicate a much higher exposure among women as men`s CI was much lower (95 % CI: 24.8, 35.8). Women had also over six times higher exposure to PP than men (GM, 95 % CIs: women 20.4; 16.0, 25.9; men 2.96; 2.33, 3.77) (107). Smith et al. (60) observed

the GM of MP to be over four times higher in women, while the GM of PP was over seven times higher in women than men ($p = < 0.1$). As the detection rates of MP and PP was observed to be quite similar among men and women (60), men are also widely exposed, but at lower concentrations. This shows it is possible to reduce paraben exposures in women, and thereby also potential exposures of fetuses and infants. Exact percentages of detections above LODs were not documented by Calafat et al. (107).

To decrease paraben exposures can be achieved by reducing the use of cosmetics, or by decreasing or removing the paraben content in cosmetic products. Today there are a highly reduced number of products for sale containing parabens, and that is likely to be a result of the attention parabens have received in media and producers removing parabens from their products. As a consequence, paraben exposures could have diminished the last year.

In conclusion, available studies show stronger evidence of a larger paraben exposure in women in general than in men. One study shows paraben exposures in newborns, which is an indication on paraben exposures of fetuses or exposures to the newborn. More studies are warranted to investigate the extent of exposure to newborns and infants, and whether exposures harm these sensitive groups.

6. METHODOLOGICAL LIMITATIONS

There are a number of limitations in studies on parabens that can decrease the reliability of the conclusion. It is therefore necessary to present these challenges and reflect upon their possible effects on the result.

The general limitations considered are:

a) The low number of RCTs;
b) Small sample size;
c) Blood/ urine;
d) Differences among study populations;
e) Different limit of detection (LOD) levels.

These limitations are separately explained and reflected upon below:

a) Low number of RCTs.

The first and maybe the most important limitation is the lack of RCTs. RCTs are considered the "golden standard" in study designs, where strong experimental control is possible; if conducted properly. Because of the low number of RCTs and the thesis focus on human studies, more studies with observational designs are used. The study designs of papers discussed in chapter 5 (also in appendix B) are shown in table 1.

STUDIES	STUDY DESIGN
Janjua et al. (2007, 2008) (97, 98)	Randomized controlled trials (RCTs). Cross- over design
Fredriksen et al. (2011) (22)	Prospective cohort
Sandanger et al. (2011) (69)	Prospective cohort
Smith et al. (2012) (60)	Prospective cohort
Calafat et al. (2010) (107)	Prospective cohort
Ye et al. (2008) (103)	Cross- sectional
Wang et al. (2013) (21)	Cross- sectional
Shirai et al. (2013) (64)	Cross- sectional
Meeker et al. (2013) (106)	Cross- sectional

Table 1: Study designs.

The larger amount of observational studies makes it impossible to study the percentage of parabens absorbed, because the dose applied is unknown. And because of parabens short half- life, and a time delay between exposure and measurement, it is neither possible to identify the exact mean and maximum concentration reached in blood. An example, when a cross- sectional or cohort study is conducted to measure native paraben concentrations in serum, the body burden of parabens may already have decreased. Another limitation in using an observational design is the lack of ability to prove causal relationships. Causality is according to Jekel et al. (112): *"a factor that produces or contributes to the production of a specified outcome"* (p. 383). For instance, a causal relationship must be shown between the use of cosmetics and the detection of parabens in blood or urine, before evidence of causality can be proven. However, because dermal exposures have been shown to be the main route of exposure (14, 64), the discussion have been based on this fact. But it is important to have in mind that 4 - 5 % of paraben exposures are estimated to occur from an oral route (9, 14). Since the exposure must be proven present before the disease (temporality) to show causality (112), cross- sectional studies cannot prove causal relationships. The reason is because cross- sectional studies collect information at the same point in time. A prospective cohort on the other hand is better showing temporality as subjects are followed over time to see how the outcome develops (112). A limitation of using cohorts on studies of parabens

is the possible influence of the Hawthorne effect. The Hawthorne effect occur when subjects recognize they are observed, and change habits during the study. For instance, such a change can be a change in cosmetic use. The different study designs have been ranged in a hierarchy based on their abilities to provide evidence of causality (figure 7):

