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Dermal absorption of triclosan following short-, and long-term exposure in an ex vivo human skin model- implications for safe use in personal care products

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Public health science

Contents

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Martine Malmer Herud

Abstract

In the every-day life, humans are surrounded by a multitude of man-made chemicals which they, intentionally or unintentionally, are exposed to through inhalation, ingestion or dermal absorption. In risk assessments of chemicals, data on dermal absorption is often sparse or lacking. Triclosan is a synthetic, broad-spectrum antimicrobial agent often added to a broad range of personal care- and household products that may come in contact with human skin, and the use of triclosan in such products have raised concerns about potential adverse health effects in humans. The aim of this study was 1) to establish an *in vitro* skin model to study dermal absorption of triclosan using human skin, 2) to obtain dermal absorption values for triclosan after short- and long-term exposure, and 3) to estimate human systemic exposure doses (SED) and a margin of safety (MoS) of triclosan after the use of selected personal care products (hand soap, shower-gel and deodorant).

An *in vitro* skin model was established according to the OECD Guideline 428 and the SCCS Notes of Guidance. Abdominal human skin obtained from donors undergoing abdominal surgery was mounted onto Franz diffusion cells and radiolabeled (^{14}C-) triclosan (0.3%) was applied on the skin surface and washed off after short- (20 minutes) and long-term (24 hours) exposures. The absorption values obtained were used to calculate SED and MoS.

^{14}C-triclosan was detected in all compartments with a recovery of the applied dose ranging from 94% to 113% for both the short- and long-term exposure. The obtained recovery of the applied dose suggests a successful establishment of the skin model. The mean (±SD) absorbed doses of ^{14}C-triclosan (epidermis, dermis and receptor fluid) after 20 minutes and 24 hours exposure were 2.06 (±2.02) % and 18.38 (±6.21) %, respectively. Based on the absorption values obtained, the SED calculated for hand soap, shower-gel and deodorant was 0.00041 mg/kg bw/day, 0.00034 mg/kg bw/day and 0.01716 mg/kg bw/day, respectively. The MoS for hand soap, shower-gel and deodorant was 29441, 35140 and 699, respectively. The total MoS for hand soap, shower-gel and deodorant was 670. The present study demonstrated that triclosan is absorbed through the human skin, but to a less extent for short-term exposure compared to the long-term exposure. The calculated MoS suggest a low risk to health of the presence of 0.3% triclosan in both short-term exposure products like hand soap and shower-gel, and for long-term exposure products as deodorants. The MoS calculated for the total use of all products was within the optimal margin of safety.

Sammendrag

I dagliglivet er mennesker omgitt av et bredt omfang av kjemikalier som de eksponeres for, bevisst eller ubevisst, gjennom inhalasjon, mat og drikke eller via hudabsorpsjon. I risikovurderinger av kjemikalier er data om absorpsjon via hud ofte mangelfull eller fraværende. Triclosan er et syntetisk, bred-spektret antimikrobielt stoff som tilsettes flere kroppspleie- og husholdningsprodukter som kan komme i kontakt med huden, og bruken av triclosan i slike produkter har vekket bekymring for potensielle helseskadelige effekter. Målet med denne studien var 1) å etablere en *in vitro* hudmodell for å studere hudabsorpsjon av triclosan ved bruk av menneskehud, 2) å beregne absorpsjon av triclosan i hud etter kort- og langtidseksponering, og 3) å estimere systemisk eksponeringdose (SED) og margin of safety (MoS) for triclosan ved bruk av utvalgte kroppspleieprodukter (håndsåpe, dusjsåpe og deodorant).

En *in vitro* hudmodell ble etablert i henhold til OECD`s retningslinje 428 og SCCS veiledningsnotat. Hud donert etter bukoperasjoner ble montert på Franz diffusions celler, og radiomerket (^{14}C-) triclosan (0.3%) ble applisert på hudens overflate og vasket av etter korttids- (20 minutter), og langtidseksponering (24 timer). Absorpsjonsverdiene ble brukt til å beregne SED og MoS.

^{14}C-triclosan ble gjenfunnet i alle deler med en recovery av applisert dose på mellom 94% og 113%. Recovery av applisert dose indikerer en vellykket etablering av hudmodellen. Gjennomsnittlig (±SD) absorbert dose av ^{14}C-triclosan (epidermis, dermis og reseptorløsning) etter 20 minutter og 24 timer var henholdsvis 2.06 (±2.02) % og 18.38 (±6.21) %. Estimert SED basert på absorpsjonstallene for håndsåpe, dusjsåpe og deodorant var henholdsvis 0.00041 mg/kg kroppsvekt/dag, 0.00034 mg/kg kroppsvekt/dag og 0.01716 mg/kg kroppsvekt/dag. MoS for håndsåpe, dusjsåpe og deodorant var henholdsvis 29441, 35140 og 699. Den totale MoS for håndsåpe, dusjsåpe og deodorant var 670.

Studien viser at triclosan absorberes gjennom hud, men i mindre grad ved korttidseksponering sammenlignet med langtidseksponering. De kalkulerte verdiene for MoS indikerer en lav helserisiko for bruk av 0.3% triclosan både for korttidseksponering som ved bruk av produkter som såpe og dusjsåpe, og for langtidseksponering som ved bruk av produkter som deodoranter. Den estimerte MoS for den totale bruken av alle produktene var innenfor den optimale MoS.

Abbreviations

BW	Body weight
CAS	Chemical Abstract System
DALY	Disability-Adjusted Life Years
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EFSA	European Food Safety Authority
EU	European Union
ER	Electrical resistance
EHC	Environmental Health Criteria
H&E	Hematoxylin and eosin
MoS	Margin of safety
NIPH	Norwegian Institute of Public Health
NOAEL	No observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
SCCS	Scientific Committee on Consumer Safety
SCCP	Scientific Committee on Consumer Products
SED	Systemic exposure dose
SD	Standard deviation
TEER	Trans-epidermal electrical resistance
TEWL	Trans-epidermal water loss
TWF	Tritiated water flux
WHO	World Health Organisation

1 Introduction

In the everyday life, people are surrounded by a multitude of man-made chemicals, which they intentionally or unintentionally, are exposed to through inhalation, ingestion and dermal absorption (Eurostat, 2010; Pruss-Ustun, Vickers, Haefliger, & Bertollini, 2011). Many chemicals that were assumed to be harmless have been found to have negative effects on both human health and the environment (Eurostat, 2010; Folkehelseinstituttet, 2016). Half of the chemical production (total 340 million ton) in EU in 2010 contained substances with presumed adverse health effects (Eurostat, 2011). The World Health Organisation (WHO) claims that 25% of the global burden of disease is associated with environmental factors including exposure to chemicals (WHO, 2010). In 2004, it was calculated that 4.9 million deaths and 86 million Disability-Adjusted Life Years (DALY) were lost due to chemical exposure (Pruss-Ustun et al., 2011). Meanwhile, millions of new chemicals are synthesized each year according to the Chemical Abstract System (CAS) registry (Figure 1). The industry is trying to supply both market and consumers demand of chemicals with desired properties, often replacing banned chemicals with new chemicals (Newshire, 2015).

