Endocrine disrupting chemicals in cotton lingerie

Assessing the influence of endocrine disrupting chemicals in cotton lingerie on women's fecundity

Final work of the bachelor course of study to get the academic degree "Bachelor of Science"

by

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Abstract

This thesis aims to provide scientific knowledge on the potential risks of exposure to and effects of endocrine disruptors, chemicals that have hormone-like activity and may affect the human hormone systems, in black cotton lingerie. Specifically, this work analyses the influence endocrine disruptors may have on fecundity, the ability for a woman to get pregnant.

In order to analyse possible exposure to chemical substances from lingerie, samples have been tested for specific chemical content. Data about commonly occurring substances in textiles in general has also been gathered, via literature and interviews.
Furthermore, the skin absorption process of these chemical substances has been studied.

Regarding effects, some substances of high concern have been selected to be evaluated via *in-silico* models (computer programs) for predicting toxicity: Polycyclic aromatic hydrocarbons (PAH), Nonylphenols (NP) and Nonylphenols ethoxylate (NPEO). Their potential to act as endocrine disruptors as found in the *in-silico* modelling was also confirmed by literature data.

The conclusion from the thesis is that both PAH, NP and NPEO could potentially affect fecundity via clothing, though lingerie is not necessarily worse than other textile in repeated skin-contact. The doses of the chemicals that would be needed to be administered to the human body from the textiles for an effect on fecundity was not able to be established within the scope of this thesis. Thus, future research is necessary in order to proof their impact on fecundity.

Acknowledgement

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Maren Duprés

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List of Acronyms

AEEA	Aminoethyl ethanolamine
B[a]P	Benzo[a]pyrene
BPA	Bisphenol A
ECHA	European Chemical Agency
EDCs	Endocrine disrupting chemicals
EPA	United States Environmental Protection Agency
FSH	Follicle Stimulating Hormone
GC-MS	Gas chromatography–mass spectrometry
GnRH	Gonadotropin-releasing hormone
GOTS	Global Organic Textile Standard
LC-MS/MS	Liquid chromatography–mass spectrometry
LH	Luteinizing hormone
NOAEL	No-observed-adverse-effect-level
NP	Nonylphenol
NPE	Nonylphenol ethoxylated
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated Biphenyl
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
QSAR	Quantitative structure–activity relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RISE	Research Institutes of Sweden
SVHV	Substances of very high concern
WHO	World Health Organisation

1 Introduction

This work's overarching purpose has been to compile scientific information about the potential health risks from substances present in dyestuff and auxiliary chemicals related to the colouring process of cotton lingerie for women. Specifically, health risks from endocrine disrupting chemicals (EDCs) have been analysed. The work has been performed in consultation with the Materials and Production Division at RISE - Research Institutes of Sweden (RISE IVF) located in Mölndal, Sweden.

Today, the wide use of chemicals in textile production and the pollution of local wastewater in the production countries is common knowledge. Regarding the chemicals that remain in the fabric, potentially exposing the consumer to health risks, regulations such as the European REACH are currently evolving to legally restrict even more potentially harmful compounds hiding in textile garments (European Chemicals Agency, 2012). Some examples are the upcoming regulations on substances in textiles with skin contact (CMR fast track / REACH entry 72) and restriction of NPEO in textiles (REACH entry 46a). Even though there is an active drive to substitute harmful chemicals for chemicals with a lower impact, some of the harmful chemicals will most likely remain in production in the nearest years, especially in products derived from Asian production countries (Muthu *et al*., 2018).

The rates of chemicals shedding from the textile garment depend on different parameters, importantly including how much of the chemical is bound to the fibre and how the dye has been applied. The concentration of many chemicals in garments is most likely to decrease after washing, nevertheless there may remain substantial amounts of the compounds in the textile with the

possibility of exposure to the skin. However, when it comes to dyestuff, these chemicals are intended to stay on the fabric for the garment's entire life span, otherwise the garment would lose its colour. The literature has shown that dermal exposure to chemicals from textiles can not only cause allergic skin reactions, but may also result in serious diseases and reproductive toxicity (Kaerlev *et al.*, 2005), (Schug *et al.*, 2011). Therefore, the potential relationship between the dermal uptake of colour-related EDCs found in women's underwear and a potential increased infertility risk will be investigated. As dark coloured underwear's potential of harming the female reproductive system can be expected to be higher than light coloured garments from a dose-perspective, the study has been limited to dark-coloured lingerie. Furthermore, it will be examined if representative classes of EDCs have the potential to decrease women's fecundity.

If the assumptions should be confirmed, the management and sustainable use of chemicals in textile garments should be enhanced. Moreover, consumers should be made more aware of the potential for endocrine disrupting-related health risks deriving from the exposure to such garments.

1.1 Outline of thesis

In the Introduction, the current state of research is analysed, followed by stating the research gap out of which the research question evolves.

Within the Materials and Methods chapter, the collection of industry data via interviews is described as well as the testing methods which have been used: *in-silico* modelling and sample testing. A comprehensive, systematic literature search and screening approach is documented in a table in the chapter of Literature Data.

The Results and Discussion and the Conclusions and Future Research chapters present the outcome of the thesis work.

1.2 Endocrine disrupting chemicals

Chemicals are a big part of peoples' daily lives, as they are exposed to them through water, food, air, hygiene products as well as textiles. As widely known, textile garments contain a number of chemical substances, from dyes to finishing agents which ensure colour fastness during washing, a stable shape or a wrinkle-free garment (Luongo, 2015).

Not only do these chemicals pose a health risk to the textile factory workers, but many of the chemicals also remain in the end product, presenting a potential threat to the customer. The dermal exposure to such chemicals caused by wearing apparel might be relatable to the occurrence of various human diseases. The

substances of very high concern (SVHC) within the European Union include endocrine disrupting chemicals (EDCs), which are able to interfere with hormonally controlled processes of humans (REACH, 2006).

The World Health Organization (WHO) defines EDCs as substances that alter one or more functions of the hormone system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub) populations (World Health Organisation , 2019). Since EDCs can lead to imbalances of the hormonal systems, they may not only be having a direct impact on the overall health of humans, but also may have especially impact on the reproductive system (Bergmann *et al.*, 2012).

Unfortunately, endocrine-related diseases and disorders are on the rise. Almost 800 chemicals are now known to be capable of interfering with hormone receptors. The majority of chemicals which are present in different consumer products such as cosmetics, food or garments have not yet been tested on endocrine disrupting properties, resulting in uncertainty about the actual extent of risks deriving from EDCs (Bergmann *et al.*, 2012). Hence, minimization of exposure to EDCs is an important aspect of consumer protection.

1.3 Fertility and fecundity

The word ‘fecundity’ refers to the ability of women to bear children, where fecundity depends on ‘fecundability’ which is the monthly probability of pregnancy among sexually active non-contraceptive couples (Leridon and Slama, 2008). Women’s fecundity is influenced by ovulation, age, menopause, and genetics (Williams, 2019). In the literature study performed as a part of this thesis (see section 3.3), it was found that the specific area of endocrine disruptors’ effects on fecundity has in principle been overlooked in the literature, especially regarding the exposure to textile garments.

This thesis is limited to the effects on women’s fecundity, where the potential influence of endocrine disruptors on the unborn child or male fertility is left out of scope. Thus, the area of interest is the female reproductive system, which actively impact fecundity.

Figure 1 shows the reproductive organs, which actively impact fecundity, including two ovaries, two fallopian tubes, the uterus, the cervix, and the vagina. The average woman’s reproductive period lasts from puberty to menopause, amounting to an average of 39 reproductive years (Weschler, 2006, p. 105).

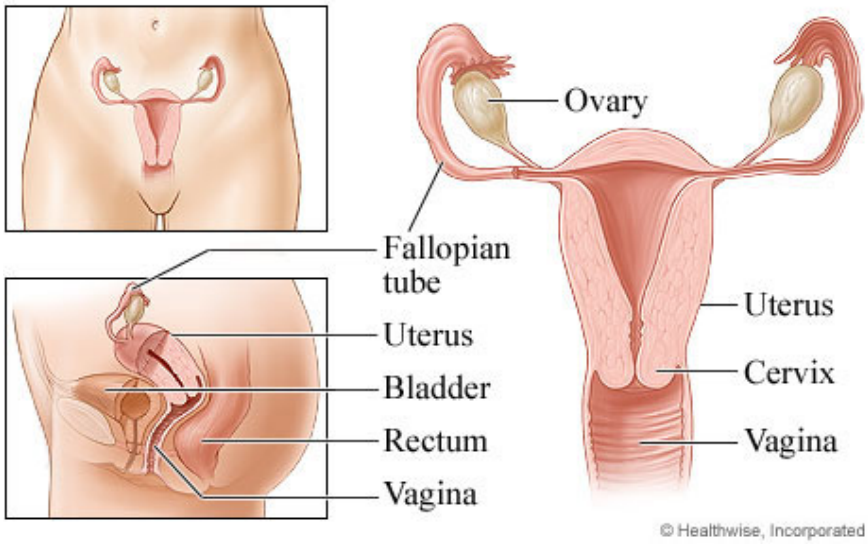

Figure 1: Female reproductive system (Health Link British Columbia, 2018)

1.3.1 Historical data on fertility

Global fertility rates have decreased by 50% since 1950. Accordingly, the 2018 worldwide fertility rate amounted only to 2.4 births per woman, however there are drastic differences between developed and undeveloped countries (The Week UK, 2018). Figure 2 clearly shows the strong decline in fertility rates in developed countries, whereby the world's average fertility rate in 1950 amounted to five children per woman, three children per woman in US and two children per woman in Germany.

In the following decades the fertility rates in developed countries strongly declined, however Figure 2 also shows that the number of seven children per woman in Mali remained rather constant, from the 1970s to the mid 90s, even increasing slightly to 7.15 children per woman (Roser, 2017). The world's average fertility rate saw its highest decline from 5.03 children per woman in 1965 to only 3.53 children per woman within two decades. A similar development can be seen in Germany and United States, with a

decrease from 2.9 children in the United States and 2.48 children in Germany to 1.85 children per woman in the United States and 1.45 children per woman in Germany in 1985.

The World's average fertility rate in 2015 numbered to 2.49 children per woman, whereas in Mali the fertility rate still remains at 6.15 children per woman (Roser, 2017). Figure 2 clearly pinpoints a decline in fertility rates for developed countries, one reasons for this development are shifting societal expectations as well as women's empowerment, technological and economic changes (The Week UK, 2018).

However, the decline in fertility rates is not always a matter of choice. Thus industrialized countries particularly, conception is becoming more difficult and miscarriages are more common (The Week UK, 2018).

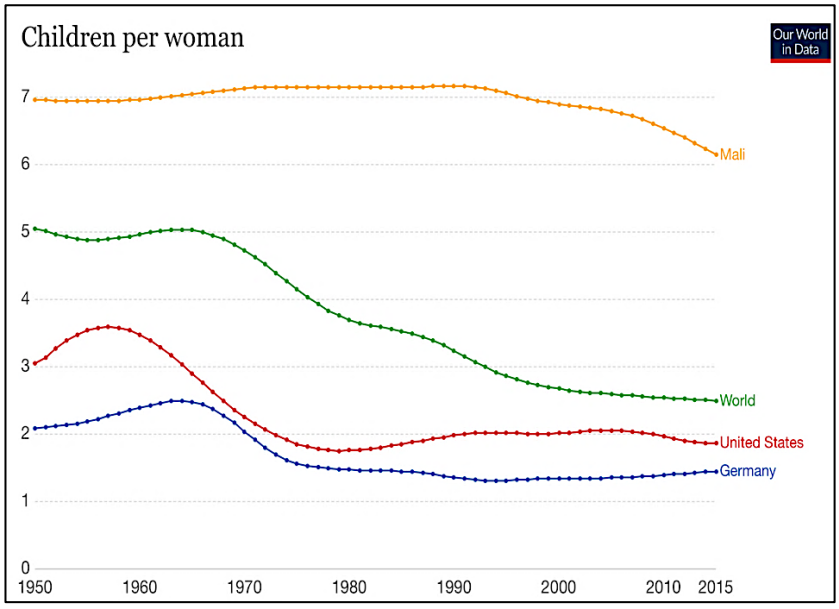

Figure 2: Children per woman; International average in comparison to Mali, US and Germany (Roser, 2017)

1.3.2 Reasons for decline of fertility

Environmental toxins can lead to infertility in many ways, but one of the emerging important aspects is by endocrine disruption. There are many classes of EDCs. Some of the most potent groups of endocrine disruptors include the organochlorines (chlorinated pesticides, polychlorinated biphenyls and dioxins), bisphenol A (BPA), and organophosphate pesticides and herbicides (Pizzorno, 2018). Other chemical compounds acting as endocrine disruptors are phthalate esters, pesticides and polychlorinated biphenyls (Leridon and Slama, 2008).

1.4 Research gap

According to information gained from the literature search regarding EDCs and hazard testing, much research on the issue of EDCs and human health has been performed. Nevertheless, the potential adverse health effects deriving from dermal exposure to EDCs are just about to be identified and investigated in animal and epidemiology studies (Anderson and Meade, 2014). In an initial overview of the literature, there appears to be a complete lack of publications about EDCs in lingerie products and their potential influence on the reproductive functions in women. Most articles focus on environmental EDCs and their influence on the health of animals and men (Dziewirska, 2018); (Harrison, 1997). One article about toxic chemicals in menstrual underwear products warned of the human health hazards but didn't refer to the specific risks to the female reproductive system (Choy, 2020). Nguyen and Saleh (2017) researched on the exposure of women to toxins by dermal absorption, though none of the articles include the exposure to EDCs but rather focus on heavy metals. Finally, a study on the connection between EDCs and the age of menarche in adolescent girls was found, however not in relation with dermal absorption or textile related chemicals (Buttke, 2012).

Hence, the work in this thesis will examine the available data points, that in combination may help to initiate the investigation as to whether and how these EDCs related to the dyeing process of cotton underwear may affect women's fecundity. It is hoped that this groundwork will increase awareness of the potential health effects resulting from dermal exposure to EDCs in dyestuffs.

1.5 Research question

In order to answer the main research question, a set of sub questions were formulated to guide the research.

Main question	To what extent does the dermal exposure to EDCs in cotton underwear garments influence women's fecundity?
Sub questions	Which chemicals used for the reactive dyeing process of cotton have the most endocrine disrupting properties?
	To what extent are EDCs in textiles subject to skin uptake?
	How do these EDCs impact women's fecundity?

2 Theoretical Framework

The theoretical framework of this thesis work is a research approach based on a hypothetical quantitative risk assessment of a case where women risk effects on their fecundity due to exposure to and dermal uptake of EDCs in dark-coloured cotton lingerie. In order to populate the chosen risk assessment model with data, three different scientific areas have been investigated: 1) textile dyeing technology, in order to determine the possible concentration of EDCs in a garment, 2) dermal exposure and uptake, and 3) fecundity and the mechanisms that will affect fecundity.

2.1 Research approach

Due to the formulation of the main research question and the desired aim of the research, the most appropriate research method was considered to be quantitative research. Through the research methods employed (see Chapter 3), information on various components of a hypothetical risk assessment has been derived. The work has included lab-testing samples of lingerie for a subset of the most hazardous EDCs found in dyestuff, auxiliary and degradation products from the dyeing process of cotton lingerie. In this study only dermal contact was considered as the route of exposure to elements derived from the contact of the textile with the skin (ECHA, 2012).