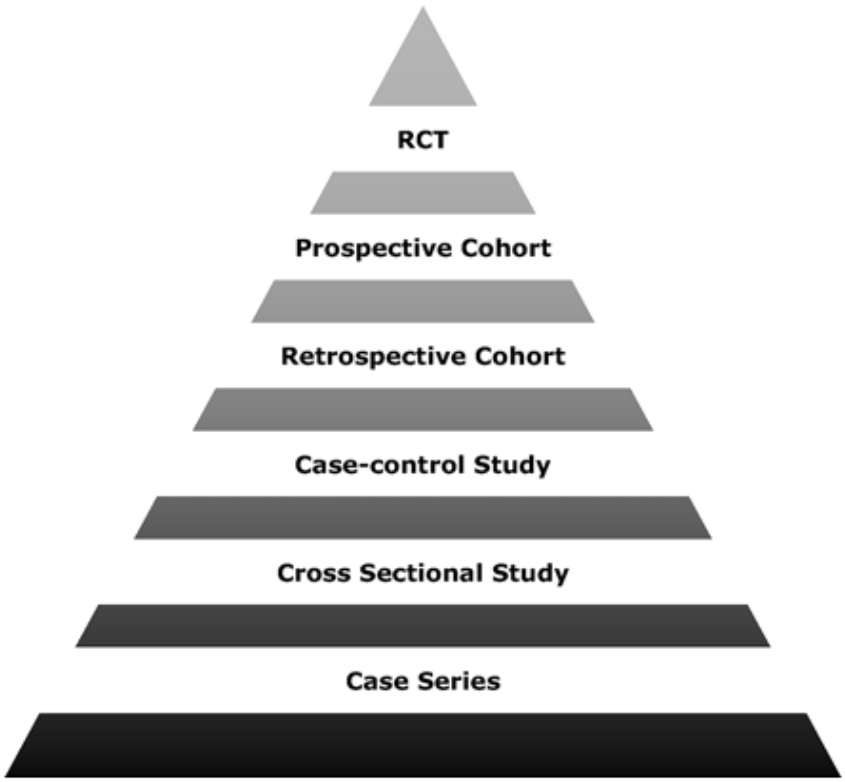

Figure 7: The evidence pyramid. Figure reused with publishers permission (113).

Designs closer to the top of the pyramid have a larger potential to provide causality. The probability of showing causal relationships occur when there is less likelihood of bias, and then better internal validity (112). Only systematic reviews and meta- analysis is placed higher than RCTs (114). Studies lower than the RCT in the design hierarchy are observational. Observational studies are used when an RCT is inappropriate to use. This can for instance be related to ethical or medical principles. For example is it both unethical and medically unjustifiably to conduct a RCT and apply parabens on pregnant women`s skin to study paraben concentrations in newborns. It is also unethical to expose humans to experiments and thereby detect no causal relationship.

Observational designs can in such circumstances be used to investigate associations before a potential experimental study. Other factors affecting the choice of study design are cost, time span and data availability. A cross- sectional study are simpler and less costly to perform than for instance prospective cohorts (112). However, when writing a literature study and there is a lack of RCTs, it is common to use animal or *in vitro* experimental studies instead. The problem of using such studies is their lack of external validity. For example is animal studies lower ability to extrapolate evidence to humans caused by differences in genetics, nutrition, size, life expectancy etc. Animal and *in vitro* experimental studies can only be used as indications before further studies on humans, if possible. Because of the decreased generalizability, both animal- and *in vitro* experiments are placed lower than case series in the evidence pyramid (115).

Despite observational studies lack abilities to identify dose and causal relationships, and there is a time delay between exposure and measurement, the reliability of the thesis conclusion is not reduced. Based on the thesis objective, this can be defended by the high detection rates of parabens, regardless of study design. The high detection rates show humans are widely and regularly exposed to parabens. Observational studies cannot be used to show exact concentrations of parabens either, as they have a very short half- life. And neither is it possible to identify absorption rates in humans by using observational designs. To identify absorption rates, the amount of parabens applied must be known, together with more precise estimates of the absorbed parabens than what is possible with observational designs. More human RCTs are therefore required. However, a common limitation with the use of RCTs is the false setting they are conducted under. A good example of false setting is seen in Janjua et al. (97, 98) studies, where unnaturally high amounts of parabens and phthalates were applied on the skin. False settings cause less generalizable results.