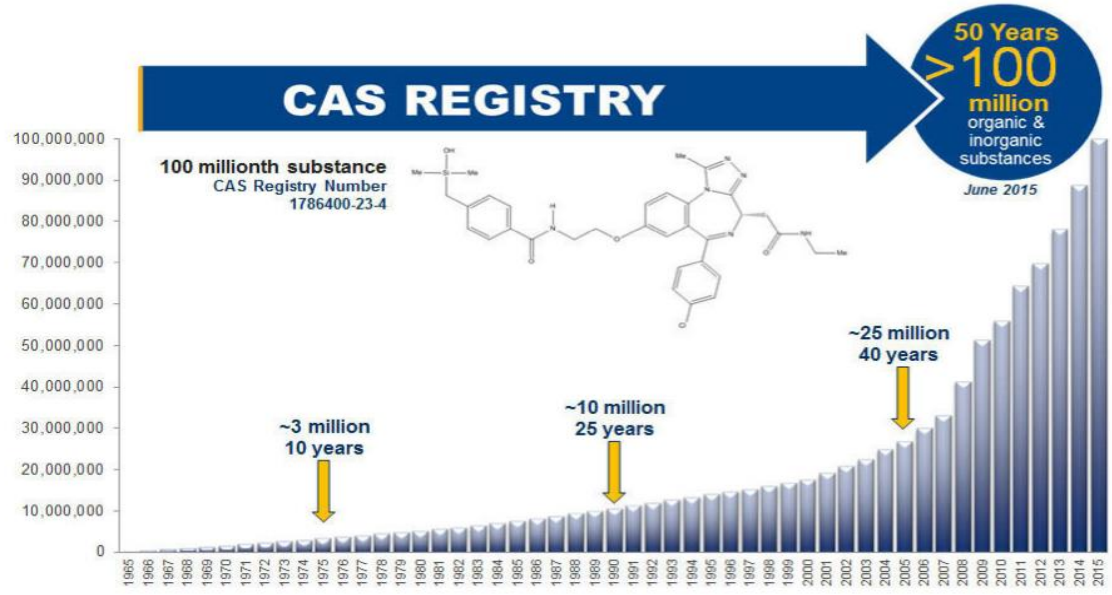

Figure 1. The number of new chemicals introduced to the marked since 1965 (Newshire, 2015).

Chemical compounds are commonly designed and selected for function, price and convenience, and they can be incorporated into various consumer products, functioning as mechanical components or raw-materials in industrial processes (Eurostat, 2010).

Triclosan is a synthetic, broad-spectrum antimicrobial agent added to a wide range of products including personal care and household products which may come in contact with the skin (Bakker et al., 2014). Use of triclosan in such products have raised concern about potential adverse health hazards (APUA, 2011). Although dermal absorption of chemicals is an important route of exposure, dermal absorption data available for risk assessment is often lacking (Buist, Schaafsma, & van de Sandt, 2009; SCCS, 2015). From a public health perspective, it is important to gain more knowledge of how triclosan may be absorbed through the skin and become systemically available and contribute to the total body burden of chemicals in humans. The aim of the present study was therefore to establish an *in vitro* skin model to study dermal absorption of triclosan and to calculate the margin of safety (MoS) and systemic exposure dose (SED) of triclosan in a selection of commonly used personal care products like hand soap, shower-gel and deodorant.

1.1 Health effects of chemicals

Although many chemicals are beneficial for the public health, such as pharmaceutical drugs, are all chemicals toxic to some degree dependent on the chemical, the physical and biological properties of the compound and the dose (Yassi, Kjellstrøm, Kok, & Guidotti, 2001). The most toxic chemicals may lead to serious negative health effects even in small doses according to the Norwegian Institute of Public Health (NIPH) (Folkehelseinstituttet, 2016). The hazard of some chemicals are organ specific, which means that they cause damage on certain organs such as liver, kidneys or nervous system, while other chemicals affects the whole body in general (Yassi et al., 2001). Exposure to certain chemicals may lead to acute poisoning, allergy, cancer, birth defects or subfertility, and some individuals may be more susceptible to these adverse health effects, referred to as vulnerable groups (Folkehelseinstituttet, 2016). These individuals may have reduced metabolism, such as elderly, or underdeveloped organs such as children, or women during pregnancy. However, the health effect on the body system are dependent on the total exposure dose, the duration of exposure and the exposure route of the chemicals (Yassi et al., 2001). The population is exposed to man-made chemicals through many sources, such as personal care products, food, textiles, electronics and household products (ECETOC, 2016).

1.2 Exposure routes of chemicals

Humans are mainly exposed to chemicals orally, by inhalation and through dermal absorption (Yassi et al., 2001). Oral exposure to chemicals is primarily through intake of food and drinking water which is excreted if insoluble. If soluble, the chemicals may cross the intestinal tract and follow the blood-stream to target organs where they can cause harm (Folkehelseinstituttet, 2016). Chemicals in form of gas, vapor or particulates are inhaled into the respiratory tract and either exhaled or deposited in the lungs. When deposited in the lungs, the chemicals can either cause local damage or absorb into the blood through the gas exchange in the smallest parts of the lungs (Yassi et al., 2001). Dermal exposure to chemicals may occur from e.g. household products, textiles, electronic equipment, toys, and personal care products such as body lotions, soaps, deodorants and shower-gels (SCCS, 2015). Chemicals with certain characteristics may cause local irritation in the skin or get systemically available through the blood-stream and can thus cause systemic effects (Folkehelseinstituttet, 2016). Data on dermal absorption constitute a key step in risk assessment of chemicals (OECD, 2011). Moreover, risk assessment are traditionally based on estimated doses from *in vivo* animal studies where inhalation and oral ingestion of chemicals are the main exposure routes (Buist et al., 2009; SCCS, 2015). Since dermal absorption is an important exposure route to consider when calculating systemic exposure doses, lack of data on dermal absorption is, therefore, an important knowledge gap to fill (OECD, 2011).

1.3 Risk assessment of chemicals

Human risk assessment is a process where information is analyzed to determine whether a chemical might cause harm to an exposed population (SCCS, 2015). This process integrates some general steps:

- Hazard identification, which is a description of adverse effects with respect to the chemical`s toxicity. Hazard data can be based on both human and animal studies, as well as *in vitro* studies.
- Dose-response assessment, where there is emphasis on dose-response relationship for a critical effect and identification of health-based guidance values.
- Exposure assessment, which involve identification of populations that may be exposed, exposure routes and estimation of exposure doses.
- Risk characterization, where the information is integrated to determine the likelihood that a chemical can cause harm to exposed individuals (SCCS, 2015).

These four steps are the foundations for developing guidelines and regulations for chemical use, in order to protect public health and environment. One of the national objectives of the Norwegian Government is to focus on minimize the risk of negative health effects to humans and environment caused by chemical use by 2020 (Regjeringen, 2015).

In the process of determining the risk and degree of exposure to a certain chemical, it is not only important to consider the products that contain the chemical, but also the multitude of chemicals we are exposed to (ECETOC, 2016). Risk assessment can be challenging because most products on today's market have several uses that can influence the pathway, magnitude and duration of exposure. Both quantitative estimation of total exposure, and data of health effects of a specific chemical is therefore a considerable research challenge (ECETOC, 2016; Huang, Ernstoff, Fantke, Csiszar, & Jolliet, 2017).

Identification of health effects and dose-response assessment in risk assessment is most commonly based on animal studies where scientists investigate the toxicological effects of the chemical of interest (SCCS, 2015). Studies of human exposure to chemicals are generally epidemiological cohort studies, cross-sectional or case-control studies (Yassi et al., 2001). Although these studies are important in mapping the exposure and possible health effects, they can often not be used alone for dose-response assessment (SCCS, 2015). When

interpreting results from animal studies to humans, there are crucial steps that have to be considered. In the dose-response assessment of animal experiments, a no adverse effect level (NOAEL) is determined (the highest dose tested where no adverse effects in the animal were observed). This NOAEL is then used to calculate margin of safety (MoS), which is the ratio between the NOAEL and the systemic exposure dose (SED) (the amount of chemical expected to enter the blood-stream). A calculated MoS above 100 is considered as safe for the use of consumer products. This default value consist of a factor of 10 for extrapolation from test animals to an average human being, and another factor of 10 taking into account the inter-individual variations within the human population, as illustrated in figure 2 (SCCS, 2015).