Equation 1, which was suggested by the European Chemical Agency (ECHA, 2012) to predict the risk of toxic chemicals in fabrics which can be taken up through direct skin contact, helps in determining the dermal exposure:

Equation 1: Dermal Exposure (ECHA, 2012)

$$\textit{Dermal Exposure mg/ kg/ day} = \frac{\boldsymbol{C}_{cloth}\, 10^{-6}\, \boldsymbol{D}_{cloth}\, \boldsymbol{A}_{skin}\, \boldsymbol{F}_{migrating}\, \boldsymbol{F}_{contact}\, \boldsymbol{F}_{penetration}\, \boldsymbol{T}_{contact}\, \boldsymbol{N}}{\textit{Body Weight (kg)}}$$

Where $\boldsymbol{C}_{\text{cloth}}$ is the concentration of element in clothes (in mg/kg), 10^{-6} is a conversion factor (kg/mg), $\boldsymbol{D}_{\text{cloth}}$ is the cloth surface density (in mg/cm$^{2)}$, $\boldsymbol{A}_{\text{skin}}$ is the area of contact between the cloth and the skin (in cm^2) which is assumed to be equivalent to female thighs surface, $\boldsymbol{F}_{\text{migrating}}$ is the fraction of substance migrating to the skin per day (1/day) which was estimated to be 0.005, $\boldsymbol{F}_{\text{contact}}$ is the fraction of contact area for skin, $\boldsymbol{F}_{\text{penetration}}$ is the penetration rate, $\boldsymbol{T}_{\text{contact}}$ is the contact duration between cloth and skin (days) and $\boldsymbol{N}$, is the mean number of events per day (ECHA, 2012), (BfR, 2012).

The aim of the first sub question is to find the most hazardous EDCs in women's lingerie by testing chemicals used during the dyeing process of the cotton underwear garments. In order to determine the EDCs present in dyestuff and auxiliary chemicals used during the dyeing process, the dyeing processes have to be examined. Therefore, Fanny Vermandel, the vice president in Global Marketing Coloration at DyStar, a textile dye producer, has been interviewed about which chemical substances could

possibly be found in the dye itself, auxiliary products as well as degradation products. The use of EDCs in dyestuff and auxiliary chemicals is not allowed in the EU, nevertheless they might still be in use for the clothing production in Asian countries (Vermandel, 2019).

To answer the last two sub questions, the substances of most concern and with the highest impact have been screened out with the help of a variety of commercially available and open-source computer-based prediction models routinely utilized with RISE.

2.2 Textile dyeing technology

In order to filter out a chemical class of very high concern, with the potential to act as an EDC, literature regarding this matter has been reviewed. Furthermore, Fanny Vermandel from DyStar has been interviewed in order to get a detailed understanding about the reactive dyeing processes and chemicals used.

Historically, many harmful chemicals have been used throughout the dyeing process of cotton. Even though the European quality standards are very high nowadays, those harmful chemicals might still be in use during the production outside the European Union. According to Fanny Vermandel, 80% of raw materials for dyeing still come from China and India where different laws apply. The rates of chemicals shedding from the textile garment depend on different parameters, importantly including how much of the chemical is bound to the fibre and how the dye has been applied.

2.3 Dermal uptake

The manner by which harmful substances penetrate into the body is various and called the ‘route of exposure’ or ‘the route of absorption’. Chemical substances can enter the body through the skin, hence dermal absorption is also a highly probable route of exposure for toxic chemicals. The amount of chemicals absorbed by the skin depends on the local concentration, the molecular weight of the molecule, the duration of the contact, the solubility of substances and the physical condition of the skin (International Program of Chemical Safety, 1987; Bos and Meinardi, 2000).

The human skin makes up 10 % of total body mass, hence it is the body’s largest organ (Hoffmann, 2019). The skin does not only protect the inner organs from the external environment, but also manages to regulate body temperature and permits the sensation of touch, heat and cold (Hoffmann, 2019). Further functions of the skin are e.g. water preservation, tactile sensation, thermal regulation, endocrine activity and vitamin D synthesis, immunological effector, and biotransformation of xenobiotics (Anderson and Meade, 2014). The penetration of harmful chemicals via the skin can result in numerous diseases and imbalances in the human body, therefore it is especially important to elaborate research and increase awareness about potential health hazards deriving from dermal exposure to chemicals of high concern.

2.3.1 Skin Anatomy

The skin is composed of several types of tissues, making it a very complex organ, see Figure 3. The skin includes two distinct layers: the outer layer called epidermis and the inner layer called dermis (Shier *et al.* , 2019, p. 179). Beneath the dermis is another layer, the subcutaneous layer.

The inner skin layer is made up of connective tissue containing collagen and elastic fibres, muscle tissue, nervous tissue and blood. The inner skin layer (dermis) is thicker than the epidermis and the two skin layers are separated by a membrane which is connected to the dermis by short fibrils (Shier *et al.* , 2019, p. 180).

The subcutaneous layer is located beneath the dermis containing different forms of loose connective tissues. The major blood vessels which supply the skin are also located in the subcutaneous layer (Shier *et al.* , 2019, p. 180). The subcutaneous layer and dermis are not sharply separated since the collagen and elastic fibres of the subcutaneous layer are continuous with those of the dermis (Shier *et al.* , 2019, p. 180).

The epidermis does not contain any blood vessels but is made up of different cell layers. The lowest cell layer is the best nourished one since it is located closest to the dermis where it is nourished by the dermal blood vessels. The older epidermal cells are moved to the skin surface. With increasing distance from the nourishing blood vessels, located in the dermis, the cells have a poorer nutrient supply. Those keratinocytes will eventually die due to the insufficient blood supply (Shier *et al.* , 2019, p. 180). The epidermis is thickest on the palms of the hands and the soles of the feet, where it varies from 0.8 to 1.4 millimetres (mm). In other body areas the epidermis is usually thinner, averaging 0.07- 0.12 mm (Shier *et al.* , 2019, p. 181).

Figure 3 shows the cross section of all skin. Beginning with the *stratum corneum* as the most outer skin layer, followed by the epidermis, dermis and hypodermis which includes the blood vessels.

The skin structure around the vaginal opening has not been studied. Hence this area of the skin which garments are exposed to is quite generic in the scope of this thesis. The study of more specific skin areas in connection with the exposure of EDCs however constitutes an important topic for future research.

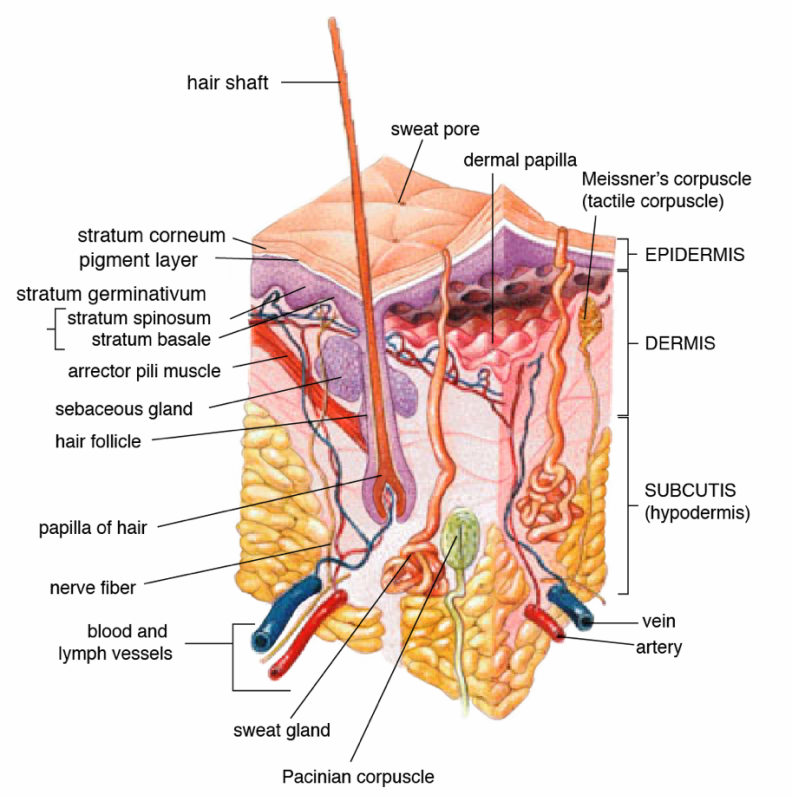

Figure 3: Cross section of all skin layers (The National Centre for Biotechnology Information, 2019)

2.3.2 Skin absorption

The skin acts as a barrier that protects the body from harmful substances in the outer environment. Nevertheless, some harmful chemicals can pass through the skin. The rates of chemical substances taken up by the skin depend on the skin properties. For example, warm, wet, sweaty and damaged skin diminishes the barrier function and facilitates chemical substance passage (International Program of Chemical Safety, 1987).

Through dermal exposure the chemical substances are transported from the outer surface of the skin into the body, passing though the tissues, eventually reaching blood vessels and ending up in the vascular system, as illustrated in Figure 4 below. Dermal absorption largely depends on the barrier function of the *stratum corneum* which is influenced by skin integrity, thickness, water content, temperature, density of hair follicles and sebaceous glands, physiochemical properties of the substance, chemical exposure concentration and duration of exposure. Sebaceous glands are microscopic exocrine glands in the skin that secrete an oily or waxy matter to lubricate and waterproof the skin (Anderson and Meade, 2014).

Chemicals of a low molecular weight with good solubility in both water and fat are able to penetrate the skin more easily than larger chemicals with a highly hydrophilic or highly lipophilic compound (Anderson and Meade, 2014). Low molecular weight means in this context less than 500 Dalton (Da, 1 Da = 1g/mol); (Bos and Meinardi, 2000).

The three most common mechanisms of absorption are via the intercellular lipid transport processes, transcellular permeation or via uptake by appendages such as hair follicles (International Program of Chemical Safety, 1987). However, the substances also move through the cell layers by passive diffusion. Passive

diffusion is a form of movement of molecular substances across cell membranes for which no cellular energy is needed (Baynes and Hodgson, 1951).

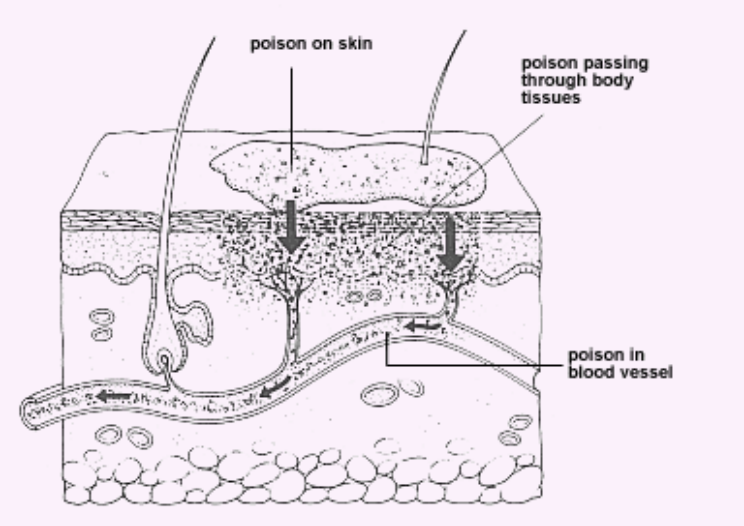

Figure 4: Dermal absorption of substances (The Chemistry of Poisons)

2.3.3 Human health risk assessment

Besides causing allergic skin reactions, dermal exposure to chemicals from textiles might also result in serious diseases and reproductive toxicity (Kaerlev *et al.*, 2005); (Schug *et al.*, 2011). The authors of an article published by the Journal of Environmental Health state that 'emerging evidence suggests that dermal exposure to some chemicals, initially thought to be safe, may result in immune, reproductive, and/or developmental effects as well as cancer, diabetes, and obesity because of their endocrine disrupting properties' (Anderson and Meade, 2014). Dermal exposure to these chemicals can occur through contact with materials contaminated with hazardous chemicals such as polycyclic aromatic hydrocarbons (PAHs) or Nonylphenol/ Nonylphenol ethoxylated (NP/NPEs). PAHs are part of the pigment carbon black, which is used in the dyeing process of

elastane, thus it derives from the dye itself. Whereas NP/NPEs are used as an auxiliary product in stabilizers for dyes. Nonylphenols are non-ionic surfactants. Nonylphenols' use as detergent has been prohibited by the European Union due to its high environmental toxicity. Nevertheless, are NP/NPEs still used as stabilizers for dyes in the textile industry, which increases the probability of its presence in cotton and elastane garments (Roos, 2019/ 2020).

In order to determine the measurement of skin absorption, the penetration rate has to be established. Once the penetration rate is known, the risk from exposure can be assessed. In the case of underwear garments, the exposure rate tends to be relatively high, since the average person wears these garment on a daily basis from the early morning till night, some people even wear their underwear during night time, leading to the assumption of an average exposure rate of 12 hours per day, day on day, week on week. Even though there may be no direct subjective symptomology, the chemicals in our clothing might have a direct impact on our health. It could take years of exposure to potentially harmful agents, even at relatively low levels in garments, in order to ensure either accumulation of agents in sensitive tissues where adversity is then triggered or to ensure constant triggering of adverse processes to a point where they become irreversible. Furthermore, the type of garment and frequency of use will naturally affect potential to cause adverse health effects. Additionally, the possibility of harmful chemicals being present in other garments besides lingerie products is presumable, leading to a multiple exposure which additionally increases health hazards (Cotgreave, 2019/2020). The consumer's exposure to such underwear garment products might have severe influences on their health since it is a potential risk of causing either local or systemic effects (Cotgreave, 2019/2020).

If a chemical has a local effect, only the part of the body, which the chemical is in direct contact with, is exposed. In the case of potentially 'toxic' lingerie, the female reproductive organs may be afflicted, since these organs are located close to the exposed body part. However, in case of a systemic effect, chemicals can not only cause harm directly to exposed organs, but also potentially all organs and functions located in the human body (International Program of Chemical Safety, 1987).

For the purpose of this thesis the argumentation is limited to the systemic scenario, as a local effect is highly unlikely since the chosen EDCs, being very lipid soluble, will probably rapidly enter the body via the skin and mucosal membranes of the vagina/ lower uterine opening, directly into the blood stream. In case of absorption via the mucosal membranes, the reproductive organs would be located too far away from the actual location of exposure, that they most likely won't have an impact on the reproductive organs (Cotgreave, 2019/2020).

Recently, a study referring to 'toxic chemicals in menstrual underwear' from the brand 'THINX' was published. The underwear has been tested positive for the presence of harmful chemicals such as per- and polyfluoroalkyl substances (PFAs), confirming once more the issue of toxic chemicals in clothing. PFAs are associated with decreased fertility, even at very low levels of exposure (Choy, 2020). 'THINX' underwear garments are made out of a nylon and elastane blend (89% Nylon, 11% Elastane), the elastane amount makes the study on 'THINX' lingerie products relevant for this work since it refers to cotton underwear garments which mostly contain an amount of elastane for higher flexibility (THINX, 2020). According to the study the crotch in the underwear garment contained 3,264 parts per million

(ppm). The Particle Induced Gamma Ray Emission (PIGE) test has been carried out by Dr. Graham Peaslee, who works as a nuclear scientist at the University of Notre Dame (Choy, 2020).

Further, a test carried out by the Commission for Environmental Cooperation, PFAs have migrated from waterproof textiles into spit, sweat and laundry wash water, confirming the leaching potential of PFAs (Commission for Environmental Cooperation, 2017). The results from those studies confirm the importance of research in the field of potentially toxic chemicals in lingerie products once more. This study however stands alone in referring to an investigation made on underwear products for women.

2.4 Fecundity

Since endocrine disruptors can disturb the hormonal balance of the human body, it is important to understand that a lot of necessary processes of the female reproductive organs are controlled by hormones. This fact underlines how enormous the impact of endocrine disruptors on women's reproductive health might be.