In conclusion, despite of the low number of RCTs, high detections of parabens in observational studies is evidence enough to prove humans are widely and regularly exposed to parabens. More human RCTs are however required to obtain information on the rate of absorption. And because of

the reduced applicability to humans, animal and *in vitro* studies can only be used to show indications of exposure.

b) Small sample size

The studies discussed in chapter 5 vary in sample size. For instance, Calafat et al. (107) has a sample size of 2548, while Ye et al. (103) only has 15. Several of the studies have populations of less than 70 subjects, and neither of the studies have shown sample size calculations. A small sample size can reduce confidence in a study's results and decrease generalizability.

A likely reason for small sample sizes in studies on parabens is the cost of analyzing parabens. One solution to increase studies power is to use within- subject, cross- over designs as Janjua et al. (97, 98). However, the reliability of the thesis conclusion is not affected by the fact that some of the studies have low sample size. As every population, regardless of sample size, show high detection rates of parabens, prove wide exposures of parabens.

c) Blood/ urine

Urine is often the preferred matrix for biomonitoring of chemicals with short– half lives. The simplicity of collecting spot urine samples and the fast excretion are two reasons (116). For dermally absorbed parabens, blood is preferred. The preference for blood is caused by the fact that native parabens better reflect dermal than oral exposures (22), and that native parabens are to a larger extent conjugated before excreted in urine. Blood is however also preferred because of individual differences in spot urine and unclear definitions of whether it is metabolites, conjugates or total levels that have been measured. In urine each void can differ in volume, and therefore also in the concentration of chemicals. Adjustments are for this reason often conducted to adjust for differences in the volume of spot urine samples (116). If unadjusted values are compared, measurement bias can occur.

However, despite more studies use urine as matrix than blood, the reliability of the conclusion is not reduced. High detection rates of parabens are observed in both blood and urine, but using urine as matrix is inadequate for determining the percentage of dose reached, and exposure route. Detection rates are neither greatly influenced by whether urine is adjusted or not, as shown by Shirai et al. (64). The conclusion of widely occurring paraben exposures is reliable.

d) Differences among study populations

The studies discussed in chapter 5, have populations that differ in for instance gender, age and origin. Differences therefore exist in the extent of cosmetic use. By comparing findings from several different and heterogeneous populations the thesis generalizability increases.

However, the use of unlike populations may render comparisons, especially if unadjusted measures are compared. But as detection rates show the extent of exposures, and high detection rates are identified among every population studied, differences among study populations' do not affect the conclusions reliability.

e) Different limit of detection- levels (LODs)

As there is different ways to calculate the LOD (19), terms such as LOD, method detection limit (MDL), limit of quantification (LOQ) etc. are used. The use of different methods to calculate LOD and high percentages below or close to LOD do however reduce comparability. Examples of different LOD- levels are observed in the studies by Wang et al. (21) and Shirai et al. (64), where LODs were 0.02 ng/ml and 0.47 ng/ml for native EP in urine, respectively. Different methods of detection and different LOD- levels are however usually described in the research papers, and can therefore be taken into consideration by the assessor. The use of different LOD- levels does not decrease the reliability of the thesis conclusion, as high detection rates of parabens can still be and are detected. This limitation will only affect comparisons of paraben concentrations detected at low levels.

Finally, the low number of RCTs, studies small sample size, more studies using urine as matrix, differences among study populations and the different LOD- levels do not decrease the reliability of the thesis conclusion of proving widely occurring paraben exposures among humans.

7. MIXTURE EFFECTS

Recently, more focus has been placed on mixture effects of EDCs and the Precautionary Principle. The main concern is related to the fact that compounds in combination or mixtures, might elicit effects that are not shown for single compounds, or enhance the effects shown for single compounds. As evidence is lacking on mixture effects in general, potential risks can be limited by enforcing the Precautionary Approach. When the Precautionary Approach enters into force, rapid response is permitted to protect human health and the environment. And as it is a fundamental principle of environmental law both nationally and internationally, it can be enforced by policy makers (17). The Stockholm Conventions framework for instance, is based on the Precautionary Approach (117). The principle applies especially to possible threats where significant data is lacking (118).