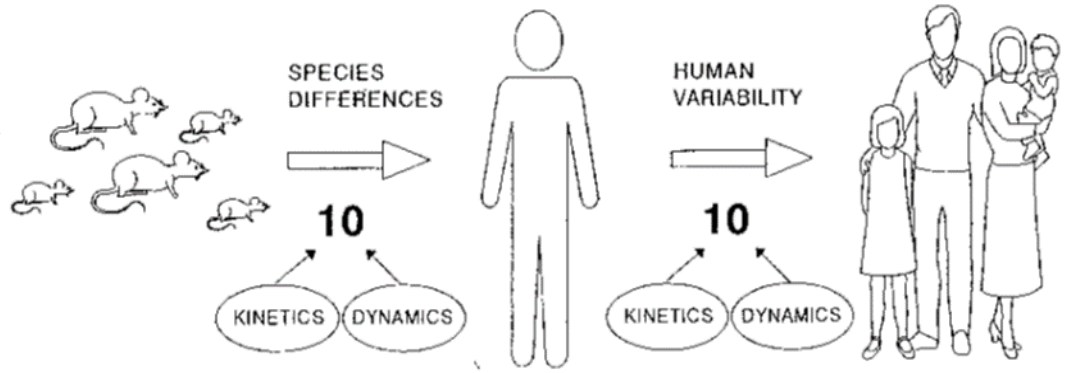

Figure 2. Schematic presentation of the extrapolation from animal to human (SCCS, 2015).

1.4 Dermal absorption of chemicals

Dermal absorption is an important route of exposure to chemicals in personal care products (SCCS, 2015). Several factors affects dermal absorption, including the chemical properties of the compound, type of vehicle, application dose, skin quality (e.g. skin diseases and skin barrier integrity) and the dermal area of absorption (Kielhorn, Melching-Kollmub, & Mangelsdorf, 2006). In risk assessments of chemicals, data on dermal absorption of chemicals is an important part of the exposure assessment (Davies, Heylings, McCarthy, & Correa, 2015). However, data on dermal absorption in human skin available for risk assessment are often lacking in the public domain (SCCS, 2015).

Most of the available data on dermal absorption of chemicals are based on studies using animals (Abdallah, Pawar, & Harrad, 2015; SCCP, 2009). Since animal and human skin have different characteristics, dermal absorption data based on animals should not be directly translated to humans. Differences in skin structure, thickness and lipid content are important characterizations that affect dermal absorption (Baki & Alexander, 2015). Animal skin, such as mouse and rat skin, is generally more permeable and consequently chemicals are more easily absorbed (Moss, Howes, & Williams, 2000). Monkey and pig skin is more similar to human skin, but are not directly comparable when study dermal absorption (Kielhorn et al., 2006; Kuchler, Struver, & Friess, 2013). Human skin is regarded as the "gold standard" in skin absorption studies and are not subjected to the same ethical concerns as for *in vivo* animal experiments (Kuchler et al., 2013). Access to human skin for *in vitro* studies is therefore preferable when testing dermal absorption (Abdallah et al., 2015).

1.5 Human skin

The skin is the largest organ in humans and functions as a barrier to the outside environment (Kielhorn et al., 2006). The skin consists of three layers; epidermis, dermis and hypodermis as shown in figure 2.

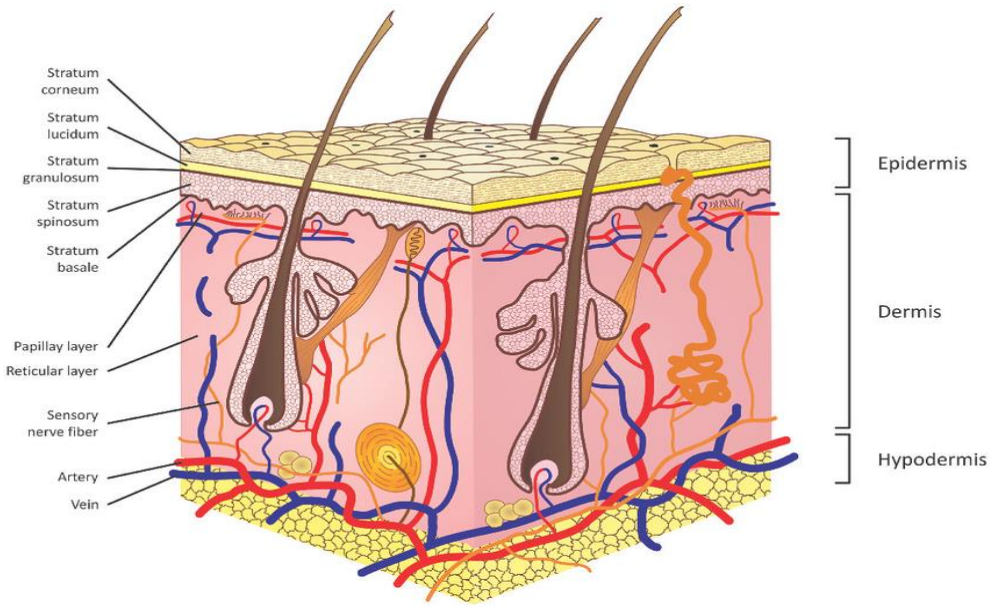

Figure 2. Human skin with the exterior layers. Retrieved from: https://www.researchgate.net/figure/282643441_fig18_Figure-1-Sketch-of-the-outermost-layers-of-the-human-skin-Moving-from-the-outside-to

The epidermis is the outermost layer of the skin and consists of distinct strata that reflect different stages of keratinocytes maturation: stratum corneum, stratum lucidium (only on palms of hands and sole of feet), stratum granulosum, stratum spinosum and stratum basale. Stratum corneum consist of nonviable keratinocytes enclosed by lipids. The keratinocytes in the stratum corneum are eliminated by desquamation after 17-70 days, as they grow outwards from the inner layers of epidermis (Kielhorn et al., 2006). The stratum corneum function as the crucial barrier of the skin, such as preventing percutaneous absorption of chemicals and regulating hydration. The layers below stratum corneum constitute the viable epidermis (living cells) and include melanocytes and Langerhans cells as well as keratinocytes (Kielhorn et al., 2006). Dermis and hypodermis has a more complex composition including nerves, hair follicles and sweat glands which are directly connected with arteries and veins. When investigating absorption of chemicals through skin, the amounts present in the viable epidermis and dermis are considered as dermally absorbed and taken into account for calculations (SCCS, 2015).

1.6 Triclosan

Triclosan is a synthetic, broad-spectrum antimicrobial agent often added to a wide range of products such as personal care- and household products (Bakker et al., 2014). The total amount of triclosan in various products in Norway in 2001 was estimated to be roughly 2300 kg (VKM, 2005), and according to the Scientific Committee of Consumer Safety (SCCS) (2011) the use of triclosan in products within EU in 2006 reached 450 tons, including 85% within personal care products. The most common products containing triclosan are soap, deodorants, mouthwashes, shampoos, toothpastes, cosmetics, toys and detergents (ECETOC, 2016). The purpose of adding triclosan is mainly to prevent or reduce bacterial contamination. The main exposure route of triclosan is through the skin, although unintentional ingestion through the use of oral products also occurs. Triclosan (CAS no.3380-34-5) has the chemical structure 5-chloro-2-[2, 4-dichlorophenoxy] phenol (Figure 3), and is easily absorbed through the skin (Bakker et al., 2014). It is a phenol and a weak acid, which, in combination with its partition coefficient (logPo/w 4.8), facilitate transfer of the protonated (non-ionized) form of triclosan across lipid membranes (SCCP, 2009).

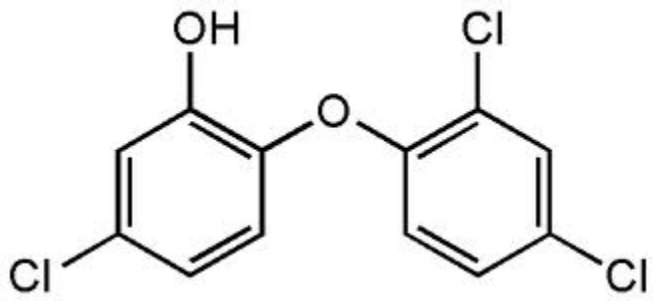

Figure 3. The chemical structure of triclosan (Bakker et al., 2014).