This chapter provides an overview about the most important hormonal processes of the female reproductive organs which are relevant for fertility. The knowledge about the reaction and development of the female sex hormones is a necessity in order to understand how the EDCs can influence fecundity.

As stated in the introduction, fecundability is defined as the probability of being pregnant in a single menstrual cycle, and fecundity is the probability of achieving a live birth within a single cycle (Berek JS, 2007) .

2.4.1 Female sex hormones

The five most important female sex hormones of the reproductive system are Follicle Stimulating Hormones (FSH), Oestrogen, Luteinizing Hormone (LH), Progesterone and Gonadotropin Releasing Hormone (GnRH) (Weschler, 2006). However, ovulation mainly involves four hormones which rise and fall over the course of a month. The FSH and the LH are produced by the brain, whereas oestrogen and progesterone are derived in the ovaries (Weschler, 2006, p. 367).

Figure 5 shows the hormonal fluctuations during the normal human menstrual cycle. Ovulation, which is characterized by a spike in levels of oestrogen (A.E.: oestrogen) and LH, occurs in the middle of the cycle. Ovulation is followed by the luteal phase, when both oestrogen and progesterone levels are high (Ihalainen, 2020).

Follicle Stimulating Hormones

The FSH is responsible for the follicle development. FSH is produced in the anterior part of the pituitary, a gland which is located between the brain stem and the hypothalamus. The produced hormones in the pituitary are absorbed by FSH receptor cells on the follicular wall. The FSH triggers a few follicles to grow and the egg inside the follicles to mature. The eggs within each follicle approaches the capacity to be ovulated (Weschler, 2006, p. 367).

Oestrogen

There are several steroid molecules which carry estrogenic activity, including oestrogen itself and its primary metabolite oestradiol. Oestrogen is responsible for maturing eggs and uterine lining. Moreover, oestrogen develops the cervical fluid. Additionally, is it in control of the maturation of female sex organs (Weschler, 2006, p. 367).

Luteinizing Hormone

The anterior picturality produces another hormone called LH, which completes the follicular growth together with FSH. Together, FSH and LH are also called the picturality or gonadotropin hormones. Eventually one follicle becomes the dominant follicle, which will be the released egg at ovulation. Ovulation is caused by the so called 'LH surge', a significant increase in LH production. The LH also triggers luteinisation which is the formation of the corpus luteum, the follicular body on the interior of the ovarian wall which is left behind by the ovulated egg (Weschler, 2006, p. 367). The human luteal phase lasts between ten and sixteen days, the average being fourteen days. A luteal phase of less than twelve days may decrease chances of a pregnancy (Marieb , 2013).

Progesterone

Progesterone is a hormone generated by the *corpus luteum*. It keeps the endometrial wall nourished and in place, hence the endometrium is able to nurture a fertilized ovum (Weschler, 2006, p. 368).

Gonadotropin Releasing Hormone

GnRH is produced in the hypothalamus as a consequence of the release of oestrogen. Stress and environmental factors may influence the lengths of menstrual cycles by affecting the hypothalamus and its production of GnRH, hence consequently also the amount of FSH and LH. GnRH causes the production of LH, which generates the development of follicles (Weschler, 2006, p. 368).

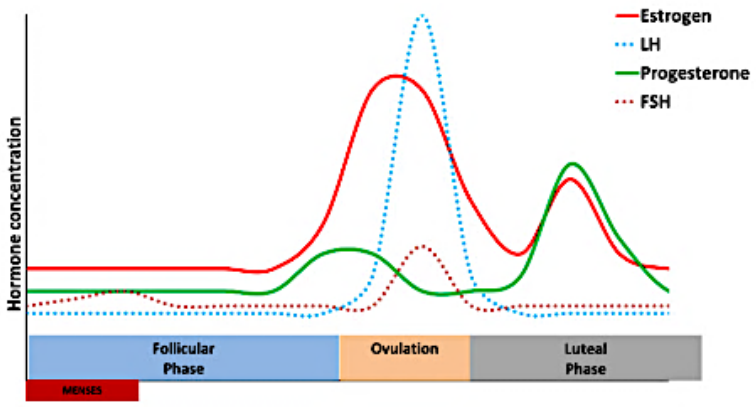

Figure 5: Hormonal fluctuations during the menstrual cycle (Senanayake, P., Potts, M. (2008).

2.4.2 The menstrual cycle

In order to provide an overview of the female reproductive system, the processes occurring during the menstrual cycle will be discussed. Therefore, the hormonal developments which occur within a prototype 28-day cycle will be analysed in chronological progression.

The menstrual cycle can be divided into two different cycle phases, preovulatory and postovulatory. The preovulatory phase, hence the processes which happen before the ovulation, includes the estrogenic, follicular and proliferative phases. Whereas the postovulatory phase, consequently all processes happening after the ovulation, consists of the pregestational, luteal and secretory phase (Weschler, 2006, p. 373).

The first day of the menstrual cycle equals the first day of menstruation. As soon as a woman starts to menstruate, the endocrine system starts producing hormones. First of all, the pituitary gland, also called hypophysis, which is an endocrine gland attached to the base of the vertebrate brain controlling other endocrine glands and influencing growth, metabolism and

maturation, creates small amounts of FSH. The FSH hormones send a signal to the ovaries, which contain the follicle with an egg, causing them to grow. The ripening of follicles results in increased oestrogen levels since oestrogen is secreted by follicles cells. The emitted oestrogen thickens the uterine lining and opens up the cervix, which is coated with a nutrient- filled fluid which protects incoming sperm.

The increased amount of oestrogen levels suppress further release of FSH, the so- called negative feedback. The increase in oestrogen levels result in a mid-cycle surge in LH which causes ovulation. The LH causes the follicle to rupture, releasing the matured egg. The so-called *corpus luteum*, which secretes progesterone and oestrogen, is left behind after the release of the egg. The progesterone supresses further release of LH. The egg is now travelling down the fallopian tube to the uterus, as progesterone levels rise, the uterine lining is built up, providing a safe environment for the implanted egg.

In case of none fertilization, the *corpus luteum* degenerates into a small white fibrous scar called the *corpus albicans*. A decline in progesterone is following, causing menstruation. The oestrogen is reduced whereas FSH increases, starting the menstrual cycle all over again (Weschler, 2006, p. 372).

2.4.3 Infertility

The World Health Organisation defines infertility as ‘a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse’ (World Health Organisation, 2018). Since this definition does not apply to single women or groups such as LGBTQ couples, doctors have changed the definition in 2017 to ‘an impairment of a person’s capacity to reproduce either as an individual or with his or her partner’ (Zegers-Hochschild *et al.*, 2017).

At least 40 % of the cases of infertility can be attributed to lack of male fecundity (Hormone Health Network, 2017). Infertility may be permanently impacted by accidental exposure to chemicals such as EDCs which change the body’s hormonal balance. If the hormonal regulation is temporally or permanently interrupted, there may be the potential to impair components of the entire reproductive system variously ovulation disorder, a damaged fallopian tube or a damaged uterine lining (Hormone Health Network, 2017).

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About 25% of women, who suffer from infertility, have infrequent or absent ovulation, which can cause irregular periods or even lead to the absence of periods. These changes in ovulation can derive from imbalances of certain hormones which are essential female sex hormones, influencing the menstrual cycle.

3 Materials and Method

In order to research into and eventually address the research question raised at the origin of this thesis, some examples of the most potentially harmful EDCs used during the dyeing process have had to be determined. Literature regarding this matter has been reviewed in order to filter out a chemical class of very high concern, with the potential to act as EDC. Furthermore, Fanny Vermandel, the vice president in Global Marketing Coloration at DyStar, a textile dye producer, has been interviewed about substances used in dyes and auxiliary chemicals during the dyeing process of cotton.

Another method is the so called 'read–across' technique, which is an approach for predicting properties of chemicals based on structural similarity. When using the read-across technique, relevant test information from one chemical ('source') is used to predict the properties for another chemical of the same group ('target substance'). Substances of a structural similarity and with similar physiochemical, toxicological and ecotoxicological properties are considered as a group. For the purpose of this thesis work, the read-across technique will be used for predicting information of one target substance by using data from another substance which belongs to the same group (Andersson, 2019).

3.1 Industry data

Industry data was retrieved to provide information about dyeing processes within the textile industry, most sold underwear products using the examples of two Swedish clothing brands, as well as testing results of underwear samples.

This work aimed at selecting two substances to be analysed in detail, one chemical substance deriving from the colourant itself and the other one from auxiliary products that are used throughout the dyeing process. PAHs were selected as a group of pigment, and NP/NPEOs were selected as auxiliary chemicals related to the colour of the lingerie.

3.1.1 Interview DyStar

An interview with Fanny Vermandel provided insight on the dyeing process within the textile industry. According to DyStar Aminoethyl ethanolamine (AEEA) is a contaminant used in auxiliary products. This is of special interest, since Fanny Vermandel assumes that this substance is still used by chemical suppliers in Asia. DyStar provides also a list of dyes which are of high relevance for this topic since they include human dangers such as reproductive toxicity and damage of fertility, these are the following dyes: Black 038, Blue 006, Red 028, Red 104 and Yellow 034 (Vermandel, 2019).

Table 1 shows the human hazards including reproductive toxicity, may damage fertility or the unborn child (H360) as well as reproductive toxic and the suspect of damaging fertility or the unborn child (H361) (RI.SE IVF , 2019).

Table 1: Overview of dyes which can cause (H360) or are suspected of causing (H361) reprotoxicity (Vermandel, 2019)

Generic	**Name**	**CAS Number**	**C.I. No.**	**Concern**	**Human danger**
Direct	Black 038		30235	carcinogenic dyestuff	H361d
Direct	Blue 006		22610	carcinogenic dyestuff	H361d
Direct	Red 028		22120	carcinogenic dyestuff	H361d
Pigment	Red 104	12656-85-8	77605	carcinogenic dyestuff	H360, H360(Df)
Pigment	Yellow 034	1344-37-2	77603	carcinogenic dyestuff, lead chromate pigment	H360, H361, H360Df

DyStar also confirmed the fact that competitor products, which contain contaminants or components which have been banned for many years, tend to still be used in Asian production countries, which emphasizes the importance of this topic once more. Fanny Vermandel confirmed that PAH, NP and NPEOs are examples of such chemicals that are still occurring. To the question of how DyStar substitutes harmful chemicals, which tend to still be used in Asian production countries, Fanny Vermandel replied that DyStar discontinues use of the harmful products and replaces them by products based on completely different chemistries. DyStar also uses alternative synthesis routes and less contaminated raw materials which lead to less by- products in the final chemical.

Fanny Vermandel also mentioned other examples of contaminants such as chlorinated phenols, toluene's and benzenes which can be generated during disperse dyestuff synthesis. However, those contaminants most likely originate from the raw material. DyStar tries to avoid contaminations by strict synthesis process, quality control and testing of critical raw materials. Moreover, are synthesis processes optimized and laboratory tests applied in order to detect contaminations (Vermandel, 2019).

3.1.2 Most sold products

Two of the most popular Swedish garments producing companies, Björn Borg and Lindex, have been interviewed about their most sold products.

Both companies offer a big section of underwear garments.

According to Björn Borg's CSR manager the 'minishort style' in cotton and polyamide in black is their most sold product (Borg, 2019).

Lindex's most sold product is also black underwear garments made out of 83% Polyamide and 17% Elastane or 79% Polyamide and 21% Elastane. The crotch lining is always made from 100% Cotton. Lindex's cotton products are made from 90 % cotton and 10 % elastane (Lindex, 2019/2020).

3.1.3 Sample testing

A well-known Swedish apparel company with a grand lingerie section offered to contribute testing samples.

Figure 6 shows the samples, which are black dyed underwear garments made from 90% Cotton and 10% Elastane; the used cotton is certified by the Global Organic Textile Standard (GOTS).

The samples are made in Bangladesh, produced within one batch and have been shipped to Europe together.

Figure 6: Underwear testing samples (Duprés, 2019)

In order to analyse the colour fastness of the testing samples, a wet and dry rub fastness test has been performed as well as a perspiration test. Both tests have been executed on the 12th of December 2019.

Colour fastness to rubbing

The test has been carried out according to the European Standard, EN ISO 105- X12:2016.

The staining was assessed visually, the used rubbing finger was of a circular shape.

In order to receive the best results, the testing has been conducted in the standard atmosphere as defined in ISO 139.

This test has been performed in order to determine the resistance of colour of the lingerie sample. Therefore, two tests have been performed, one with a dry rubbing cloth and one with a wet

rubbing cloth, each in vertical and horizontal direction (Technical Committee ISO/TC 38 and Technical Commit, 2019).

The test specimens are clamped to the baseboard of the testing device, so the long direction of the specimen follows the track of the device. For the dry rubbing, the test specimen is rubbed at a rate of one cycle per second, rubbing to and fro in a straight line 20 times, 10 times to and 10 times fro with a downward force of 9 N, as demonstrated in Figure 7 below. Any extraneous fibrous material is removed for an exact rating.

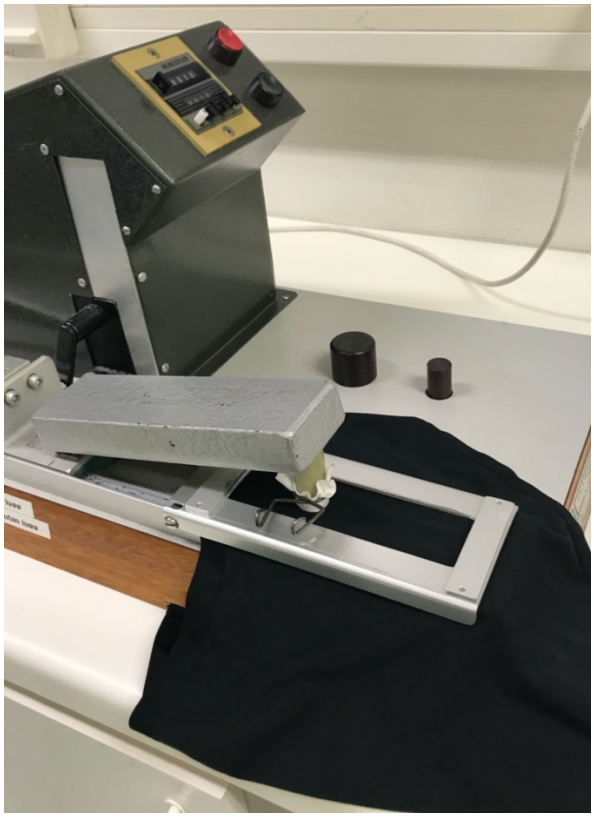

Figure 7: Dry rubbing test with lingerie specimen (Duprés, 2019)

For the wet rubbing, the rubbing cloth has to be prepared. The piece of cloth is soaked in distilled water and reweight to ensure a take up of 95% to 100%. Then, the same procedure, which has been applied to the dry rubbing, is applied to the wet rubbing test.

Afterwards the rubbing cloth are air dried (Technical Committee ISO/TC 38 and Technical Commit, 2019).

Table 2 presents the results from the rubbing test, especially the results for dry staining are very good, which proof the colour fastness in dry state with a value from 4-5, where 5 is the best.

The results for wet staining always tend to be worse than for dry staining, nevertheless, are the results in total satisfying (Rockström, 2019).

Table 2: Colour fastness to rubbing, rating. Scale 1-5, where 5 is the best (Duprés, 2019).

Test material/ Colour	**Dry staining**		**Wet staining**	
	Length	Width	Length	Width
Black	4-5	4-5	2	2

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Colour fastness to perspiration

Colour fastness to perspiration was determined according to SS-EN 105- E04:2013 standard. The lingerie specimen are treated in two different solutions, one alkaline solution and one acid solution. Both solutions were freshly prepared, the alkaline solution contains per litre 0.5 g of L- histidine monohydrochloride monohydrate ($C_6H_9O_2N_3$ HCL H_2O) and 5g of sodium chloride (NaCl). The solution is brought to pH 8 with 0.1 mol/l sodium hydroxide solution (Technical Committee ISO/TC 38 and Technical Commit, 2019).