One study where mixtures of EDCs were shown to increase the overall effect of hormonal influences, identified that mixtures of parabens stimulated proliferation of MCF- 7 breast cancer cells (83). Even though parabens estrogenic potential or anti- androgenic potential is low by themselves (78), they come in addition to the already existing body burden of persistent EDCs (see chapter 4.1 on persistent chemicals). Parabens are therefore likely to increase the overall effects of estrogenic influences. However, despite of a lack of knowledge on mixture effects, the EU considers most parabens safe to use in cosmetics, as long as they are below maximum recommended concentrations (86). And as the EUs calculation of MoS do not account for mixture effects of parabens in combinations with other EDCs in either children or adults, the likelihood of an underestimation of endocrine effects is expected.

It is however possible for consumers to make choices in order to reduce the number of EDCs they are exposed to. One approach is to avoid purchasing EDC- containing cosmetics, but this requires knowledge about EDCs. Increased focus and knowledge on parabens among consumers is one likely reason for the highly reduced number of cosmetics containing parabens, and is an example

on that increased awareness and knowledge among consumers can give positive result in reducing exposures to EDCs. An easier approach to determine which cosmetics that contain EDCs however, are to download the Norwegian Consumer Councils hormone check application for iPhones and android mobile devices. The application scans cosmetic products and show whether products contain EDCs or not.

In summary, there is lack of research on mixture effects in general, but potential risks can be limited by enforcing the Precautionary Approach. The main concern to mixture effects are related to the fact that compounds in combination or mixtures, might elicit effects that are not shown for single compounds, or enhance the effects shown for single compounds. As the EUs calculation of MoS do not account for mixture effects of parabens, an underestimation of endocrine effects is expected. But, it is possible for consumers to make choices in order to reduce the number of EDCs they are exposed to.

8. CONCLUSION

Paraben exposures in humans have been studied by discussing and comparing evidence of human concentration, dermal absorption, metabolism and excretion. The results show high detection rates of native and total parabens in blood and urine, and provide strong evidence of regular or constant exposures to widely occurring parabens. Evidence from the literature show a higher cosmetic use and higher concentrations of parabens among women than men, and point to cosmetic products being responsible for elevated paraben exposures. This is supported by one study that shows strong association of elevated paraben levels with cosmetic use. As evidence also point towards higher exposures to native parabens if dermally absorbed, dermal exposures are more likely to affect health compared to oral exposures.

Parabens were only used as an example of EDCs in cosmetics, and it is important to be aware that there are numerous other EDCs in use. And because the unborn child and breastfeeding infant has been identified as sensitive groups to the effects of EDCs and thus parabens, use of EDCs in products to pregnant women and mothers with newborns should have special attention. It is however possible for consumers to influence the use of parabens and other EDCs in cosmetics. One example of the positive effects of caring is the highly reduced number of products for sale containing parabens. And this may have reduced exposures to parabens the last year. It is nevertheless a need for better knowledge in order to know more about the chemicals in replacement products, alone and in combination with other EDCs (mixture effects).

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APPENDIX A: Literature search

URINE CONCENTRATIONS OF PARABENS

SCOPUS

Urine excretion

#5 ((TITLE-ABS-KEY(urinary excretion*) OR TITLE-ABS-KEY(urinary concentration*) OR TITLE-ABS-KEY(urine excretion*) OR TITLE-ABS-KEY(urine concentration*))) AND ((TITLE-ABS-KEY(cosmetic*) OR TITLE-ABS-KEY(body care product*) OR TITLE-ABS-KEY(personal care product*))) AND ((TITLE-ABS-KEY(parahydroxybenzoic acid*) OR TITLE-ABS-KEY(p- hydroxybenzoic acid*) OR TITLE-ABS-KEY(paraben*) OR TITLE-ABS-KEY(phenol*))) AND ((TITLE-ABS-KEY(human*) OR TITLE-ABS-KEY(people*) OR TITLE-ABS-KEY(adult*) OR TITLE-ABS-KEY(grown up*) OR TITLE-ABS-KEY(adolescent*) OR TITLE-ABS-KEY(youth*) OR TITLE-ABS-KEY(teenager*) OR TITLE-ABS-KEY(child*) OR TITLE-ABS-KEY(toddler*) OR TITLE-ABS-KEY(infant*) OR TITLE-ABS-KEY(newborn*))) AND (LIMIT-TO(PUBYEAR, 2013) OR LIMIT-TO(PUBYEAR, 2012) OR LIMIT-TO(PUBYEAR, 2011) OR LIMIT-TO(PUBYEAR, 2010)) AND (LIMIT-TO(DOCTYPE, "ar")) **RESULTS: 11**