Triclosan has low solubility in water (0.001 g/100 g water) compared to solvents such as aceton, ethanol, isopropanol, propylene glycol and polyethylene glycol (SCCP, 2009). Upon oral intake, absorption of triclosan from the gastrointestinal tract is extensive in both humans and animals. As mentioned above, triclosan is easily absorbed through the skin. After uptake, triclosan is rapidly distributed in the organism. The half-life of elimination after oral intake of triclosan range from 13 to 29 hours in humans compared to 10 to 15 hours in rats, 8-12 hours in mice and 25 to 32 hours in hamsters. The main excretion route in humans is via urine, with excretion via faeces being of secondary importance. The reverse situation is observed in rats

and mice where biliary excretion is more important than excretion via urine. There is no evidence for a bioaccumulation potential for triclosan in humans (SCCP, 2009).

A Swedish study measured high levels of triclosan in breastmilk (Adolfsson-Erici, Pettersson, Parkkonen, & Sturve, 2002) and high levels of triclosan have been detected in urinary samples from pregnant women (Weiss et al., 2015). Evidently, elevated levels of triclosan in urine, is associated with the use of increasing numbers of triclosan containing products (Weiss et al., 2015). This is supported by Toms et al. (2011), who demonstrated that the inter-individual differences in the use of triclosan containing products reflects the levels of triclosan measured in human breast milk.

1.7 Triclosan and health

According to the Norwegian Scientific Committee for Food Safety (VKM, 2005), triclosan has toxic effects on the environment and can lead to adverse health effects. Triclosan has been on the priority list of the Norwegian Environment Agency since 2008 together with over thirty other pollutants, and is controlled by the regulations of cosmetics and the law of product control. The priority list consists of chemicals that constitute a serious threat to health and environment (Miljødirektoratet, 2016).

It has been demonstrated that dermal exposure of triclosan increases immune-related responses in mice (Marshall et al., 2015). One study suggested that high exposure of triclosan have an impact on the development of allergies (Anderson, Meade, Long, Lukomska, & Marshall, 2016). This is supported by Bertelsen et al. (2013), who showed in an epidemiological study that high urinary levels of triclosan were associated with allergic sensitization in 10-year old Norwegian children.

A recent review suggests there is evidence that triclosan has endocrine-disrupting effects (Wang & Tian, 2015). Another study showed an association between internal concentrations of endocrine disrupting chemicals, including triclosan, and subfertility in men (Den Hond et al., 2015). The impact of decreased fertility has economic consequences for the society. Male infertility is estimated to cost 4.71 billion dollars annually because of the need for assisted reproductive procedures (Den Hond et al., 2015). However, there are conflicting results on triclosan as an endocrine disruptor, and one case study of triclosan claims there is no evidence that triclosan is an endocrine disruptor (Mihaich, Capdevielle, Urbach-Ross, & Slezak, 2017).

The findings that elevated levels of triclosan in urine, is associated with the use of increasing numbers of triclosan containing products (Weiss et al., 2015) support the notion that triclosan exposure is a public health issue. Knowing that triclosan is a component of several consumer products and that it can be dermally absorbed, the total exposure, described as aggregated exposure, are an important contribution when performing risk assessment (ECETOC, 2016).

Both the Norwegian Scientific Committee for Food Safety (2005) and the SCCS (2011) have considered triclosan to potentially contribute to antimicrobial resistance (co- and/or cross–resistance). Antimicrobial resistance is a serious threat to public health and a high governmental concern, and the Norwegian Scientific Committee for Food Safety (2005) recommended to restrict the use of triclosan in 2004. Since antimicrobial resistance is outside the scope of this thesis, this topic is not further discussed.

1.8 Regulation of triclosan in consumer products

In EU and EEC countries, triclosan is allowed to be used as a preservative in concentrations up to 0.3% in toothpaste, hand soaps, body soaps/shower gels, deodorants, face powders, blemish concealers and nail products for cleaning the fingernails and toenails before the application of artificial nail systems, and 0.2% in mouthwashes (EUR-Lex, 2014).

The SCCS evaluated triclosan as a preservative in 2009 and 2011, and concluded that the maximum allowed concentration of 0.3% for triclosan in all cosmetic products was not safe for the consumer (SCCP, 2009; SCCS, 2011). This was reasoned by the lack of knowledge on the magnitude of aggregated exposure. However the maximum concentration at 0.3% were considered safe in common-use products defined as toothpaste, hand soaps, body soaps/-, shower-gels and deodorants (SCCS, 2011).

2 Research objectives

From a public health perspective, it is important to gain more knowledge on exposures contributing to the body burden of chemical exposures, in particular chemicals in personal care products commonly used on an every-day basis (e.g. soap, toothpaste, moisturizer, deodorant and shower-gel) that may be absorbed through the skin and become systemically available. It is also important to identify all relevant exposure routes and the doses that can give adverse health effects in order to prepare risk assessment and guidelines to protect public health. In the present study, the main goal was to investigate the absorption of triclosan in human skin by establishing an *in vitro* skin model.

The specific goals of the study was: 1) to establish an *in vitro* model to investigate dermal absorption of ^{14}C-labeled triclosan, using human donor skin, 2) to obtain a dermal absorption value for triclosan after short- and long-term exposure, and 3) to estimate human systemic exposure doses of triclosan (SED) and a margin of safety (MoS) value after the use of selected personal care products (hand soap, shower-gel and deodorant).

3 Materials and methods

3.1 Guidelines and guidance documents

To investigate to what extent triclosan is absorbed through human skin, the *in vitro* dermal absorption experiments in the present study was conducted in line with the Guideline 428 for determination of skin absorption (OECD, 2004b), the Guidance Document for the conduct of skin absorption studies No.28 (OECD, 2004a) and the Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation (SCCS, 2015). These guidelines and guidance documents are developed to estimate both beneficial and hazardous effects of compounds that humans are exposed to via the skin.

3.2 Human skin donors

Human skin was obtained from the biobank DermaTox at NIPH. The biobank contain abdominal skin from patients undergoing cosmetic surgery at two clinics in Oslo, Norway; Akademiklinikken AS and Aleris Helse AS. Abdominal skin samples was received shortly after surgery with the only information of gender and age. The skin was donated anonymously and a written consent was obtained from the donors allowing the use for research (Appendix I).

3.3 Skin integrity

The upper layer of the skin, stratum corneum, can be compromised or damaged during both storage and preparation. To evaluate the integrity of the skin before and after freezing, the trans-epidermal water loss (TEWL) method was used to eliminate potentially damaged skin. The TEWL instrument (Cortex technology ApS, Denmark) measures the quantity of water that passes through the skin in terms of gram water per m^2. The TEWL instrument is sensitive to air flow and requires a constant room temperature at 20-25°C as well as relative humidity of 40-45% to perform adequately. The TEWL measurements took place in a dedicated cabinet with minimum of air flow. The room temperature and relative humidity was manipulated to obtain values close to the optimal range. Before storage by freezing, TEWL of all skin

samples was measured. After packing each donor skin in aluminum foil, the skin was stored in the biobank DermaTox, at -20° C according to OECD Guideline 428 (OECD, 2004b).

3.3.1 Exclusion criteria of skin samples

The high variability in TEWL values due to the conditions mentioned in the previous chapter (3.3), makes the comparisons of TEWL measurements between laboratories challenging and also makes it difficult to define a cut-off value were the skin barrier is too poor. Thus, to give an estimate of a normal range of TEWL in *ex vivo* abdominal human skin, the mean and SD of TEWL from all skin donors (n=29) in the DermaTox biobank was calculated, and the skin samples with a TEWL-value higher than the mean +1SD of the TEWL value for all donors (4.53+1.70 g/m^2 = 6.23 g/m^2) was excluded. Also, skin samples with visible stretch marks, scars, tattoos or other damage were rejected.