The acidic solutions contains per litre 0.5g of L- histidine monohydrochloride monohydrate ($C_6H_9O_2N_3$ HCl H_2O), 5g of

sodium chloride (NaCl) and 1.95g of sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_42H_2O$). The solution is brought to pH 5.5 with 0.1 mol/l sodium hydroxide solution.

In an attempt to determine the colour fastness to perspiration, a total of eight specimen have been tested, whereas four have been treated with the acidic solution and the other four with the alkaline solution since sweat can be both, acidic or alkaline, depending on the geographical location and human health (Rockström, 2019).

After the specimen have sat in the flat- bottomed dish, containing the solution, for 30 minutes under room temperature, the solution is poured off and excess liquor is wiped off the specimen between two glass rods. Then, the specimen will be placed into test devices, whereas the specimen from the acidic and alkaline solutions will be put in two separate test devices.

The test devices, showed in Figure 8, consist of a frame of stainless steel into which a weight- piece of mass of 5kg and base 60 mm x 115mm is closely fitted, so that a pressure of 12.5 kPa can be applied on the test specimens measuring 40 mm x 100 mm. The specimen are placed between glass plates measuring approximately 60 mm x 115 mm x 1.5 mm (Technical Committee ISO/TC 38 and Technical Commit, 2019).

Figure 8: Test devices in which the specimen are placed between (Duprés, 2019)

The test devices, containing the specimen, are placed in the oven for four hours at 37 ° C degrees.

Table 3: Colour fastness to perspiration (Duprés, 2019)

Test material/ Colour	Staining												Change in colour
	Triacetate		Cotton		Polyamide		Polyester		Acrylic		Viscose		
	pH 5.5	pH 8.0	pH 5.5	pH 8.0	pH 5.5	pH 8.0	pH 5.5	pH 8.0	pH 5.5	pH 8.0	pH 5.5	pH 8.0	
Black	4-5	4-5	5	5	5	4-5	5	4-5	5	5	4-5	4-5	4-5

Table 3 shows the rating from the colour fastness test to perspiration. The rating varies from 1 to 5, whereas 5 is the best. Since the test results only vary between 4 and 5, the outcome is very satisfying.

The test results show that the lingerie samples have a strong colour fastness which leads to the overall assumption of a low leaching potential of the dye stuff since the dye seems to be bound to the fibre quite strongly.

Nevertheless, is future research required in order to proof the release of any EDCs from the dye stuff. Direct analysis of PAHs and Nonylphenols might be a possible activity in the future.

3.2 *In-silico* modelling

The term '*in-silico*' is used in regard to research which has been performed on a computer, as opposed to *in-vitro* (in cells or organs) and *in- vivo* (in intact organisms) (Quinion, 1996).

In furtherance of determining the properties of chemical substances, an *in-silico* analysis of the chemical substances has been run, testing tumorigenic and reprotoxic behaviour as well as skin irritating properties.

Firstly, a list with harmful chemicals has been created, summarizing more than 50 chemicals with potential endocrine disrupting properties which might cause adverse health outcomes in humans.

After consultation with Ian Cotgreave, who is specialized in human toxicology, a gross list has been created, including chemicals which fulfilled the following criteria's:

Reprotoxicity, presence in the cotton dyeing process, substance of very high concern.

The Gross list includes the following substances:

Nonylphenols, aryl amines which are banned from cleavable azo dyestuffs, Lead, Copper and Polycyclic aromatic hydrocarbons (PAHs).
This list has been used to derive the target substance group, Nonylphenols and Polycyclic aromatic hydrocarbons.

Furthermore, every chemical substance listed has been associated to the simplified molecular-input line-entry system (SMILES), which describes the structure of the chemical substance.

After consultation with Ian Cotgreave, the following substances have been filtered out as the most potentially harmful to the reproductive system: 4- Nonylphenol, 2- Nonylphenol, 4- (2,6-Dimethyl heptyl) phenol and Lead di (acetate).
The nonylphenols are all xenoestrogens and may act on fecundity. Moreover, do these substances have good exposure data, both in animals and humans (Cotgreave, 2019/2020); (ECHA, 2013).

In facilitating this read-across approach, various *in-silico* (computer-based) predictive models were employed to characterize potential hazard alerts which may be associated with particular chemical groups chose to study. This is rapidly becoming an important part of modern risk assessment, and there are many commercial and open-source software's with specific prediction modules, selective for various toxicity hazards, such as elicitation of oestrogen like activity, effects on reproduction etc, both relevant to the present work (Cotgreave, 2019/2020). The *in-silico* software has run an analysis of 4- Nonylphenol with the following models:

- Developmental Toxicity model (CAESAR) 2.1.7
- Developmental/Reproductive Toxicity library (PG) 1.0.0

- Oestrogen Receptor Relative Binding Affinity model (IRFMN) 1.0.1
- Oestrogen Receptor-mediated effect (IRFMN/CERAPP) 1.0.0
- Skin Sensitization model (CAESAR) 2.1.6
- Hepatotoxicity model (IRFMN) 1.0.0

Additionally, those chemical structures have also been compared in the quantitative structure- activity relationship analysis (QSAR).

Lead or lead acetate disturbs follicular implantation and is well established from this perspective (Al-Juboori, Hamdan and Al-Salihi, 2016).

After all, lead or lead acetate are not commonly found in textile garments due to high regulations hence they have been excluded from further research (Roos, 2019/ 2020).

Furthermore, are lead/ lead acetate due to their in- organic property out of scope of predictive models since the models have not yet been trained on in- organic compounds.

3.3 Literature data

The data was obtained using different databases such as

Scopus, Google Scholar, Research Gate, Web of Science, National Centre for Biotechnology Information and Bielefeld Academic Search Engine (BASE).

In contemplation of finding appropriate books, reports, journal articles or websites, the terms mentioned in the table below have been combined into search phrases.

Table 4: Literature data overview

Keywords	Database	Date
Benzo[a]pyrene	Google scholar	January 2020
Decreased fertility	Google scholar	November 2019
Endocrine disrupting chemicals	Scopus	September 2019
Environmental toxins	Google Scholar	December 2019
Exposure rate	Web of Science	January 2019
Fecundity	Research Gate	September 2019
Fecundability	Google Scholar	November 2019
Fertility	Scopus	September 2019
Health risk assessment	Scopus	October 2019
Human toxicity	Google Scholar	September 2019
Nonylphenol	ECHA	January 2020
4- Nonylphenol	ECHA	January 2020
Nonylphenol ethoxylated	ECHA	January 2020
Reprotoxicity	Scopus	December 2019
Reproductive organs	Web of Science	November 2019
Reproductive health	Google Scholar	October 2019
Polycyclic aromatic hydrocarbons	BASE	January 2020
Skin anatomy	Google scholar	November 2019
Substances of very high concern	ECHA	September 2019
Textile dyeing process	Research Gate	October 2019

Moreover, exclusion criteria has been applied conductively in order to find the most suitable literature.

Hence, the literature must include research about black cotton lingerie, endocrine disruptors, chemical substances which are used throughout the textile manufacturing process. Additionally, it should include risk assessment of chemical substances with endocrine disrupting properties, consequently the primary focus lies on the study of female reproductive organs in order to determine the risk of infertility.

4 Results and Discussion

The media has placed considerable focus on the uptake of hazardous chemicals from industrial produced products such as Bisphenol A in plastic products (Bergman *et al.*, 2012).

Although most scientific literature in this area addresses oral uptake, it is not the only route of exposure to such substances and plastic products are not the only source of exposure.

Harmful chemicals with endocrine disrupting properties, derived from finished textile products, represent an immense hazard for human health. The considerable potential exposures, due to the daily skin contact of underwear garments with the body, strongly suggests this topic important to research in the future.

This thesis work has developed a theoretical framework for a risk assessment of EDCs commonly present in black lingerie products.

More than 50 chemical compounds have been identified by literature searching as being a potential health hazard.

After consultation with an experienced toxicologist, Ian Cotgreave, 4 out of the 50 EDC substances have been analysed for their relationship to reproductive toxicity, using state of the art *in-silico* tools. These investigations have been initially focussed on Nonylphenols (NP/NPEs), but could be extended to other classes of relevant chemicals such as PAHs.

4.1 Testing results

This chapter summarizes the results of the in-silico investigations of various NPEs and lead acetate.

The models used are largely based on quantitative structure-activity relationships (QSAR) between structure and a particular hazard and include suites specifically for reproductive toxicity and hormone disruption. The *in-silico* predictive models confirm oestrogen-mimetic activity for the 3 NPEs studied, and toxicity alert for reproductive toxicity (not specifically related to fertility changes) were revealed. Lead acetate was not found to be in scope for the predictive models utilized.

Despite this concordance with the general literature on the EDC nature of the NPEs tested, human hazard prediction for these agents could not be confirmed by interrogating the ECHA database, which publishes a report for each chemical substance of very high concern. This is likely due to the fact that ECHA did not include the potential human health hazard in their report, largely basing their definitions on environmentally derived effects, perhaps due to the lack of information on relevant human exposure, such as via clothing (ECHA, 2018).

This indicates a research gap and should strongly motivate future research of this matter.

4.2 Toxicity data

This chapter summarizes the relevant toxicity data of NP/NPEs and PAHs, with respect to hormone disruption.

Both classes contain members which have been shown to act via endocrine disruption (Agency for Toxic Substances and Disease Registry, 2012); (ECHA, 2012a). NP/NPEs end up in garments due to their use as auxiliary products, mostly as stabilizers in dyes and as surfactants in textiles processing (Roos, 2019/ 2020); (U.S. Environmental Protection Agency, 2010).

PAHs are a component of the carbon black pigment; thus, PAH is the chemical compound deriving from the dye itself.

4.2.1 Polycyclic aromatic hydrocarbons

It is important to mention that cotton lingerie products are often blended with elastane, such as in the tested underwear samples, which have been made out of 90% cotton and 10% elastane.

The use of Elastane introduces PAHs into the material, which is a group of chemicals considered to contain many carcinogenic and reprotoxic members (Agency for Toxic Substances and Disease Registry, 2012).

PAHs belong to the group of hydrocarbons and are produced by incomplete combustion or high pressure processes under high temperatures (Agency for Toxic Substances and Disease Registry, 2012).

As stated above, example PAHs have not been analysed with the *in- silico* software utilized in this work but have been intensively studied by the use of literature data.

The rationale for the selection of PAHs as the second group of potentially harmful agents is its presence in the carbon black pigment, which is used for dyeing. Hence, the analysis of PAHs

as a contaminant in carbon black creates a link to the colour of textiles, referring to the potentially higher human health risks of black textile garments.

In the textile industry, carbon black is used as a pigment for the dyeing process of Elastane (Roos, 2019/ 2020).

Industrially manufactured carbon black is produced by pyrolysis of hydrocarbons at high temperatures under controlled process conditions. Pyrolysis denotes the process of decomposition caused by high temperatures and an absence of oxygen.

Unfortunately, it is clear that this process will result in the formation of organic impurities such as PAHs (Bergman *et al.*, 2012). Thus, carbon black pigments all tend to contain PAHs.

As components of synthetic dyes PAHs are also present as contaminants in textile dyeing sludge due to the recalcitrance in wastewater treatment processes, which may pose a threat to environment in the process of sludge disposal (Ning *et al.*, 2014).

Moreover, PAHs are amenable to considerable dermal exposure and transdermal uptake, due to their hydrophobicity.

If the washing process should not be carried out appropriately, for reasons such as the production of less toxic wastewater, the PAHs might still be present in the final garment and may result in considerable dermal exposure.

Thus, these facts all indicate that PAHs in carbon- black coloured lingerie may present risks for adverse health outcomes in women (Agency for Toxic Substances and Disease Registry, 2012); (Bergman *et al.*, 2012). This will of course depend on the leaching rates, exposure levels and duration of exposure.

Nevertheless, depends the probability of adverse health outcome on the exposure rate and the extraction of the PAHs from the carbon black particles. The adverse health outcomes can only be caused if those PAHs leach easily from the carbon black.

As a corollary, an investigation at the University of Düsseldorf tested if PAHs would leach from the carbon black surface if it came into contact with body fluids, leading to an interaction with the tissue and possible uptake of PAHs into the human body.

The study showed that the PAHs did not leach from the carbon black surface when it came into contact with artificial lung fluid.

According to the trade manufacturer ’Cabot Corporation’ carbon black is embedded and bound into a polymeric matrix, for example ink, which would make migration out of the final product impossible (Cabot, 2019).

However, the study, conducted by the University of Düsseldorf, only refers to pulmonary exposure in the lining fluid when referring to the non- leaching behaviour of PAHs. The lung lining fluid is aqueous whereas PAHs are very lipid- soluble. This property of PAHs could possibly lead to direct penetration of the dermis.

Hence the probability of exposure to PAHs through carbon black pigments in lingerie products cannot yet be fully appraised, but future research into this question is clearly necessary.

4.2.2 Benzo[a]pyrene

The following section focuses on the impact PAHs can have on the human reproductive system using Benzo[a]pyrene as a prototypical PAH toxicant.

Benzo[a]pyrene is considered to be amongst the most toxic PAH compound (Cave *et al.*, 2010). Benzo[a]pyrene is listed as a hazardous substance, additionally it is ranked 8th out of 275 chemicals on the Priority List of Hazardous Substances for CERCLA, Comprehensive Environmental Response, Compensation, and Liability Act (Agency for Toxic Substances and Disease Registry, 2009).

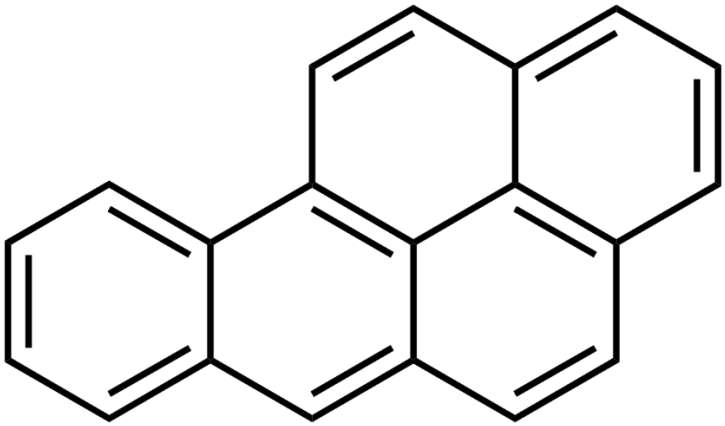


Figure 9: Chemical structure of Benzo[a]pyrene (Agency for Toxic Substances and Disease Registry (ATSDR), 2012)

Figure 9 shows the chemical structure of Benzo[a]pyrene, containing five fused benzene rings.

Human studies on Benzo[a]pyrene report developmental, neurobehavioral, reproductive, and toxicological effects, which all provide qualitative evidence for hazards associated with benzo[a]pyrene exposure (Burgoon *et al.*, 2017).

However, Benzo[a]pyrene is primarily classified as carcinogenic (EPA, 1993).

In their report on Benzo[a]pyrene Burgoon *et al.* (2017) state that ’carcinogenicity studies in animals by the dermal route of exposure are available for benzo[a]pyrene and are supportive of the overall cancer hazard’.

Routes of exposure include ingestion, inhalation (especially cigarette smoke) and dermal contact. Animal studies demonstrate various effects including changes of the reproductive organs and the hormonal system deriving from the above mentioned routes of exposure of PAHs (Burgoon *et al.*, 2017); (Ning *et al.*, 2014).