#4 (TITLE-ABS-KEY(cosmetic*) OR TITLE-ABS-KEY(personal care product*) OR TITLE-ABS-KEY(body care product*)) **RESULTS: 63 066**

#3 (TITLE-ABS-KEY(urinary excretion*) OR TITLE-ABS-KEY(urinary concentration*) OR TITLE-ABS-KEY(urin excretion*) OR TITLE-ABS-KEY(urin concentration*)) **RESULTS: 109,503**

#2 (TITLE-ABS-KEY(p- hydroxybenzoic acid*) OR TITLE-ABS-KEY(parahydroxybenzoic acid*) OR TITLE-ABS-KEY(paraben*)OR TITLE-ABS-KEY(phenol*)) **RESULTS: 218,0172**

#1 (TITLE-ABS-KEY(human*) OR TITLE-ABS-KEY(people*) OR TITLE-ABS-KEY(adult*) OR TITLE-ABS-KEY(grown up*) OR TITLE-ABS-KEY(adolescent*) OR TITLE-ABS-KEY(youth*) OR TITLE-ABS-KEY(teenager*) OR TITLE-ABS-KEY(child*) OR TITLE-ABS-KEY(toddler*) OR TITLE-ABS-KEY(infant*) OR TITLE-ABS-KEY(newborn*)) **RESULTS: 16, 952, 082**

Included articles:

1. Urinary concentrations of four parabens in the U.S. Population: NHANES 2005-2006. Calafat, A.M., Ye, X., Wong, L.-Y., Bishop, A.M., Needham, L.L. 2010. Environmental Health Perspectives 118 (5) , pp. 679-685
2. Parabens in urine, serum and seminal plasma from healthy Danish men determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Frederiksen, H., Jørgensen, N., Andersson, A.-M. 2011. Journal of Exposure Science and Environmental Epidemiology 21 (3) , pp. 262-271
3. Predictors and variability of urinary paraben concentrations in men and women, including before and during pregnancy. Smith, K.W., Braun, J.M., Williams, P.L., Ehrlich, S., Correia, K.F., Calafat, A.M., Ye, X., (...), Hauser, R. 2012. Environmental Health Perspectives 120 (11) , pp. 1538-1543
4. Urinary excretion of parabens in pregnant Japanese women. Shirai, S., Suzuki, Y., Yoshinaga, J., Shiraishi, H., Mizumoto, Y. 2013. Reproductive Toxicology 35 (1) , pp. 96-101
5. Characteristic profiles of urinary p -hydroxybenzoic acid and its esters (Parabens) in children and adults from the United States and China. Wang, L., Wu, Y., Zhang, W., Kannan, K. 2013. Environmental Science and Technology 47 (4) , pp. 2069-2076
6. Distribution, variability, and predictors of urinary concentrations of phenols and parabens among pregnant women in puerto rico. Meeker, J.D., Cantonwine, D.E., Rivera-González, L.O., Ferguson, K.K., Mukherjee, B., Calafat, A.M., Ye, X., (...), Cordero, J.F. 2013. Environmental Science and Technology 47 (7) , pp. 3439-3447

Excluded articles:

1. Development of a quantitative analytical method for determining the concentration of human urinary paraben by LC-MS/MS. Lee, S.-Y., Son, E., Kang, J.-Y., Lee, H.-S., Shin, M.-K., Nam, H.-S., Kim, S.-Y., (...), Rhee, G.-S. 2013. Bulletin of the Korean Chemical Society 34 (4) , pp. 1131-1136
2. The contribution of diet to total bisphenol A body burden in humans: Results of a 48hour fasting study. Christensen, K.L.Y., Lorber, M., Koslitz, S., Brüning, T., Koch, H.M. 2012. Environment International 50 , pp. 7-14
3. Lifestyle behaviors associated with exposures to endocrine disruptors. Martina, C.A., Weiss, B., Swan, S.H. 2012. NeuroToxicology 33 (6) , pp. 1427-1433
4. Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. Meeker, J.D., Yang, T., Ye, X., Calafat, A.M., Hauser, R. 2011. Environmental Health Perspectives 119 (2) , pp. 252-257
5. Triclosan is a potent inhibitor of estradiol and estrone sulfonation in sheep placenta. James, M.O., Li, W., Summerlot, D.P.,Rowland-Faux, L., Wood, C.E. 2010. Environment International 36 (8) , pp. 942-949

PUBMED

Urine excretion

#5 ((((((cosmetic*) OR body care product*) OR personal care product*)) AND ((((p- hydroxybenzoic acid*) OR parahydroxybenzoic acid*) OR paraben*) OR phenol*)) AND ((((((((((((human*) OR people*) OR adult*) OR grown up*) OR teenager*) OR youth*) OR adolescent*) OR toddler*) OR child*) OR infant*) OR newborn*) NOT animal*) NOT rat*) NOT rabbit*)) AND ((((urinary concentration*) OR urinary excretion*) OR urin concentration*) OR urin excretion*) Filters: Publication date from 2010/01/01 to 2013/04/23 **RESULTS: 4**

#4 (((p- hydroxybenzoic acid*) OR parahydroxybenzoic acid*) OR paraben*) OR phenol* **RESULTS: 74,684**

#3 (((urinary concentration*) OR urinary excretion*) OR urin concentration*) OR urin excretion* **RESULTS: 28,499**

#2 ((cosmetic*) OR body care product*) OR personal care product* **RESULTS: 57,140**

#1 ((((((((((((human*) OR people*) OR adult*) OR grown up*) OR teenager*) OR youth*) OR adolescent*) OR toddler*) OR child*) OR infant*) OR newborn*) NOT animal*) NOT rat*) NOT rabbit* **RESULTS: 6,685,607**

Included articles:

1. Urinary concentrations of four parabens in the U.S. population: NHANES 2005-2006. Calafat AM, Ye X, Wong LY, Bishop AM, Needham LL. Environ Health Perspect. 2010 May;118(5):679-85. doi: 10.1289/ehp.0901560. Epub 2010 Jan 4.
2. Urinary excretion of parabens in pregnant Japanese women. Shirai S, Suzuki Y, Yoshinaga J, Shiraishi H, Mizumoto Y. Reprod Toxicol. 2013 Jan;35:96-101. doi: 10.1016/j.reprotox.2012.07.004. Epub 2012 Jul 10.
3. Distribution, variability, and predictors of urinary concentrations of phenols and parabens among pregnant women in Puerto Rico. Meeker JD, Cantonwine DE, Rivera-González LO, Ferguson KK, Mukherjee B, Calafat AM, Ye X, Anzalota Del Toro LV, Crespo-Hernández N, Jiménez-Vélez B, Alshawabkeh AN, Cordero JF. Environ Sci Technol. 2013 Apr 2;47(7):3439-47. doi: 10.1021/es400510g. Epub 2013 Mar 19.

Excluded articles:

1. Bisphenol A and other phenols in urine from Danish children and adolescents analyzed by isotope diluted TurboFlow-LC-MS/MS. Frederiksen H, Aksglaede L, Sorensen K, Nielsen O, Main KM, Skakkebaek NE, Juul A, Andersson AM. Int J Hyg Environ Health. 2013 Mar 11. doi:pii: S1438-4639(13)00016-3. 10.1016/j.ijheh.2013.01.007 [in press].