3.4 Franz diffusion cell system

In order to investigate dermal absorption, the Franz diffusion cell (PermeGear, Hellertown, PA/USA) was used. Franz diffusion static cell (Figure 4) is an apparatus designed to perform *in vitro* skin permeation studies. In the apparatus, distilled water connected via a water bath and pump circulates continuously in the outer chamber (water jacket) to maintain a constant temperature of 32°C ±1, similar to the human skin physiological temperature. The skin sample is mounted between the donor chamber and receptor chamber, with a permeation area of 1.76 cm^2/cell.

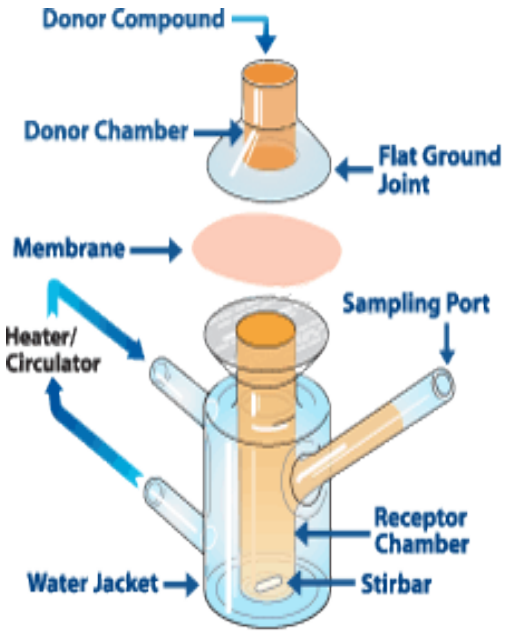

Figure 4. Franz diffusion cell. Retrieved from: http://permegear.com/franz-cells/

3.5 Chemical substances

The test substance used in this study was ^{14}C-labelled triclosan, purchased from American Radio-labelled Chemicals Inc. St. Louis, USA (Appendix II). The compound was determined

by the manufacturer to have a radiochemical purity of > 98%. Propylene glycol ($C_3H_8O_2$) purchased from Fluka Chemie AG, Buchs was used as vehicle. UltimaGold scintillation cocktail (4 ml per vial) was used for all samples as counting fluid (PerkinElmer Inc. Waltham, USA). Solvable^{TM} (PerkinElmer Inc. Waltham, USA) was used to dissolve the epidermis and dermis sample before scintillation counting.

3.6 Experimental protocol

Two separate experiments were conducted, one with a duration of 20 minutes exposure and one experiment with a duration of 24 hours exposure to ^{14}C-triclosan. This was done to imitate realistic product-use for rinse-off products such as shower-gel and hand soap, and a leave-on product such as deodorant. In both the experiments, the skin samples remained on the Franz diffusion cells for 24 hours and each experiment included skin samples from 4 donors in duplicate.

3.7 Short-term and long-term exposure

The donor skin was thawed in room temperature before start of the experiments and TEWL measurements were repeated after skin samples were cut to 500 µm thickness by using a dermatome (Aesculap AG, B. Braun Company, Tuttlingen, Germany). Skin samples were also visually inspected on a light-box, and skin samples with visual damage or TEWL > 6.23 g/m^2 were excluded from the experiments.

Receptor chambers were filled with sodium chloride (0.9%) to a level of convexity to ensure the donor skin being in contact with the receptor fluid during the experiment. A magnetic stirrer was placed in the receptor chamber to ensure proper mixing of the receptor fluid within the receptor chamber. The skin samples were randomized to the Franz diffusion cells, named A to - H, and secured between the donor and receptor chamber using clamps. After fixing the donor skin samples to the Franz cells, the skin samples were equilibrated with the receptor fluid for approximately 10 minutes before application of ^{14}C-triclosan. Concentrations of 0.3% ^{14}C-triclosan in propylene glycol were applied as a single dose of 30 µl, by a pipette, at the top of the skin. The donor chamber and sampling port were covered with parafilm after application of ^{14}C-triclosan to avoid contamination and evaporation. After 24 hours, vapor

assembled on the parafilm surface were carefully dried by filter paper and collected in scintillation vials. The skin samples were dismantled from the Franz diffusion cells and the epidermal skin surface was washed three times with filter paper soaked in sodium chloride (0.9%) followed by wiping both sides of the skin with dry filter paper. The protocol for short-, and long-term exposure was similar, except that after 20 minutes in the short-term exposure, the skin surface was dried with one dry filter paper and washed with two filter paper soaked in sodium chloride (0.9%) after 30 µl ^{14}C-triclosan was applied.

3.8 Tissue separation

After 24 hours, the exposed area of the skin samples was separated from the excessive skin (underneath the clamps) and the surplus skin was removed before the tape stripping procedure. To remove the stratum corneum from the viable epidermis, the skin samples were tape stripped with five Corneotape strips using a pressure of approximately 225 grams per square centimeter for three seconds by a Cuderm D-Squame disc applicator (CuDerm Corporation, Dallas, USA). Each tape was separately collected in scintillation vials and assayed for ^{14}C-triclosan.

The number of tapes necessary to remove stratum corneum was decided based on a pilot study made earlier in the lab at NIPH. In the pilot study, human donor skin samples were taped with 0-8 Corneotapes, embedded in paraffin, sectioned by a microtome at 0.5 mm, stained with H&E (hematoxylin and eosin) and visually inspected in a light microscope to study when the stratum corneum was removed. Based on the results from the pilot study, it was concluded that five tapes were necessary to remove stratum corneum. Figure 5 shows the stratum corneum almost fully removed from epidermis after five tape strips.

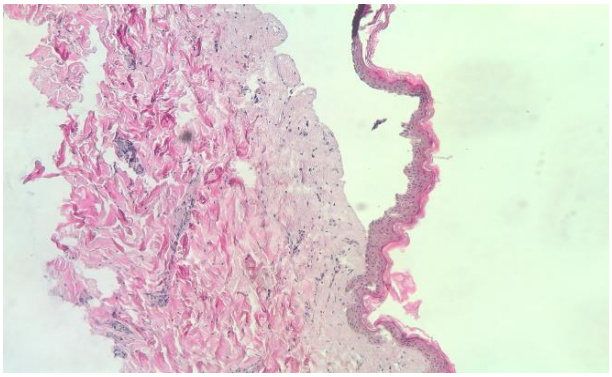

Figure 5. Skin sample after five tape strips.

To separate the viable epidermis from dermis, the procedure included heating of the skin samples in an empty glass in a water bath at 60°C for two minutes. This step was followed by manually separation of the viable epidermis from dermis by forceps. Epidermis was separated from dermis by digestion using 1 ml of the tissue solubilizer Solvable^{TM} with the mechanical shaker Belly dancer (Alfa-lab AS, Oslo, Norway), overnight.

3.9 Scintillation counting

Samples from all the different compartments (skin wash, stratum corneum, epidermis, dermis, receptor fluid and wash of equipment) were separately collected in scintillation vials containing 4 ml UltimaGold scintillation cocktail for determination of ^{14}C-triclosan. The countings from a blank vial (containing only the scintillation fluid) were automatically subtracted from all the other vials.

The amount of ^{14}C-triclosan in stratum corneum included the sum of five tape strips from each skin sample. Epidermis and dermis were transferred to scintillation vials by pipettes after being digested in Solvable overnight. The detection of ^{14}C-triclosan in the receptor fluid included all the samples taken from the sampling port during the experiments. The skin wash included wash of the upper part of the skin with three filter paper soaked in sodium chloride (0.9%) and one dry filter paper on both side of the skin. The amount of ^{14}C-triclosan in equipment included all the washing steps of apparatus (receptor chamber, donor chamber, needles used and petri dish), parafilm, evaporation from parafilm and excessive skin summarized. The amount of applied dose detected in the receptor fluid, the viable epidermis and dermis was used to summarize the total skin absorption of ^{14}C-triclosan.