Carcinogenicity potency estimates for PAHs have been published for the oral and inhalation routes of exposure. However, no such data been established by a regulatory agency for dermal exposures (Hussain *et al.*, 1998); (Tolbert, 1997).

It is interesting to note, however, that skin painting experiments with PAHs, where both local and systemic effects are feasible, record tumours both in the skin and in other organs, supporting the idea that PAHs can be absorbed into the body via the skin and produce their adverse reactions distally from the point of absorption (Godschalk *et al.*, 1998); (Knafla *et al.*, 2006).

This supports the idea that absorbed PAHs from lingerie/carbon black may also be able to elicit hormone disruptive effects in the body via systemic exposure after dermal absorption.

4.2.3 Nonylphenols/ Nonylphenols ethoxylated

Nonylphenols and Nonylphenol ethoxylated (NP/NPEs) are organic compounds consisting of phenols with a 9-carbon tail, the chemical structure is shown in Figure 10 below (Soares *et al.*, 2008). The degradation product of Nonylphenol ethoxylate is Nonylphenol, which causes harmful effects to the environment (Soares *et al.*, 2008).

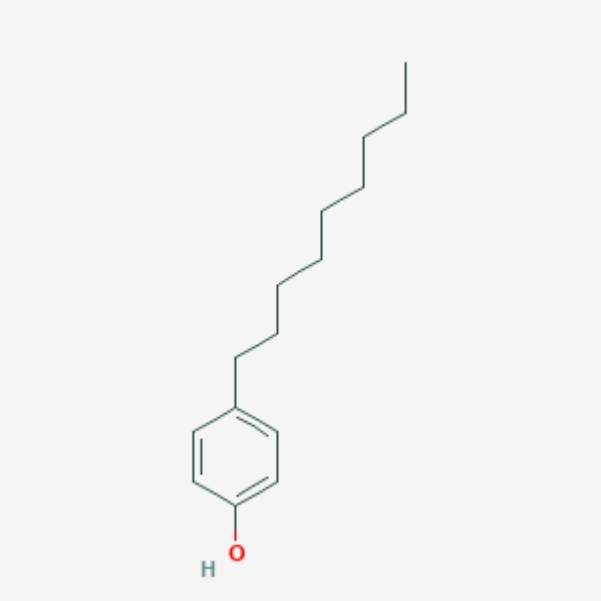


Figure 10: Chemical structure of 4- Nonylphenol (PubChem, 2019)

Due to their hazardous nature, the use and production of NPs has been banned in the EU, as well as other countries like Japan and Canada (ECHA, 2018); (Soares *et al.*, 2008).

In the EU, nonylphenols used for production are being slowly replaced by alcohol ethoxylates, which are able to degrade faster than nonylphenols, causing less pollution to the environment.

NPEs are also persistent and subject to poor degradation in the environment (Organisation for Economic and Development, 2019).

Despite this, NPs are almost certainly still being used in Asian production countries, where 80% of textile garments are still produced (Luongo, 2015).

Nevertheless, neither are NPEs able to achieve full degradation, resulting in the production of hardly degradable substances (Organisation for Economic and Development, 2019).

Neither, NPs nor NPEs, occur in the environment naturally and are released into nature only by human activity (Organisation for Economic and Development, 2019).

The main release sources of NPs/NPEs in 1996, according to a survey carried out by Environment Canada/ Health Canada were: Into rivers by surfactant producers (25-60 tons/year) and into air, land surface or underground milieu, by industrial users of detergents (25-60 tons/year). Moreover, paint producers are a considerable source of release (Organisation for Economic and Development, 2019).

The total release of NPEs has been estimated at about 108 tons/day in the EU, according to a survey conducted by the European Commission, with 50% of the total release being caused by industrial users of detergents. The textile industry is estimated to release 15% total NPEs (Organisation for Economic and Development, 2019).

NP/NPEs are used in the production of paints, plastics, coatings, detergents and in textile processing formulations (U.S. Environmental Protection Agency, 2010).

In the European Union NP/NPEs are no longer allowed to be used as detergents, but are they still used as a stabilizer in dyes (Roos, 2019/ 2020).

NP/NPEs are identified as a substances of very high concern (SVHC) by the REACH regulation, due to their endocrine disrupting properties (ECHA, 2012a); (REACH entry 46a).

Nonylphenols are also known to give rise to cancer and reproduction hazards in animals, as well as humans (Luongo, 2015).

As stated above, in their classification by the EU, there is strong scientific evidence for endocrine- mediated adverse effects for NP/NPEs in fish species. These studies also reveal effects on fecundity, as well as sexual development and growth, especially in female populations (ECHA, 2012b). This assessment is also compounded by the chemical stability of these agents, and their ability to accumulate in the environment.

Moreover have epidemiological studies shown its ability to act with oestrogen- like activity, making NP a xenoestrogen (ECHA, 2012b).

Xenoestrogens belong to the group of xenohormones which show oestrogen- like properties and can interfere within normal hormone function.

This group of chemicals includes Bisphenol A (BPA), polychlorinated biphenyls (PCBs) and phthalates (Bergman *et al.*, 2012).

The estrogenic effects of xenoestrogens on a living organisms, including humans, are due to the ability of the agents to mimic the normal action of endogenous oestrogen, via structural similarity (ECHA, 2018).

By mimicking the effects of endogenous oestrogen, xenoestrogens can lead to severe disorders of the reproductive system (Aksglaede *et al.*, 2006).

Indeed, clinical and experimental data have shown that xenoestrogens possibly lead to impairment of female reproductive functions.

Further, epidemiological data correlates exposure to xenoestrogens and the development of polycystic ovaries, which is the most common cause of infertility in women (Götz, 2001); *(*Khan*, et al.*, 2019).

Moreover, nonylphenol has been detected in human breast milk, blood, and urine, according to a report by the U.S. Environmental Protection Agency (EPA) on Nonylphenol is it also associated with reproductive and developmental effects in rodents (U.S. Environmental Protection Agency, 2010).

The European Chemical Agency (ECHA) has investigated the hazards presented by 4- nonylphenol has on the environment and to animals in their report. However, as stated previously, the report lacks a human health risk assessment.

The report indicates that 4- nonylphenols are indeed endocrine active in invertebrate species also, however this assumption cannot be currently confirmed due to a lack of knowledge about the exact endocrine mechanisms in invertebrates and test systems (ECHA, 2012a).

The presence of nonylphenols in dyes is concerning due to their xenoestrogenic properties. The lack of research on the human health hazards due to chemical residues from textiles including compounds such as nonylphenols emphasises the importance of further investigations into this matter.

4.3 Risk assessment

Risk assessment aims at assessing chemicals in order to indicate adverse effects which may not be obvious to an unskilled observer, such as consumers of textile garments.

According to van Leeuwen and Vermeire (2007), the method of risk assessment of chemicals consists of four steps, starting with the hazard identification (van Leeuwen and Vermeire, 2007).
The hazard identification process helps defining potential hazards such as the use of chemicals known to be toxic.
This thesis work has identified hazards by searching for chemicals which are known to potentially act as an endocrine disruptor. Therefore, a list with more than 50 chemicals has been created, which eventually has been used to derive the target substance group, Nonylphenols and Polycyclic aromatic hydrocarbons.

The second step in the risk assessment of chemicals is the so called exposure assessment (van Leeuwen and Vermeire, 2007). The word 'exposure' is defined as a concentration or amount of a particular stressor that reaches an individual target or population at a specific frequency for a defined duration (van Leeuwen and Vermeire, 2007).

In the third part of risk assessment of chemicals, called the dose-response assessment, the effects for risk assessment purposes are quantified.
Effects are defined as changes in an individual or population caused by exposure to a stressor (van Leeuwen and Vermeire, 2007). Adverse effects include reduction of survival, growth and reproduction, which environmental species potentially

experience due to the exposure to specific chemicals (Stephan, 1986).

The purpose of the fourth part of the chemical risk assessment, the risk characterization, is to evaluate the risk deriving from the exposure to chemicals. The risk is evaluated by comparing the predicted environmental concentration (PEC) and the predicted no risk environmental concentration (PNEC). If the PEC is higher than the PNEC, that is in the case of the quotient being higher than one, it indicates risk (van Leeuwen and Vermeire, 2007).
This ratio is also applicable to textiles, speaking that if the predicted concentration of the chemical in the textile is higher than the predicted no risk concentration of the chemical in the textile, a risk is given.

Due to time limitations for the creation of this thesis, a dose-response assessment could not be performed.

4.3.1 PAHs/ NPs content in samples

The measured amounts of PAHs/NPs in this test will lead, together with the times of exposure, to the beginnings of an exposure scenario, which will hopefully be investigated in future research.

The test has been conducted in order to determine the content of PAHs/ NPs in the black cotton underwear sample, the same samples which have also been tested on colour fastness.
The tests have been carried out on the 14th of January 2020 in the laboratory at the Research Institute of Sweden, in Mölndal, according to OEKO-TEX®.

In order to determine the PAHs content, the sample is put in an ultrasonic bath with an organic solvent, followed by detection with a gas chromatography–mass spectrometry (GC-MS), the quantification has been carried out with certified reference material. The limit of quantification is 0.20 mg/kg.

23 different PAHs, where 9 of them are identified as a substance of very high concern (SVHC), have been tested, all resulting in a negative outcome, below the limit of quantification.

The same testing procedure applies to the alkylphenols and alkylphenol ethoxylated compounds, only that they have been additionally detected with a liquid chromatography–tandem mass spectrometry (LC-MS/MS).

NP/NPEs was quantified using the technical mixture Igepal Co-720/ Imbentin- N/060. The test results in the same outcome, all alkylphenols as well as alkylphenols ethoxylated are below detection limit, which is 20mg/kg for NP/NPEs and Octylphenol/ Octylphenol ethoxylated, 2.5 mg/kg for 4-tert-butylphenol, 4-pentylphenol, 4-heptylphenol, 4-octylphenol, 4-nonylphenol.

Despite the negative results, substances of very high concern are frequently found in imported textile products (Swedish Chemicals Agency, 2016).

The possibility of finding any content of hazardous chemicals in garments which have been imported to Sweden from serious suppliers has been low, thus the negative results are not surprising. Nevertheless, is the presence of harmful chemicals in textile garments still a problem which has the potential of leading to adverse health effects in humans.

4.3.2 Adverse health effects

The work described in this thesis has mainly been based on fecundity in regard to adverse health effects deriving from dermal exposure to black textile underwear garments.

PAHs and NP/NPEs have both been the subject of risk- related studies.

Studies in humans and animals provide evidence for PAH- and Benzo[a]pyrene- to induce male and female infertility. However, refers this study to highly exposed human populations who are exposed to B[a]P by inhalation of cigarette smoke or due to their occupation (here: coke- oven workers); (Hsu *et al.*, 2006); (Soares and Melo, 2008).

Nevertheless, is it difficult to ascribe adverse health effects in epidemiological studies to specific PAHs because most exposures occur to PAH mixtures (Agency for Toxic Substances and Disease Registry, 2012).

A large body of mechanistic data, including *in-vivo* and *in-vitro*, leads to the overall assumption that PHAs, especially referring to Benzo[a]pyrene, impact fertility through the disruption of folliculogenensis, the process of maturation of the ovarian follicle.

A study on humans could link the amount of B[a]P with a decreased ability to conceive, B[a]P levels were quantified in the serum and follicular fluid of women who are exposed to mainstream smoke (Neal *et al.*, 2007).

Studies on female rats have shown altered oestrous cyclicity and hormone levels through inhalation or oral exposure to B[a]P (Burgoon *et al*., 2017).

Further animal studies indicate fertility-related effects such as decreases in ovarian follicle populations and decreased fecundity (Burgoon *et al*., 2017).

Furthermore, has a decreased amount of oestradiol in rats been reported as a consequence of orally exposure and inhalation of B[a]P, leading to altered oestrous cyclicity.

Oestradiol is the primary metabolite of oestrogen, which is responsible for maturing eggs and uterine lining (Kummer *et al.*, 2008).

Another research proposal suggests the impairment of folliculogenensis from reactive metabolites or through a decreased sensitivity to FSH- stimulated follicle growth after the exposure to B[a]P (Takizawa *et al.*, 1984); (Mattison and Thorgeirsson, 1979); (Neal *et al.*, 2007).

Another assumption is the interaction of B[a]P with oestrogen receptors (ER) (Kummer *et al.*, 2008).

In order to receive exact toxicity data regarding the dermal exposure to B[a]P via black garments an exposure scenario must be known, which will hopefully be researched in the future.

In conclusion, the toxic effect on fecundity associated with B[a]P exposure are also relevant to the dermal uptake by humans through garments since the exposure rate might be relatively high through multiple exposure to different garments on a daily basis, triggering imbalances of the hormone system, potentially leading to a decrease in fecundity.

As already mentioned in the previous chapter of ‘Human health hazard’ has the impact of nonylphenol on human health not been investigated yet. ECHA states in their report on NPs/NPEs, where the identification as a substance of very high concern is justified, that the human health hazard has not been assessed (ECHA, 2012b).

However, have studies of fish species shown that there is strong evidence for endocrine mediated adverse effects. Results for amphibians provide indication that effects could have been caused by an oestrogen-like mode of action (ECHA, 2012b).

The exposure to 4- Nonylphenol has the potential to reverse sex in female fish populations as studies have shown (ECHA, 2012b). Further, have the studies investigated that exposure during sensitive life stages could possibly result in life long effects, even impacting following generations, adverse health outcomes might even occur after short term exposure and are likely to cause systemic exposure (ECHA, 2012b).

Based on the available mechanistic information it can be concluded that 4-nonylphenol possesses the potential to exert oestrogen-like effects and disrupt endocrine homeostasis, which is the state of steady internal physical and chemical conditions, *in-vivo* (ECHA, 2012b). Further has been examined if 4-nonylphenols potentially bind to the oestrogen receptor. ECHA has analysed 7 different studies to clarify this matter, all 7 studies have shown that NPs displace specifically bound 17β-oestradiol (E2) from the oestrogen receptor (ER) (ECHA, 2012b). The binding of 4- nonylphenols to the ER leads to activation of the ER- mediated pathway and consequently to transcriptional activation of typically oestrogen- responsive genes (ECHA, 2012b).

Another study, published in the Journal of Molecular Endocrinology, showed that 4-nonylphenol was sufficient to displace 50 % of specifically bound E_2 (Petit *et al.*, 1997).

Interestingly, was the affinity of NP to the human oestrogen receptor higher than the one for the oestrogen receptor in fish, which again leads to the assumption that the exposure to

NP/NPEs can potentially be a reason for a decline in fecundity in humans (Tollefsen e*t al*. 2002).

Consequently, several *in- vivo* studies have proven the oestrogen like activities of 4- nonylphenol, resulting in a disrupt endocrine homeostasis.

In conclusion, Benzo[a]pyrene as a prototypical PAH toxicant, as well as Nonylphenols, represented by 4- Nonylphenol, have been identified as EDCs, acting on important hormone systems involving oestrogen, the most important hormone for folliculogenensis, immediately influencing female fecundity, and by showing oestrogen like activities.

Moreover, are both compounds chemically suited to dermal absorption and systemic distribution.

It is therefore plausible that dermal exposure to B[a]P and 4-Nonylphenol through textile garments could lead to decreased fecundity.

4.4 Exposure level

The exposure level is one of the two basic components of any risk assessment.

In order to determine the risk of black underwear garments, the exposure rates to target chemicals must be established. As we have already performed a hazard assessment for two chemical groups with endocrine disrupting properties, the risk can only be evaluated properly once the exposure rates to these are known. Due to practical and time limitations in this work, a quantitative analysis of the exposure rate is not possible, but some theoretical considerations are.