APPENDIX B: Literature matrices

Matrix 1: Literature matrix of observational human studies reporting parabens in serum and plasma

Country	n	Population	Median blood paraben concentrations				Units	Reference
			Native parabens:					
			MP	**EP**	**PP**	**BP**		
Norway	332	Postmenopausal women[a]	9.4	< 3	< 2	-	ng/ml	Sandanger et al. (2011) (69)
		Detection rate (%)	63	22	29			
USA	15	11 women, 4 male[b]	0.2 (< 0.1–9.8)	< 0.1(< 0.1)	< 0.2 (< 0.2–2.3)	-	ng/ml	Ye et al. (2008) (103)
		Detection rate (%)	60	0	47			
			Total parabens (free + conjugated; glucuronidated and sulfated):					
			MP	**EP**	**PP**	**BP**		
USA	15	11 women, 4 male[b]	10.9 (0.4–301)	0.2 (< 0.1–5.4)	1.4 (< 0.2–67.4)	-	ng/ml	Ye et al. (2008) (103)
		Detection rate (%)	-	-	-			
Denmark	60	Healthy, young men[b]	1.53 (< 0.36–59.6)	< 0.35(< 0.35–20.8)	0.32(< 0.1–5.4)	< 0.33	ng/ml	Frederiksen et al. (2011) (22)
		Detection rate (%)	100	53	80			

[a] p = < 0.001 [b] range (minimum- maximum)

Matrix 2: Literature matrix of observational human studies reporting unadjusted values of parabens in urine

Country	n	Population (age)	Geometric mean (GM) urine paraben concentrations				Units	Reference
			Native parabens:					
			MP	**EP**	**PP**	**BP**		
USA	40	Children 3-10[a]	0.32 (< 0.3–8.74)	NA	NA	NA	ng/ml	Wang et al. (2013) (21)
		Detection rate (%)	78	33	48	8		
China	70	Children 9–10[a]	1.00 (0.29–11.0)	0.19 (0.03–5.56)	0.32 (0.10–4.25)	0.01 (0.004–0.03)		
		Detection rate (%)	100	100	100	98		
	26	Adults[a]	3.15(< 0.03–312)	NA	0.46 (< 0.03–119)	NA		
		Detection rate (%)	92	46	92	23		
Japan	111	Pregnant women[b]	6.99 (< 0.57–544)	0.62 (< 0.47-63.2)	0.60 (< 0.48–14.3)	-	ng/ml	Shirai et al. (2013) (64)
		Detection rate (%)	88	49	46			
			Total parabens (free + conjugated; glucuronidated and sulfated):					
			MP	**EP**	**PP**	**BP**		
Japan	111	Pregnant women[b]	62.6 (<0.57-1361)	5.59 (<0.47-593)	19.7 (<0.48-2690)	0.89 (0.46-22.8)	µg/L	Shirai et al. (2013) (64)
		Detection rate (%)	94	81	89	54		
Denmark	60	Healthy, young men[b]	1.53 (< 0.36–59.6)	< 0.35(< 0.35–20.8)	0.32 (< 0.1–5.4)	< 0.33	ng/ml	Frederiksen et al. (2011) (22)
		Detection rate (%)	100	53	80			

USA	40	Children 3-10[a]	62.4 (3.30–4510)	0.10 (<0.02-2.69)	0.92 (<0.03-365)	<0.02 (<0.02-0.48)	ng/ml	Wang et al. (2013) (21)
		Detection rate (%)	100	60	83	10		
China	70	Children 9–10[a]	5.28 (0.85–54.7)	0.97 (0.11–31.7)	1.89 (0.24–71.5)	0.04 (<0.02–0.41)		
		Detection rate (%)	100	100	100	99		
	26	Adults[a]	30.5 (1.85–3420)	0.34 (<0.02-119)	5.07 (0.07–515)	NA (<0.02-11.0)		
		Detection rate (%)	100	50	100	35		
Puerto Rico	105	Pregnant women[c]	140 (117,167)	-	30 (24.1-37.5)	1.0 (0.8-1.3)	ng/ml	Meeker et al. (2013) (106)
		Detection rate (%)	100	-	99.3	58.4		
USA	653	Male and females (mean 36)[b]	100 (<1- 23,200)	-	17.9 (<0.2-2870)	1.08 (<0.2-998)	µg/L	Smith et al. (2012) (60)
		Detection rate (%)	99.7	-	96.5	65.4		
USA	2548	Male and females (>6)[c]	56.4 (46.9-97.9)	<0.01 (<0.01)	7.91 (6.41-9.77)	Male: NA Women:0.904 (0.760-1.07)	µg/L	Calafat et al. (2010) (107)
		Detection rate (%)	*>60*	*<60*	*>60*	*<60*		

[a] 5th-95th percentile [b] range (minimum- maximum) [c] 95 % CI