All components of the test system were assayed to determine the total recovery in a scintillation counter purchased from Tri-Carb 2810TR, PerkinElmer, Oslo. The results obtained from the scintillation counter were calculated in amounts (%) of applied dose for each Franz diffusion cell by using the counting result from a positive control (a single dose of 30 µl ^{14}C-triclosan applied in a vial) as reference of applied dose.

3.10 Calculation of systemic exposure dose (SED) and margin of safety (MoS)

By using the dermal absorption values of triclosan obtained by the method developed in the present study, the human systemic exposure doses of triclosan was estimated according to the formula in SCCS (2015) Notes of Guidance. The systemic exposure dose was calculated by using the following formula:

SED = A (mg/kg bw /day) x C (%) /100 x Dap (%)/100

SED (mg/kg bw/day) = Systemic Exposure Dose

A (mg/kg bw/day) = Estimated daily exposure to a cosmetic product per kg body weight, based upon the amount applied and the frequency of application

C (%) = Concentration of the substance under study in the finished cosmetic product on the application site

DAp (%) = Dermal Absorption expressed as a percentage of the test dose assumed to be applied in real-life conditions (SCCS, 2015, p. 62).

The values presented in table 1 for hand soap, shower-gel and deodorant are taken from the Notes of Guidance (SCCS, 2015, pp. 76-79) and the calculated relative daily exposure for the different product types was used for the calculations of SED in the formula presented.

Table 1. Levels of daily exposure for different products.

Default values from Notes of Guidance v9	**Hand soap**	**Shower-gel**	**Deodorant**
Estimated daily amount applied (g)	20,00	18,67	1,50
Retention factor*	0,01	0,01	1,0
Calculated daily exposure (g/day)	0,20	0,19	1,50
Calculated relative daily exposure (mg/kg bw/day) (A)**	3,33	2,79	22,08

*A factor taken into account rinsing off and dilution of finished products by application on wet skin or hair

** Estimated daily exposure per kg body weight used to calculate SED

The current NOAEL set for triclosan by SCCP (2009) was 12 mg/kg bw/day. This dose was calculated due to haematotoxicity and decreased absolute and relative spleen weights from long term studies in rats. This NOAEL were chosen for MoS calculations in the present study. The (MoS) value was estimated according to the formula in SCCS (2015) Notes of Guidance. The margin of safety was calculating by using the following formula:

$$\text{Margin of Safety(MoS)} = \frac{\text{NOAEL}}{\text{SED}}$$

3.11 Calculations

The results from the scintillation counter were transferred to Microsoft Office Excel 2016 and calculated in percentage triclosan, using the positive control as a reference for total dose applied. Descriptive statistics as mean and standard deviation, were used to present the mean skin absorption for short- and long-term exposure.

3.12 Ethical approvals

The present study utilized skin samples from the biobank DermaTox, approved by the Regional Committee for Medical and Health Research Ethics (REK approval id 2015/1032) (Appendix III), REK did also approve the application for using human skin in the present study (REK approval id 2015/1522) (Appendix IV).

4 Results

4.1 Skin donors and TEWL

Abdominal skin was obtained from both male (n=1) and female (n=7) donors, age 27-68 years. To assess the skin barrier integrity for each donor skin, the TEWL was measured both before and after storage at -20°C. Table 2 present the mean ± standard deviation (SD) of repeated TEWL measurements for each skin sample (and the corresponding Franz cell, A-H) before and after freezing (-20°C), total days in freezer, and age and gender of the donors.

Table 2. Mean trans-epidermal water-loss (TEWL) ± SD in g/m^2 before and after storage, total days in freezer, age (year) and gender (Female (F)/Male (M)).

	Franz cell	TEWL ± SD Before storage	TEWL ± SD After storage	Storage (Days in freezer at -20°C)	Age(year)/gender
24 hors exposure					
23Y	A	3.67 ± 0.49	5.47 ± 0.31	14	45/F
23Y	B	3.70 ± 0.40	4.63 ± 0.23	14	45/F
25Y	C	4.13 ± 0.91	3.33 ± 0.38	14	44/F
25Y	D	3.93 ± 0.80	3.33 ± 0.80	14	44/F
24Y	E	2.90 ± 0.50	4.97 ± 0.70	14	36/M
24Y	F	3.00 ± 0.35	5.33 ± 0.23	14	36/M
11X	G	N. D.*	5.43 ± 0.25	93	29/F
11X	H	N. D.*	3.97 ± 0.06	93	29/F
20 minutes exposure					
27Y	A	3.67 ± 0.8	4.20 ± 0.20	28	68/F
27Y	B	3.73 ± 0.25	4.83 ± 0.38	28	68/F
28X	C	4.40 ± 0.96	3.87 ± 0.29	21	27/F
28X	D	4.27 ± 1.27	4.90 ± 0.10	21	27/F
29X	E	5.30 ± 0.56	4.57 ± 0.23	20	35/F
29X	F	4.60 ± 0.10	5.33 ± 0.35	20	35/F
26X	G	4.10 ± 0.30	4.10 ± 0.44	33	48/F
26X	H	4.23 ± 0.47	3.43 ± 0.23	33	48/F

*N.D: Not Determined

The mean TEWL values (before freezing) ranged from 2.90 to 5.30 g/m^2, and after thawing, the TEWL values ranged from 3.33 to 5.47 g/m^2. All skin samples were still within the acceptable range after thawing (TEWL < 6.23 g/m^2) and were included in the experiments. TEWL measurements before storage were not obtained for donor 11X due to technical issues,

but because the TEWL results after thawing was satisfactory the donor skin was used in the experiment.

4.2 Recovery of ^{14}C-triclosan

4.2.1 Recovery after 20 minutes exposure

The skin samples at Franz cell G (24.12%) and H (199.35%) in the 20 minutes exposure, as shown in table 3, did not satisfy the recovery limit within 85-115% and were consequently excluded from further calculations. The total mean and the total skin absorption presented is thus calculated without the skin samples from Franz cell G and H. The ^{14}C-triclosan was present in both epidermis (mean 0.66 ± 0.50%), dermis (mean 0.43 ± 0.36%) and receptor fluid (mean 0.96 ± 1.17%). The skin surface was wiped with one dry filter paper and washed with 2 filter papers soaked in sodium chloride (0.9%), 20 minutes after 30 µL ^{14}C-triclosan was applied. These steps were included in amount calculated for the skin wash, which was 88.42 ± 6.25%. The total skin absorption included epidermis, dermis and receptor fluid and was (mean ± SD) 2.06 ± 2.02%.

Table 3. Short-term (20 minutes) exposure of ^{14}C-triclosan. Amounts of applied dose in percentage (%) for stratum corneum, epidermis, dermis, receptor fluid, skin wash, equipment, total recovery, total skin absorption and mean of all compartments ± SD in Franz cell A – F.