Women are exposed to chemicals on a daily basis, ranging from heavy metals such as mercury and lead to toxin pollutants in the air (Choy *et al.*, 2002). Exposure to endocrine disrupting chemicals via lingerie may represent yet another risk for negative impact on fertility, especially due to the potentially chronic nature of the exposure.

In the case of exposure to harmful chemicals from textile garments, the effects are influenced by the concentration of element in clothes, the cloth surface density, the area of contact between the cloth, the fraction of substance migrating to the skin per day, the fraction of contact area for skin, the penetration rate of the chemical, the contact duration between cloth as well as the mean number of events per day (ECHA, 2012), (BfR, 2012).

In regard to underwear, a daily exposure level is presumed which, coupled to dermal absorption, may allow an internal dose estimation, if appropriate blood level measurements were to be available. Moreover, the exposure level per day is likely to be quite long, since underwear are worn for an average of 12 hours. The concentration of elements have been investigated in samples testing for the purpose of this thesis.

4.4.1 Relevant exposure levels

In order to rationalise the extent to which exposures may need to occur to PAHs and NP/NPEs in order to define a clear risk for impact on fecundity, a survey of available animal experimentation data was performed.

According to one study in female rats published in the journal 'Biology and Reproduction', fecundity was reduced by 35% if female adult rats were orally exposed to a dose of 160mg/kg body weight B[a]P daily (Mackenzie and Angevine, 1981).

Whereas in another study, in which female rats were exposed to 10 mg/kg body weight daily by oral intubation for 9 days, no effects on fecundity were recorded (Kristensen *et al.*, 1995).

This might suggest rather facilely that the no observed adverse level (NOAEL) may lie between these two dose values, but the studies are no equivalent in terms of dose term and other important factors.

A similar argument can be applied to NPs/NPEs, but this time using available human exposure estimates.

As stated previously the main sources for the exposure of NP to the human population are via industrial produced products such as detergents, cleaners, agricultural and indoor pesticides, food packaging and cosmetics. Moreover, NP has been confirmed to be present in breast milk (Ademollo, et al., 2008), umbilical cord blood (Chen, *et al*., 2008) and urine (Carwile *et al.*, 2009).

Human exposures have been estimated in microgram per body weight daily for several of the above-mentioned sources:

Hair dye – 0.1µg/kg/day, food container- 2µg/kg/day, indoor pesticides- 0.35 µg/kg/day, environmental sources 5 µg/kg/day with 70% – 80 % of this due to fish and shellfish consumption (EU, 2002).

Interestingly, some data has shown extremely high human exposure at 4.4 mg/kg/day from living near a textile factory that used NP and NPEs (EU, 2002).

The potentially high exposure to NP/NPEs from this factory underlines the importance of analysing the health hazards from the garments which have been produced in such industries. Textile garments are not just intimately in contact with the body, but exposures are likely over long periods of time. Furthermore, there is the probability that most garments are treated with a high amount of different chemicals, leading to multiple exposures of the individual, and the potential for additive and/or synergistic hazards.

4.4.2 Multiple exposure

On the subject of multiple exposures, one study in mice comparing single and combined exposures to B[a]P and lead, clearly demonstrated synergy with respect to effects on fecundity (Kristensen *et al.*, 1995).

Experimental evidence suggests that inorganic lead and B[a]P suppress the development of primordial oocytes during early fetal life. However, Mice exposed to both lead and B[a]P had a significantly longer gestation period compared to mice exposed only to B[a]P or lead (Kristensen *et al.*, 1995).

It remains to be determined if these data are relevant for the situation with potential exposure to these chemicals in garments.

5 Conclusion and future research

The primary aim of this thesis work was to contribute to the risk assessment of EDCs deriving from dark-coloured underwear garments by analysing the potential dermal exposure to EDCs in cotton lingerie and determining their potential influence on women's fecundity.

Substances identified as EDCs present in black lingerie, PAHs have been focused on, and B[a]P as archetypal example, as well as NP/NPEs, have been studied in depth.

In addition to literature review, testing on lingerie samples has been performed. The samples have been tested with respect to their colour fastness to rubbing and perspiration, in order to determine how strongly the dye is bound to the textile fibre.

Moreover, the samples have been analysed for their PAHs and NP/NPEs content.

The chemicals' overall potential impact on the reproductive system is suggested by *in- vivo* studies, which however focus on the oral rather than the dermal route of exposure.

Thus, the relationship between dermal up take and EDCs from textiles could not be proven since most literature data based their results on inhalation or oral exposure. Nevertheless, dermal uptake potentially provides an important route of exposure, which might indeed, depending on the chemical's molecular properties, result in the penetration of harmful chemicals, causing systemic effects.

This thesis mainly investigated the following aspects:

- The EDCs deriving from an auxiliary product used in the dyeing process is represented by NP/NPEs.
  These compounds are still used as stabilizers for dyes in the textile production processes, possibly remaining in the garment due to neglected washing processes

- PAHs are present in carbon black pigments as contaminants. PAHs represent the EDC deriving from the dye stuff itself

- The used *in- silico* software confirmed the possibility of NP/ NPEs acting as endocrine disruptors. However, a human hazard, related to fecundity and any other adverse outcome could not be confirmed by literature searching

- It is feasible that systemic rather than local exposures may underly potential adversity on fecundity following dermal exposure to EDCs in garment.

5.1 Recommendations for future research

Currently it is nearly impossible to estimate which exposure levels of Nonylphenols and PAHs should be regarded as safe for the female reproductive system, owing to the lack of knowledge of the endocrine system as well as test systems. Despite this, there is a clear need to initiate further research in this area. These future investigations will hopefully take the field even closer to this goal by either confirming or dismissing the connection between exposure to chemicals in textiles and negative effects on human health.

A number of issues for future research have been identified in this thesis:

In order to analyse the total exposure, not only clothes, but several other textile articles present in the indoor environment should be analysed.

- The leaching behaviour of the chemical substances must be analysed further

- The study of more specific skin areas in connection with the exposure of EDCs and other chemicals

- A toxicokinetic model should be established to determine the leaching behaviour

6 Bibliography

Ademollo, N. o.a., 2008. Nonylphenol and octylphenol in human breast milk. *Environment International,* 34(7), pp. 984-987.

Anderson and Meade, 2014. Potential Health Effects Associated with Dermal Exposure to Occupational Chemicals. *Environmental Health Insights,* 8(1), p. 51–62.

Andersson, N., 2019. *ECHA- Grouping of substances and read-across.* Available at: https://echa.europa.eu/documents/10162/13628/09_read_across_webinar_en.pdf/4dbb2e64-408c-4d12-a605-e9f9b75615d8 (Accessed:15 September 2019)

Agency for Toxic Substances and Disease Registry (2009) *Substance Priority List*.

Agency for Toxic Substances and Disease Registry (2012) *Toxicity of Polycyclic Aromatic Hydrocarbons (PAHs)*, *Agency for Toxic Substances and Disease Registry (ATSDR)*.

Aksglaede, L. *et al.* (2006) 'The sensitivity of the child to sex steroids: Possible impact of exogenous estrogens', *Human Reproduction Update*, 12(4), pp. 341–349. doi: 10.1093/humupd/dml018.

Al-Juboori, B., Hamdan, F. and Al-Salihi, A. (2016) 'Paternal exposure to low-dose lead acetate: Effect on implantation rate, pregnancy outcome, and sex ratio in mice', *Turkish Journal of Medical Sciences*, 46(3), pp. 936–941. doi: 10.3906/sag-1412-62.

Baynes, R. E. and Hodgson, E. (1951) *Absorption and Distribution of Toxicants*, *The Lancet*. doi: 10.1016/S0140-6736(51)92508-1.

Bergman, Å. *et al.* (2012) *State of the science of endocrine disrupting chemicals, 2012*, *Toxicology Letters*. doi: 10.1016/j.toxlet.2012.03.020.

Bergmann, A. *et al.* (2012) *Endocrine Disrupting Chemicals*, *The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC)*.

BfR, 2012. *Bundesinstitut für Risikobewertung (BfR).* [Online] Available at: http://www.bfr.bund.de/en/health_assessment_of_textiles-531.html
(Accessed: 11 October 2019).

Borg, B., 2019. [Interview] 2019.

Bos, J. D. and Meinardi, M. M. H. M. (2000) 'The 500 Dalton rule for the skin penetration of chemical compounds and drugs', *Experimental Dermatology*, 9(3), pp. 165–169. doi: 10.1034/j.1600-0625.2000.009003165.x.

Burgoon et al., L. (2017) *Toxicological Review of Benzo [a] pyrene*.

Buttke, D. E. e. a., 2012 . *Environmental health perspectives ,* 120 (11), pp. 1613- 8 .

Cabot (2019) *Presence of PHAs in carbon black products*. doi:

.1037//0033-2909.I26.1.78

Carwile, J. L. *et al.* (2009) 'Polycarbonate bottle use and urinary bisphenol A concentrations', *Environmental Health Perspectives*, 117(9), pp. 1368–1372. doi: 10.1289/ehp.0900604.

Cave, M. R. *et al.* (2010) 'Comparison of ratch mode and dynamic physiologically rased rioaccessibility tests for pahs in soil samples', *Environmental Science and Technology*, 44(7), pp. 2654–2660. doi: 10.1021/es903258v.

Chen, J.-a., Liu, H., Qiu, Z. & Shu, W., 2008. Analysis of di-n-butyl phthalate and other organic pollutants in Chongqing women undergoing parturition. *Environmental Pollution,* 156(3), pp. 849- 853.

Choy, C. M. Y. *et al.* (2002) 'Infertility, blood mercury concentrations and dietary seafood consumption: A case-control study', *BJOG: An International Journal of Obstetrics and Gynaecology*, 109(10), pp. 1121–1125. doi: 10.1016/S1470-0328(02)02984-1.

Choy, J. (2020) 'The madrid statement on poly- and perfluoroalkyl substances (PFASs)', *Environmental Health Perspectives*, 123(5). doi: 10.1289/ehp.1509934.

Commission for Environmental Cooperation (2017) *Migration of Chemicals from Consumer Products- A Study of Per- and Polyfluoroalkyl Substances (PFASs) in Clothing, Apparel, and Children's Items*. Montreal, Canada. Available at: http://www3.cec.org/islandora/en/item/11777-furthering-understanding-migration-chemicals-from-consumer-products.

Cotgreave, I., 2019/2020. *Personal communication.* Göteborg

Dziewirska, E. e. a., 2018. Environmental non persistent endocrine disrupting chemical exposure and reproductive hormone level in adult men. *International journal of occupational medicine and environmental health,* 31(5), pp. 551-573 .

ECHA, 2012 . *Guidance on Information Requirements and Chemical Safety Assessment,* u.o.: ECHA, European Chemical Agency.

ECHA (2012a) *4- Nonylphenol*.

ECHA (2012b) *4-Nonylphenol, branched and linear SVHC Support document*.

ECHA (2013) *4- Nonylphenol, branched and linear ethoxylated*.

ECHA (2018) *Candidate List of substances of very high concern for Authorisation - ECHA*, *ECHA Website*. Available at: https://echa.europa.eu/candidate-list-table.

EPA (1993) *Risk assessment of polycyclic aromatic hydrocarbons*.

European Chemicals Agency (2012) 'Guidance on Information Requirements and Chemical Safety Assessment', *European Chemicals Agency*, 2008(May), pp. 1–58.

EU, 2002. *European Union Risk Assessment 4- Nonylphenol (Branched) and Nonylphenol,* u.o.: u.n.

Godschalk, R. W. L. *et al.* (1998) 'Aromatic DNA adducts in human white blood cells and skin after dermal application of coal tar', *Cancer Epidemiology Biomarkers and Prevention*, 7(9), pp. 767–773.

Götz, F., 2001. Female infertility-effect of perinatal xenoestrogen exposure on reproductive functions in animals and humans. *The National Center for Biotechnology Information.*

Harrison, P. e. a., 1997. Reproductive health in humans and wildlife: Are adverse trends associated with environmental chemical exposure?. *Science of the total environment ,* 205(2), pp. 97- 106 .

Hayes, B. B. (2000) 'Evaluation of Percutaneous Penetration of Natural Rubber Latex Proteins', *Toxicological Sciences*, 56(2), pp. 262–270. doi: 10.1093/toxsci/56.2.262.

Hoffmann, M., 2019. *WebMD.* [Online] Available at: https://www.webmd.com/skin-problems-and-treatments/picture-of-the-skin#1
(Accessed: Retrieved 4 November 2019).

Hormone Health Network (2017) *Female infertility*, *Journal of the American Medical Association*. Available at: https://www.hormone.org/diseases-and-conditions/infertility-female (Accessed: 12 June 2019).

Hsu, P. C. *et al.* (2006) 'Sperm DNA damage correlates with polycyclic aromatic hydrocarbons biomarker in coke-oven workers', *International Archives of Occupational and*

Environmental Health, 79(5), pp. 349–356. doi: 10.1007/s00420-005-0066-3.

Hussain, M. *et al.* (1998) 'Lifetime health risk assessment from exposure of recreational users to polycyclic aromatic hydrocarbons', *Archives of Environmental Contamination and Toxicology*, 35(3), pp. 527–531. doi: 10.1007/s002449900412.

Ihalainen, J. (2020) *The menstrual cycle and female athletic performance*, *Mittuniversitetet, Mid Sweden University*. Available at: https://www.miun.se/en/Research/research-centers/swsrc/news/2019-2/the-menstrual-cycle-and-female-athletic-performance/ (Accessed: 6 January 2020).

International Program of Chemical Safety (1987) *Management of poisoning for primary health care agents*. Available at: http://www.bvsde.paho.org/tutorial4/i/credits.html (Accessed: 20 November 2019).

Kaerlev, L. *et al.* (2005) 'Cancer incidence among Danish seafarers: A population based cohort study', *Occupational and Environmental Medicine*, 62(11), pp. 761–765. doi: 10.1136/oem.2005.020818.

Khan, M. J., Ullah, A. and Basit, S. (2019) 'Genetic Basis of Polycystic Ovary Syndrome (PCOS): Current Perspectives', p. 32.

Knafla, A. *et al.* (2006) 'Development of a dermal cancer slope factor for benzo[a]pyrene', *Regulatory Toxicology and Pharmacology*, 45(2), pp. 159–168. doi: 10.1016/j.yrtph.2006.02.008.

Kristensen, P. *et al.* (1995) 'Fertility in mice after prenatal exposure to benzo[a]pyrene and inorganic lead', *Environmental Health Perspectives*, 103(6), pp. 588–590. doi: 10.1289/ehp.95103588.

Kummer, V. *et al.* (2008) 'Estrogenic activity of environmental polycyclic aromatic hydrocarbons in uterus of immature Wistar rats', *Toxicology Letters*, 180(3), pp. 212–221. doi: 10.1016/j.toxlet.2008.06.862.

Lakna (2018) *Difference Between Areolar and Adipose tissue What is Areolar Tissue*, *PEDIAA*. Available at: https://pediaa.com/difference-between-areolar-and-adipose-tissue/ (Accessed: 19 November 2019).

van Leeuwen, C. J. and Vermeire, T. G. (2007) *Risk Assessment of Chemicals: An Introduction*.

Leridon, H. and Slama, R. (2008) 'The impact of a decline in fecundity and of pregnancy postponement on final number of children and demand for assisted reproduction technology', *Human Reproduction*, 23(6), pp. 1312–1319. doi: 10.1093/humrep/den106.

Lindex, 2019/2020. [Interview] 2019/2020.

Luongo, G. (2015) *Chemicals in textiles and environmental pollution*.

Mackenzie, K. M. and Angevine, D. M. (1981) 'Infertility in mice exposed in utero to benzo(a)pyrene', *Biology of Reproduction*, 24(1), pp. 183–192. doi: 10.1095/biolreprod24.1.183.

Marieb , E. N., 2013 . *Anatomy & Physiology.* u.o.:Benjamin-Cummings

Mattison, D. R. and Thorgeirsson, S. S. (1979) 'Ovarian aryl hydrocarbon hydroxylase activity and primordial oocyte toxicity of polycyclic aromatic hydrocarbons in mice', *Cancer Research*, 39(9), pp. 3471–3475.

Muthu, S. S. (2018) *Textile Science and Clothing Technology*. doi: 10.1007/978-981-10-4780-0.

Neal, M. S. *et al.* (2007) 'Follicle growth is inhibited by benzo-[a]-pyrene, at concentrations representative of human exposure, in an isolated rat follicle culture assay', *Human Reproduction*, 22(4), pp. 961–967. doi: 10.1093/humrep/del487.

Nguyen, T. &. S. M. A., 2017. Exposure of women to trace elements through the skin by direct contact with underwear clothing. *Journal of Environmental Science and Health,* 52(1), pp. 1-6.

Ning, X. A. *et al.* (2014) 'Levels, composition profiles and risk assessment of polycyclic aromatic hydrocarbons (PAHs) in sludge from ten textile dyeing plants', *Environmental Research*, 132, pp. 112–118. doi: 10.1016/j.envres.2014.03.041.

Organisation for Economic and Development, C. and (2019) 'Report on the Test Results of Endocrine Disrupting Effects of Nonylphenol on Fish', *Organisation for Economic Co-operation and Development*, (1), pp. 1–32.

Petit, F. *et al.* (1997) ‘Two complementary bioassays for screening the estrogenic potency of xenobiotics: Recombinant yeast for trout estrogen receptor and trout hepatocyte cultures’, *Journal of Molecular Endocrinology*, 19(3), pp. 321–335. doi: 10.1677/jme.0.0190321.

Pizzorno, J. (2018) ‘Environmental toxins and infertility’, *Integrative Medicine: A Clinician’s Journal (IMCJ)*, 17(2), pp. 8–11. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6396757/.

Quinion, M. (1996) *In silico*, *World Wide Words*. doi: 10.1134/s0132342318040097.

REACH, 2006. *European Commission Regulation (EC),* u.o.: European Chemical Agency .

RI.SE IVF , 2019. *Kemikaliengruppen,* Gothenburg: RI.SE IVF.

Swedish Standards Institute , 2019. *Colour fastness to rubbing*

Rockström, M., 2019. u.o., u.n.

Roos, S., 2019/ 2020 . *Personal communication.* Göteborg, u.n.

Roser, M. (2017) *Fertility Rate Rank*, *Our World in Data*.

Schug, T. *et al.* (2011) ‘Endocrine disrupting chemicals and disease susceptibility’, *The Journal of Steroid Biochemistry and Molecular Biology*, 127(3–5), pp. 204–215.

Senanayake, P. & Potts, M., 2008. *Atlas of Contraception.* London: CRC Press.

Shier et al. , 2019. *Hole's Human Anatomy & Physiology.* 15th red. New York: McGraw- Hill Education.

Soares, A. *et al.* (2008) 'Nonylphenol in the environment: A critical review on occurrence, fate, toxicity and treatment in wastewaters', *Environment International*, 34(7), pp. 1033–1049. doi: 10.1016/j.envint.2008.01.004.

Soares, S. R. and Melo, M. A. (2008) 'Cigarette smoking and reproductive function', *Current Opinion in Obstetrics and Gynecology*, 20(3), pp. 281–291. doi: 10.1097/GCO.0b013e3282fc9c1e.

Stephan, C., 1986. Proposed goal of applied aquatic toxicology. Aquatic toxicology and environmental fate. *American Society for Testing and Materials International ,* Volume 9.

Swedish Chemicals Agency (2016) *Supervision report*.

Takizawa, K. *et al.* (1984) 'Murine strain differences in ovotoxicity following intraovarian injection with benzo(a)pyrene', *Cancer Research*, 44(6), pp. 2571–2576.

Technical Committee ISO/TC 38 and Technical Commit, 2019. *Colourfastness to perspiration,* Stockholm: Swedish Standards Institute.

Technical Committee ISO/TC 38 and Technical Commit, 2019. *Colour fastness to rubbing,* Stockholm: Swedish Standards Institute.

The Week UK (2018) *Why global fertility is in decline*, *The Week*. Available at: https://www.theweek.co.uk/96241/why-global-fertility-is-in-decline.

THINX (2020) *Super Hiphugger*. Available at: https://www.shethinx.com/collections/thinx-classic/products/thinx-super-hiphugger.

Tinkle, S. S. *et al.* (2003) 'Skin as a route of exposure and sensitization in chronic beryllium disease', *Environmental Health Perspectives*, 111(9), pp. 1202–1208. doi: 10.1289/ehp.5999.

Tolbert, P. E. (1997) 'Oils and cancer', *Cancer Causes and Control*, 8(3), pp. 386–405. doi: 10.1023/A:1018409422050.

Tollefsen, K. E., Mathisen, R. and Stenersen, J. (2002) 'Estrogen Mimics Bind with Similar Affinity and Specificity to the Hepatic Estrogen Receptor in Atlantic Salmon and Rainbow Trout (Oncorhynchus mykiss)', *General and comparative endocrinology*, 126, pp. 14–22. doi: 10.1006/gcen.2001.7743.

U.S. Environmental Protection Agency (2010) *Nonylphenol (NP) and Nonylphenol Ethoxylates (NPEs) Action Plan*.

Vermandel, F. (2019) *Fanny Vermandel DyStar*. Göteborg.

Williams, Y. (2019) *Fecundity vs . Fertility : Definition & Overview*. Available at: https://study.com/academy/lesson/fecundity-vs-fertility-definition-lesson-quiz.html (Accessed: 5 December 2019).

Weschler, T., 2006. *Taking Charge of Your Fertility.* 10th red. u.o.:HarperCollins Publishers.

World Health Organisation (2018) *Infertility definitions and terminology*. Available at: https://www.who.int/reproductivehealth/topics/infertility/definitions/en/ (Accessed: 6 January 2020).

World Health Organisation , 2019 . *World Health Organisation-Endocrine Disrupting Chemicals (EDCs).* [Online] Available at: https://www.who.int/ceh/risks/cehemerging2/en/ (Accessed: 8 June 2019).

World Health Organisation , 2019 . *Wolrd Health Organisation-International Programme on Chemical Safety.* [Online] Available at: https://www.who.int/ipcs/en/ (Accessed: 8 June 2019).

Zegers-Hochschild, F. *et al.* (2017) 'The international glossary on infertility and fertility care', *Human Reproduction*, 32(9), pp. 1786–1801. doi: 10.1093/humrep/dex234.

Statement of Authorship

I hereby declare that I am the sole author of this bachelor thesis and that I have not used any sources other than those listed in the bibliography and identified as references. I further declare that I have not submitted this thesis at any other institution in order to obtain a degree.

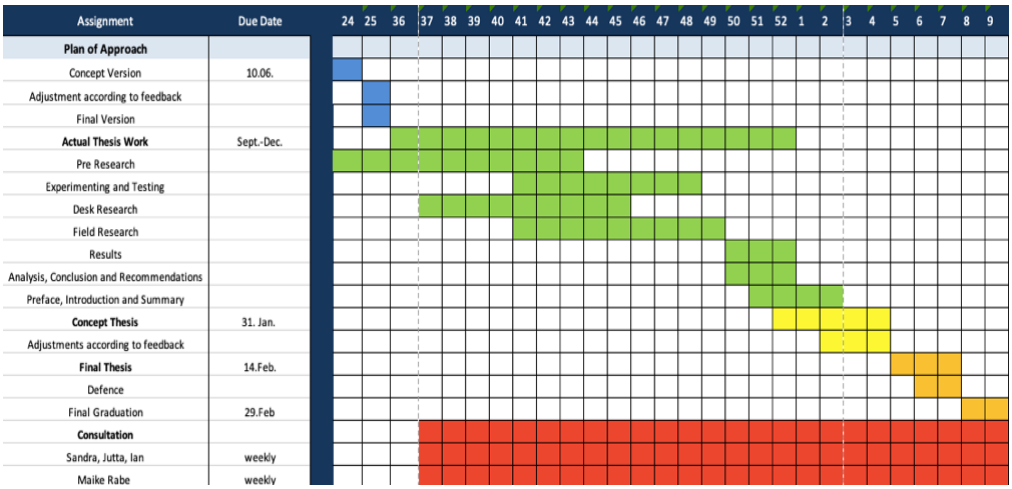
_____ _____

(Place, Date) (Signature)

Appendix

Appendix A

Time Overview Bachelor Thesis by Maren Duprés



Assignment	Due Date
Plan of Approach	
Concept Version	10.06.
Adjustment according to feedback	
Final Version	
Actual Thesis Work	Sept.-Dec.
Pre Research	
Experimenting and Testing	
Desk Research	
Field Research	
Results	
Analysis, Conclusion and Recommendations	
Preface, Introduction and Summary	
Concept Thesis	31. Jan.
Adjustments according to feedback	
Final Thesis	14.Feb.
Defence	
Final Graduation	29.Feb
Consultation	
Sandra, Jutta, Ian	weekly
Maike Rabe	weekly

Appendix B

Research design by Maren Duprés

7th of November 2019

Research design

Approach	TO - DO's
Define research question	- Reference of decreasing fertility - State importance of topic
Define the Oestrus cycle	- Chapter has already been written ➔ Detailed enough?
Identify relevant AOPs*	- **List the mechanisms for fecundity adverse effects**
What are the harmful substances?	- Make Gross list with SMILES code for known substances
Do the substances fit into the mechanism?	- Analyze in silico results ➔ Create list with substances + mechanism
Determine substance examples A+B	- Ian chooses the two most hazardous ones from the list in order to get clear results
Skin absorption: Anatomy - Can local exposure be excluded? - Is it rather local or systemic? -> 2 outcomes are possible	- Literature review (Anatomy) ➔ Contact with mucosa/ epidermis ➔ **Uptake via skin from fabric Make a connection there !** ➔ Skin circulation - Metabolism might influence the uptake as well (sweat, …)
Collect toxicity data from substance A+B	- Literature review
Collect data about hazardous level	- Determine exposure for toxicity effects
Discuss multiple exposure	- Research risks of other garments - Determine concentration of hazardous chemicals (mg/ kg fabric)

*AOP= adverse outcome pathway/ model that identifies the sequence of biochemical events required to produce a toxic effect when an organism is exposed to a substance.

Necessary properties of the hazardous substance

- Deriving from dye stuff
- Present in cotton underwear (100% CO or CO/Elastane blend 90-10%)
- Reprotoxic
- In women underwear garment

Appendix C

Report of Kemikaliengruppen; summary of chemicals which are suspected to potentially harm the reproductive system

English name	Swedish name	CAS	Synonyms	Category
1,3-Naphthalenedisulfonic acid, 6,6-[(3,3-dimethyl[1		314-13-6	Direct Blue 53	Effect chemical: Arylam
4,4´-oxydianiline	4,4-oxidianilin	101-80-4	Aniline, 4,4-ox	Reaction product: Aryla
4-methyl-m-phenylenediamir	Toluen-2,4-diamin	95-80-7	2,4-Toluylendia	Reaction product: Aryla
4-Nonylphenol (an example	4-Nonylfenol	104-40-5	4-Nonylphenol	Reaction product: Brea
4-Nonylphenol, branched	4-Nonylfenol, grenad	84852-15-3		Reaction product: Brea
Aniline	Anilin	62-53-3		Effect chemical: Other
Bis(tributyltin)oxide	Tributyltennoxid	56-35-9	EC# 200-268-(	Effect chemical: Biocid
C.I. Direct Black 38	C.I. Direct Black 38	1937-37-7	C.I. Number 3(	Effect chemical: Arylam
C.I. Direct Blue 6	C.I. Direct Blue 6	2602-46-2	C.I. Number 2(	Effect chemical: Arylam
C.I. Direct Red 28	C.I. Direct Red 28	573-58-0	C.I. Number 2(	Effect chemical: Arylam
DDD	p,p DDD	53-19-0	53-19-0 (o,p´-[	Process chemical: Pest
DHTDMAC	DHTDMAC	61789-80-	N,N-Dimethyl-N	Effect chemical: Soften
Diazo Component 48		91-91-8	C.I.Azoic Diazo	Effect chemical: Arylam
Di-Cyclohexylphthalate (DCH	Di-cyklohexylftalat (DCH	84-61-7		Effect chemical: Plastic
Dimethyl methylphosphonat	Dimetyl metylfosfonat (I	756-79-6		Effect chemical: Flame
Lead	Bly	7439-92-1		Effect chemical: Pigme
Methoxychlor	p,p-Metoxiklor	72-43-5	30667-99-3 (o	Process chemical: Pest
Mirex	Dodekakloropentacyklo	2385-85-5		Process chemical: Pest
Nonylphenol ethoxylates (NI	Nonylfenoletoxilater (NF	9016-45-9	Alkylphenol etl	Process chemical: Surf
Nonylphenol, mixed isomers	Nonylfenol, isomerblan	25154-52-3		Reaction product: Brea
Octamethyl cyclo tetra siloxane (D4)		556-67-2		Effect chemical: Soil ar
Phosphonium tetrakis(hydro	Tetrakis(hydroximetyl)fo	124-64-1		Effect chemical: Flame
Phosphonium tetrakis(hydro	Tetrakis(hydroximetyl)fo	55566-30-8		Effect chemical: Flame
Potassium permanganate	Kaliumpermanganat	7722-64-7	PP spray	Process chemical: Oxid
Tebuconazole	Etyltrianol	107534-9(	Ethyltrianol, Pr	Effect chemical: Biocid
Thiacloprid	Thiacloprid	111988-49-9		Process chemical: Pest
triphenyltin compounds	trifenyltenn föreningar	900-95-8,	Triphenyltin ch	Effect chemical: Biocid

Appendix D

Interview with Fanny Vermandel from DyStar

1. Which EDCs are most likely to be found during the dyeing process?
   She will come back to that.

   Which contaminants are found?

   Skin sensitizers, carcinogens, ecotoxic, etc. There are many different reasons for exclusion

2. How can impurities in dye stuff be controlled?

- Input control
- Lots of contaminated cargo in the textile industry
- Using Colour Index is not enough to prevent hazardous substances, because they occur as an impurity/ through process
- CAS number: same information content as the Colour Index, impurities and contaminations can occur
- The quality of a product linked to that number (CI/CAS) can be absolutely good/ bad
- Reactive Black 5 as an example: many dyes for cotton are based on that substance, which is known to be broken down to an amylamine: p-Chloroaniline
- Between 5000- 50 ppm can be found in the dyestuff (much lower concentrations on fabric
- Legal standard is 20 ppm on fabric (30 ppm in EU REACH/ 20 ppm in China
- Blue Sign has stringent requirements on amylamines
- 1400 products from DyStar on blue sign finder – relevant starting point

- Give list of substances with highest risks (cause to reject products)
- Rejection of products due to: … MRSL/ZDHC lists
- Contamination can come from all sides
- China/ India: most industry for dyeing. > 80% of raw material come from those countries

- Legislation for contamination of wastewater has become more stringent in the past year; suppliers use less water to purify the products in order to avoid wastewater; as a consequence, products are more contaminated.
- Examples are Heavy metals (can come from catalysts), Quinoline, chlorinated aromatics, amylamines; Chlorinated phenols, toluene, benzene
- Concentration is the big issue of harmful chemicals for toxicity in general, see Paracelsus

3. Could you name two substances (from the dye stuff or auxiliary products) which are of a very high concern?

   3.1 Auxiliaries: We recommend checking this contaminant as we are quite sure that it is still used by 'low end' chemical suppliers in Asia.