Franz cell	Stratum corneum (%)	Epi-dermis (%)	Dermis (%)	Recep-tor fluid (%)	Skin wash (%)	Equip-ment (%)	Recov-ery (%)	Total skin absorption (%)*
A	5.72	0.61	0.27	0.48	82.13	4.32	94.00	**1.36**
B	9.34	1.64	1.16	3.34	79.79	4.05	99.00	**6.14**
C	4.90	0.20	0.16	0.24	94.76	1.38	101.65	**0.60**
D	4.96	0.48	0.32	0.60	93.90	2.82	103.00	**1.40**
E	6.86	0.53	0.32	0.55	91.74	5.34	105.34	**1.49**
F	5.01	0.55	0.36	0.53	88.20	2.33	97.00	**1.44**
G	*6.19*	*1.19*	*0.34*	*0.79*	*14.22*	*1.39*	*24.12*	-
H	*15.49*	*6.53*	*3.19*	*4.35*	*164.82*	*4.97*	*199.35*	-
Mean	**6.13**	**0.66**	**0.43**	**0.96**	**88.42**	**3.37**	**99.99**	**2.06**
±SD	**±1.74**	**±0.50**	**±0.36**	**±1.17**	**±6.25**	**±1.46**	**±4.15**	**±2.02**

*Total skin absorption= epidermis + dermis + receptor fluid

4.2.2 Recovery after 24 hours exposure

For the 24 hours exposure, ^{14}C-triclosan was detected in all the compartments (skin wash, stratum corneum, viable epidermis, dermis, receptor fluid and wash of equipment) as shown in table 4. The ^{14}C-triclosan was present in both epidermis (mean 6.93 ± 2.93%), dermis (mean 5.57 ± 2.23%) and receptor fluid (mean 6.55 ± 1.68%). The recovery ranged from 99.73%-113.58%, with a mean (±SD) recovery of 106.03 ± 4.40%. The total skin absorption included epidermis, dermis and receptor fluid, and were (mean ± SD) 18.38 ± 6.21%.

Table 4. Long-term (24 hours) exposure of ^{14}C-triclosan. Amounts of applied dose in percentage (%) for stratum corneum, epidermis, dermis, receptor fluid, skin wash, equipment, total recovery, total skin absorption and mean of all compartments ± SD in Franz cell A - H.

Franz cell	Stratum corneum (%)	Epi-dermis (%)	Dermis (%)	Recep-tor fluid (%)	Skin wash (%)	Equip-ment (%)	Recov-ery (%)	Total skin Absorption (%)*
A	22.14	7.78	4.78	6.00	46.84	13.90	101.44	**18.56**
B	31.42	11.78	8.47	8.56	33.66	11.16	105.10	**28.81**
C	23.54	5.34	6.86	8.52	40.29	22.49	107.10	**20.72**
D	27.19	9.17	7.73	7.10	35.57	12.96	99.73	**24.00**
E	25.67	4.14	2.86	4.20	60.74	15.13	112.73	**11.20**
F	29.99	6.84	5.33	6.58	48.52	11.15	108.40	**18.75**
G	18.98	3.03	2.68	8.01	66.72	14.16	113.58	**13.72**
H	17.24	3.47	2.93	4.85	68.04	11.19	107.71	**11.25**
Mean	**25.32**	**6.93**	**5.57**	**6.55**	**47.66**	**14.00**	**106.03**	**18.38**
±SD	**±4.85**	**±2.93**	**±2.23**	**±1.68**	**±12.81**	**±4.05**	**±4.40**	**±6.21**

* Total skin absorption = epidermis + dermis + receptor fluid

4.3 Skin absorption

The total skin absorption ranged from 0.6%-6.14% and 11.20%-28.81% after 20 minutes exposure and 24 hours exposure, respectively. The total skin absorption for short- and long-term exposure was summarized by adding the amount in the viable epidermis, dermis and receptor fluid plus 1 standard deviation:

Total skin absorption, short-term exposure (20 minutes): 0.66 ± 0.5% (epidermis) + 0.43 ± 0.36% (dermis) + 0.96 ± 1.17% (receptor fluid) = 4.08 %

Total skin absorption, long-term exposure (24 hours): 6.93 ± 2.93% (epidermis) + 5.57 ± 2.23% (dermis) + 6.55 ± 1.68% (receptor fluid) = 25.9 %

4.4 Calculation of systemic exposure dose (SED) for hand soap, shower-gel and deodorant

The SED calculations are based on default values taken from Notes of Guidance (SCCS, 2015). The total skin absorptions of triclosan, from short-, and long-term exposure in the present study, are expressed as the percentage of the amount of substance applied. The concentration (C%) of triclosan used was 0.3% and the DAp(%) values used was 4.08% for hand soap and shower-gel (short-term exposure), and 25.9% for deodorant (long-term exposure). The calculated SED for hand soap, shower-gel and deodorant was 0.00041 mg/kg bw/day, 0.00034 mg/kg bw/day and 0.01716 mg/kg bw/day, respectively, as shown in table 5.

Table 5. Daily amount applied (g), retention factor and relative daily exposure (mg/kg bw/day) are default values from notes of guidance. Triclosan amount absorbed and SED is calculated based on the results obtained in the present study.

Product type	Daily amount applied (g)	Retention factor*	Relative daily exposure (mg/kg bw/day) (A)	Triclosan concentration (%) (C)	Triclosan amount absorbed (%) (DAp)	SED (mg/kg bw/day)**
Hand soap	20	0.01	3.33	0.3	4.08	0.00041
Shower-gel	18.67	0.01	2.79	0.3	4.08	0.00034
Deodorant	1.5	1.0	22.08	0.3	25.9	0.01716

*A factor taking into account rinsing off and dilution of finished products by application on wet skin or hair

**SED = A (mg/kg bw/day) x C (%)/100 x DAp (%)/100

4.5 Calculation of margin of safety (MoS)

The MoS values presented in table 6 for hand soap, shower-gel and deodorant, and for the aggregated exposure, were calculated based on the SED values obtained in the present study (Table 5), and the NOAEL of 12 mg/kg bw/day defined by SCCS (2011). The MoS for hand soap, shower-gel and deodorant was 29441, 35140 and 699, respectively. The total MoS (aggregated exposure) for hand soap, shower-gel and deodorant was 670.

Table 6. Calculation of margin of safety (MoS) for single and aggregate exposure for triclosan through cosmetic use based on systemic exposure dose (SED) calculated from absorption values for short- and long term exposures generated in the present study and a NOAEL of 12 mg/kg bw/day defined by SCCS (2011).

Product type	SED (mg/kg bw/day)	Margin of satefy (MoS)
Hand soap	0.00041	29441
Shower-gel	0.00034	35140
Deodorant	0.01716	699
Aggregated exposure	0.01791	670

5 Discussion

In the present study, an *in vitro* method on dermal absorption was established to obtain data on dermal absorption of triclosan, applied on *ex vivo* skin from human donors. The criteria for dermal absorption experiments as presented in the OECD Guideline 428 (OECD, 2004b), the Guidance document No.28 (OECD, 2004a) and the Notes of Guidance (SCCS, 2015) were followed, and the obtained recovery of the applied dose of radiolabeled ^{14}C-triclosan in the test system suggested a successful establishment of the method. Furthermore, the data obtained on triclosan absorption after short- and long-term exposure was used to calculate systemic exposure doses, and the margin of safety for a selection of three commonly used personal care products. The calculations indicated, within the methodological limitations of the study, a safe use of triclosan (0.3%) in rinse-off products like hand soap and shower-gel, and for leave-on products like deodorants. Regarding aggregated exposure, the margin of safety calculated for combined use of hand soap, shower-gel and deodorant is also considered as safe.

5.1 Development of method and methodological considerations

The first aim in the present study was to establish an *in vitro* skin model to study dermal absorption, based on the Guideline 428 for determination of skin absorption (OECD, 2004b), the Guidance document for the conduct of skin absorption studies No.28 (OECD, 2004a) and the SCCS (2015) Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation, using the Franz diffusion cell system. The advantages of using these guidelines and guidance documents as baseline for establishment of an *in vitro* method are that the documents are well accepted by regulatory agencies. The use of these documents reduces the variation of the *in vitro* methodology carried out by researchers (Davies, Heylings, Gayes, McCarthy, & Mack, 2017; Desmedt et al., 2015) and provides a possibility to compare results and reproduce dermal absorption data (Kuchler et al., 2013). The *in vitro* method is also in accordance with the aim to reduce *in vivo* animal testing, as the Cosmetic Regulation forbids the use of animal studies. Since March 2013, the import and sale of cosmetic products tested in animal studies has been forbidden in the EU (Guth, Schafer-Korting, Fabian, Landsiedel, & van Ravenzwaay, 2015; Kuchler et al., 2013). These guidelines and guidance documents are

beneficial for establishing *in vitro* methods, but as discussed below, there are some methodological considerations that must be taken into account to increase the reliability and reproducibility.