AEEA (Aminoethyl ethanolamine)	CAS number: 111-41-1	H 360 DF	Used to produce softeners

3.2 Dyes: The dyes below might be relevant for your topic. The relevant H phrases are added for your information

Generic	Name	CAS Number	C.I. No.	Concern	Human danger
	Black 038		30235	carcinogenic dyestuff	H361d
Direct	Blue 006		22 610	carcinogenic dyestuff	H361d
Direct	Red 028		22120	carcinogenic dyestuff	H361d
Pigment	Red 104	12656-85-8	77605	carcinogenic dyestuff	H360, H360(Df)
Pigment	Yellow 034	1344-37-2	77603	carcinogenic dyestuff, lead chromate pigment	H360, H361, H360Df

4. Which auxiliary products are needed for reactive dyeing?

- Salt
- Recipe from Eliot via website of DyStar
- Technical datasheet from products
- Recipes that have been phased out.

5. Have you ever heard of the myth that dark coloured underwear garments are more harmful than lighter ones? If so, do you have an explanation for that assumption?
   (more pigments are needed; therefore, more chemicals are necessary to make the pigments last)

   Never heard of it

6. Which chemicals are most likely to still be present in the end product/ not washed out during production?

   That depends on the chemicals/dyes which are used and how contaminated the products are, which the textile industry is using.

7. Are these harmful chemicals still used in Asian production countries?

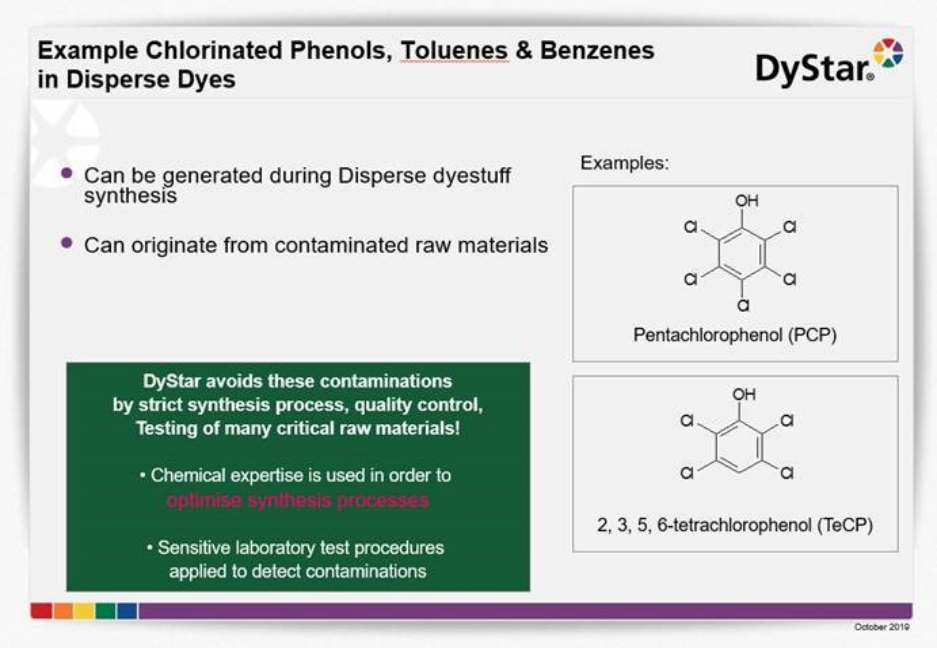
   Yes, we find quite often 'competitor' products which contain contaminants or components which we have banned since many years. This to our big surprise.

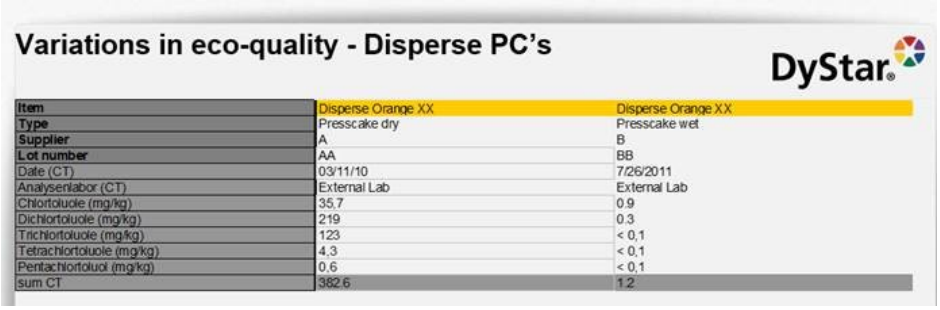
8. How did you substitute the harmful chemicals at DyStar?

   We have 2 options:

   1. We cancel the products and replace them by products based on completely different chemistry

   2. IF possible, we use cleaner/alternative synthesis routes and/or cleaner (less contaminated) raw materials which lead to less by-products or contaminants in the final chemical. See examples in the 2 pictures below. The second picture shows contamination variations in different lots of one and the same dyestuff. It demonstrates how important it is to select 'clean' raw materials and the correct synthesis routes.





Item	Disperse Orange XX	Disperse Orange XX
Type	Presscake dry	Presscake wet
Supplier	A	B
Lot number	AA	BB
Date (CT)	03/11/10	7/26/2011
Analysenlabor (CT)	External Lab	External Lab
Chlortoluole (mg/kg)	35,7	0.9
Dichlortoluole (mg/kg)	219	0.3
Trichlortoluole (mg/kg)	123	< 0,1
Tetrachlortoluole (mg/kg)	4,3	< 0,1
Pentachlortoluol (mg/kg)	0,6	< 0,1
sum CT	382.6	1.2

9. How would you rank the danger deriving from carbon black? And different grades of impurities?

   Pigment are not used for dyeing, in Elastane (spun dyed)

Appendix F

Test results colour fastness

Colour fastness to rubbing was determined according to SS-EN ISO 105-X12:2016.
The staining was assessed visually.
Used rubbing finger: Circular

Downward force of the rubbing finger: (9± 0,2) N
Type of rubbing performed: Dry and wet
Up-take of soak: (95-100) %
Conditioning time before testing: Minimum 4 h
Date of test: 2019-12-12

Table 1 **Colour fastness to rubbing**, rating

Test material/ Colour	**Dry staining**		**Wet staining**	
	Length	Width	Length	Width
Black	4-5	4-5	2	2

Scale 1-5, where 5 is the best.

Measurement uncertainty: ± 1/2 grade.

Uncertainty of measurement is based on the method of dissemination of interlaboratory trials and is stated as standard deviation.

Colour fastness to perspiration was determined according to SS-EN ISO 105-E04:2013.
Adjacent fabric: Multifibre TV, Testfabrics.
Position of the test device: Horizontal
Colour assessment: Visual
Date of test: 2019-12-12

Table 2 **Colour fastness to perspiration**, rating

Test material/ Colour	**Staining**												**Change in colour**
	Triacetate		**Cotton**		**Polyamide**		**Polyester**		**Acrylic**		**Viscose**		
	pH 5.5	pH 8.0	pH 5.5	pH 8.0	pH 5.5	pH 8.0	pH 5.5	pH 8.0	pH 5.5	pH 8.0	pH 5.5	pH 8.0	
Black	4-5	4-5	5	5	5	4-5	5	4-5	5	5	4-5	4-5	4-5

Scale 1-5, where 5 is the best.

Measurement uncertainty: ± 1/2 grade.
Uncertainty of measurement is based on the method of dissemination of interlaboratorytrials and is stated as standard deviation.

Appendix G

Gross list

Systematic name	SMILES
Nonylphenols	
2- (2-(4-Nonylphenoxy) ethoxy) ethanol	CCCCCCCCCc1ccc(cc1) OCCOCCO
2- (4-Nonylphenoxy) ethanol	CCCCCCCCCc1ccc(cc1) OCCO
1 - Ethoxy- 4- (7-methylocytl)benzene	CCOc1ccc(cc1) CCCCCCC(C) C
4- Nonylphenol	CCCCCCCCCc1ccc(cc1) O
2- Nonylphenol	CCCCCCCCCc1ccccc1O
4- (2,6-Dimethylheptyl) phenol	CC(C)CCCC(C)Cc1ccc(cc1)O
Arylamines (banned) from cleavable azo dyestuffs	
2,4,5-trimethylaniline	Cc1cc(c(cc1C)N)C
2,4 – Xylidine	
2,6 Xylidine	Cc1cccc(c1N)C
Naphthylamine	c1ccc2c(c1)cccc2N

3,3'-Dichlorobenzidine	c1cc(c(cc1c2ccc(c(c2)Cl)N)Cl)N
4,4- bi- o-toluidine	
4,4-Methylene-bis[2chloro-aniline]	
4,4-Methylenedianiline (MDA)	c1cc(ccc1Cc2ccc(cc2)N)N
4,4-Methylenedi-otoluidine	
4-Aminoazobenzene	
4-chloroaniline	c1cc(ccc1N)Cl
4-Chloro-o-toluidine	Cc1cc(ccc1N)Cl
4-methoxy-mphenylenediamine	COc1ccc(cc1N)N
4-methyl-mphenylenediamine	Cc1ccc(cc1N)N
5-Nitro-o-toluidine	Cc1ccc(cc1N)[N+](=O)[O-]

Benzidine	c1cc(ccc1c2ccc(cc2)N)N
Biphenyl-4-ylamine	c1ccc(cc1)c2ccc(cc2)N
o-Aminoazotoluene	Cc1ccccc1/N=N/c2ccc(c(c2)C)N
o-Anisidine	COc1ccccc1N
o-Dianisidin	COc1cc(ccc1N)c2ccc(c(c2)OC)N
o-Toluidine	Cc1ccccc1N
p-Cresidine	Cc1ccc(c(c1)N)OC
Lead	
Lead	[Pb]
Lead di(acetate)	[Pb+2].CC([O-])=O.CC([O-])=O
Copper	
Copper	[Cu]
Polycyclic aromatic hydrocarbons (PAHs)	
N-(4-Aminobenzoyl) glycine	c1cc(ccc1C(=O)NCC(=O)O)N
Anthracene	c1ccc2cc3cccc c3cc2c1
Phenanthrene	c1ccc2c(c1)ccc3c2cccc3
Tetracene	c1ccc2cc3cc4ccccc4cc3cc2c1
	c1cc2ccc3ccc4ccc5ccc1c6c2c3c4 c56

Dibenzo[ghi,mn o]fluoranth ene	
Benzo[pqr]tetra phene	c1ccc2c(c1)cc3ccc4cccc5c4c3c2 cc5
Pentacene	c1ccc2cc3cc4cc5ccccc 5cc4cc3cc2c1
Pyrene	c1cc2ccc3cccc4c3c 2c(c1)cc4
Coronene	c1cc2ccc3ccc4ccc5ccc6ccc1c7c2c3c4c5c67
Triphenylene	c1ccc2c(c1)c3ccccc 3c4c2cccc4
Benzo[ghi]peryl ene	c1cc2ccc3ccc4ccc5cccc6c5c4c 3c2c6c1
Chrysene	c1ccc2c(c1)ccc3c2c cc4c3cccc4
Ovalene	c1cc2ccc3cc4ccc5ccc6ccc7cc8ccc1c9c2c3c1c 4c5c6c7 c1c89
Benzo[*c*]fluroen e	c1ccc2c(c1)ccc3c2- c4ccccc4C3
Phenalene	c1cc2cccc3c2c(c 1)CC=C3

Appendix H

Content test of PAHs/NP/NPEs in underwear sample

Polycyclic aromatic hydrocarbons (PAH) were determined according to OEKO-TEX®

Sample weight: 1.00 g

Extraction with organic solvent in an ultrasonic bath, followed by detection with GC-MS.

Quantification with certified reference material.

Limit of quantification: 0.20 mg/kg

Date of test: 2020-01-14

Table 1. Polycyclic aromatic hydrocarbons (mg/kg)

Analysed compounds	CAS no	Test Material 1
Acenaphthene[3]	83-32-9	< 0.20
Acenaphthylene[3]	208-96-8	< 0.20
Anthracene[2, 3]	120-12-7	< 0.20
Benz(a)anthracene[1, 2, 3]	56-55-3	< 0.20
Benzo(b,j)fluoranthene[1, 3]	205-99-2 / 205-82-3	< 0.20
Benzo(k)fluoranthene[1, 2, 3]	207-08-9	< 0.20
Benzo(g,h,i)perylene[2, 3]	191-24-2	< 0.20
Benzo(a)pyrene[1, 2, 3]	50-32-8	< 0.20
Benzo(e)pyrene[1, 3]	192-97-2	< 0.20
Chrysene[1, 2, 3]	218-01-9	< 0.20
Cyclopenta(c,d)pyrene	27208-37-3	< 0.20
Dibenzo(a,h)anthracene[1, 3]	53-70-3	< 0.20
Dibenzo(a,e)pyrene	192-65-4	< 0.20
Dibenzo(a,h)pyrene	189-64-0	< 0.20
Dibenzo(a,i)pyrene	189-55-9	< 0.20
Dibenzo(a,l)pyrene	191-30-0	< 0.20
Fluoranthene[2, 3]	206-44-0	< 0.20
Fluorene[3]	86-73-7	< 0.20
Indeno(1,2,3-c,d)pyrene[3]	193-39-5	< 0.20
Naphthalene[3]	91-20-3	< 0.20
Phenanthrene[2, 3]	85-01-8	< 0.20
1-Methylpyrene	2381-21-7	< 0.20
Pyrene[2, 3]	129-00-0	< 0.20

[1]Substance included in REACH Annex XVII and restricted in plastic and rubber parts that may come into direct as well as prolonged or short-term repetitive contact with the human skin or the oral cavity. The restriction limit is 0.5 mg/kg for toys and childcare articles and 1.0 mg/kg for other articles.

Identified as Substances of Very High Concern and is included in the Candidate List. EU and EEA suppliers of articles which contain substances in the Candidate List in a concentration above 0.1% (w/w) have certain obligations according to the REACH regulation.

[2] Substances included in the ProdSG (Product Safety Act), Germany.

Alkylphenol ethoxylates (APEO) and alkylphenols (AP) were determined according to OEKO-TEX®.

Sample weight: 1.0 g

Extraction with organic solvent in an ultrasonic bath, followed by detection with LC-MS/MS and

GC-MS.

$NP(EO)_{1-20}$ was quantified using the technical mixture Igepal CO-720/Imbentin-N/060. $OP(EO)_{1-20}$ was quantified using the technical mixture Triton X-45/Igepal CA-720. The other analysed compounds were quantified using certified reference materials.

Analysed compounds

4-nonylphenol ethoxylate (NPEO), degrees of ethoxylation: 1-20, analysed with LC-MS/MS 4-octylphenol ethoxylate (OPEO), degrees of ethoxylation: 1-20, analysed with LC-MS/MS 4-tert-butylphenol, 4-pentylphenol, 4-heptylphenol, 4-octylphenol and 4-nonylphenol analysed with GC-MS

Limit of quantification

$NP(EO)_{1-20}$ and $OP(EO)_{1-20}$: 20 mg/kg

4-tert-butylphenol, 4-pentylphenol, 4-heptylphenol, 4-octylphenol, 4-nonylphenol: 2.5 mg/kg

Date of test: 2020-01-14.

Table 1. Alkylphenol ethoxylates and alkylphenols (mg/kg).

Test Material	$NP(EO)_{1-20}$	$OP(EO)_{1-20}$	4-tert-butyl phenol	4-pentyl phenol	4-heptyl phenol	4-octyl phenol	4-nonyl phenol
1	< 20	< 20	< 2.5	< 2.5	< 2.5	< 2.5	< 2.5