5.1.1 Human skin versus animal skin

In the present study, human skin was obtained from patients undergoing abdominal surgery. There are several benefits of having access to *ex vivo* human skin compared to animal skin when studying dermal absorption of chemicals and to perform risk assessments.

The use of human skin gives no need for extrapolation from species to species when predicting dermal absorption values in humans (Kielhorn et al., 2006). Animal skin are generally more permeable, and risk assessments based on results from dermal absorption studies using animal skin therefore require quantitative adjustments when translating to humans to avoid over-predicting of the dermal absorption (Abdallah et al., 2015; OECD, 2004a). As Moss et al. (2000) demonstrated in their study, the total amount of dermal absorption in human skin versus rat skin was 30% and 41%, respectively, after 24 hours exposure of triclosan using an *in vitro* method. They suggested that dermal absorption of triclosan in human skin is approximately one third less than the absorption as observed using rat skin. Pig (porcine) skin is more similar to human skin when comparing the skin characteristics, with ears and flanks as the preferable parts when investigate dermal absorption. Consequently, porcine skin is often used since human skin are generally difficult to obtain (Kuchler et al., 2013).

Reconstructed human skin is another alternative for studying dermal absorption. There are different types of reconstructed skin, all with the aim to mimic the physiology of human skin (Kielhorn et al., 2006). It has been shown that the reproducibility of data is higher when using reconstructed skin compared to *ex vivo* human or animal skin (Kuchler et al., 2013). On the other hand, it has been shown that reconstructed skin has differences in barrier function compared to human skin (Kielhorn et al., 2006). However, there is lack of validated studies on reconstructed skin and it`s use is consequently not recommended for *in vitro* studies (Kielhorn et al., 2006; Kuchler et al., 2013).

5.1.2 Skin integrity

Establishment of the *in vitro* method included control of the integrity of the skin before and after storage and preparation. Measurements of the skin integrity are crucial to be able to eliminate damaged skin and to demonstrate that the barrier function is maintained, and a necessary procedure according to SCCS (2015) and OECD (2004b). In the present study, the TEWL method was used before and after storage for evaluation of the skin integrity. TEWL is one of the accepted methods for integrity evaluation listed by OECD (2004a).

Different methods can be used for evaluating the integrity of the skin. The most commonly used are electrical resistance (ER), trans-epidermal electrical resistance (TEER), trans-epidermal water-loss (TEWL) and tritiated water flux (TWF). A study investigated these 4 integrity tests and aimed to identify the most useful method for evaluating the skin barrier integrity. Their findings showed high validation of all the integrity tests with the highest validity for the TEWL method (Guth et al., 2015). It has also been demonstrated that TEWL is a valid method to distinguish between damaged and undamaged skin (Desmedt et al., 2016; Pineau, Guillard, Favreau, Marrauld, & Fauconneau, 2012).

It is not possible to investigate metabolic activity when using skin that has been frozen because the metabolic capacity changes quickly after being excised from the body (Kuchler et al., 2013). Since the aim of the present study was to investigate dermal absorption as a passive diffusion process, freezing of the skin was accordingly not an issue. However, it has been reported that no changes occur in skin permeability during freezing the skin at a temperature of -20 °C for up to 466 days according to OECD (2004a). The donor skin used in the present study was frozen at -20 °C for 14-93 days. The TEWL values ranged from 2.90 to 5.30 g water/m^2 before freezing, and after thawing the TEWL values ranged from 3.33 to 5.47 g water/m^2 confirming that changes in skin integrity due to freezing was minimal.

5.1.3 Biological differences of donor skin

A factor that may affect the absorption when using human skin is the biological variations between donors (Desmedt et al., 2016). Individual differences in the skin`s ability to absorb drugs have been reported (Larsen, Nielsen, Sorensen, & Nielsen, 2003). Age is another factor that may affect the permeability of the skin due to decreased amount of lipids, transformation of the barrier function and reduced hydration (Kielhorn et al., 2006). However, there is lack of data on the consequences of age on dermal absorption of chemicals (Kielhorn et al., 2006) and since the aim of the present study was to investigate dermal absorption as a passive diffusion process, potentially changes in the skin due to age was not investigated. The age of the donors ranged from 27 to 67 years and the TEWL measurements were similar independent on age.

As females are overrepresented in abdominal plastic surgeries, most donors in the present study were women, and skin sample from only one male donor was included in the experiments. According to Kielhorn et al. (2006) however, gender have no impact on permeability of the skin.

5.1.4 The Franz diffusion cell system

The method in the present study used the Franz diffusion static cell system to investigate dermal absorption of triclosan. Static diffusion cells, is together with Flow-through diffusion cells acceptable to study skin absorption (OECD, 2011). Although the principle and compartments of the two diffusion cell systems are the same, there are some differences to consider.

The static diffusion cells allows a larger area for skin exposure than the flow-through diffusion cells, and are simpler in design and cheaper compared to the flow-through cells (OECD, 2011). In the static cell, the receptor fluid is not replaced during the experiment, in contrast to the flow-through cell where the receptor fluid is constantly replaced. Hence, the flow-through cell mimics the blood-flow better compared to the static cell. The flow-through cell are therefore suggested to be more appropriate to investigate metabolism (Kielhorn et al., 2006). However, a study demonstrated that there was no differences in the ability to measure the skin permeability using two test chemicals when comparing static cells and flow-through cells (Clowes, Scott, & Heylings, 1994). Since the aim of the present study was to study skin

absorption as a passive diffusion process, it can be assumed that the use of static diffusion cell did not influence the absorption values obtained, compared to the use of Flow-through cell.

5.1.5 Split thickness of the skin

According to Guidance Notes on dermal absorption (OECD, 2011) a split thickness of 200-400/500 µm is recommended for *in vitro* studies on dermal absorption. Different thickness of the human skin is used in different *in vitro* studies on dermal absorption; 1 mm (Larsen et al., 2003) 400 µm (Baert, Vansteelandt, & De Spiegeleer, 2011) and 230 µm (Moss et al., 2000). The donor skin in the present study was dermatomed to a thickness of 500 µm. Chemical absorption occurs via a passive diffusion process through the skin dependent on the skin temperature according to SCCS (2015). It has been shown in a recent study that when comparing 340 µm (split thickness), 450 µm (split thickness) and 1,560 µm (full thickness), the thickness of 450 µm held the most constant temperature close to 32 degrees when using a water bath of 32 ± 1°C circulating around the outer chamber in Franz diffusion cells (Desmedt et al., 2016). The water bath was thermostatically controlled in the present study, but measurements directly at the skins surface were not performed for practical reasons. However, it can be assumed that the thickness of the skin (500 µm) used in the present study was acceptable to ensure a temperature of approximately 32 degrees considering the findings presented above.

5.1.6 Removal of stratum corneum by tape stripping

In the present study, the amounts of the applied dose remaining in the stratum corneum was 6.13 ± 1.74% for short-term exposure, and 25.32 ± 4.85% for the long-term exposure. Hence, the removal (partially or totally) of the stratum corneum will affect the calculation of the absorbed dose. Furthermore, the number of tapes used to remove the stratum corneum is an important matter of discussion. After the skin samples were disassembled from the Franz diffusion cell in the present study, the tape stripping procedure was performed. The number of tape strips (five) used in this method was based on the pilot study completed at NIPH. Previous studies report removal of stratum corneum by six tape strips (Moss et al., 2000), 1-20 tape strips (SCCP, 2009) and eight tape strips (Desmedt et al., 2016). In addition to the number of tape strips, there are other important factors that must be taken into account regarding the tape stripping method. For instance has the brand of tape, type of disc

applicator, and amount and time of pressure applied an impact on the removal of stratum corneum (Kielhorn et al., 2006). It is a considerable research challenge to compare tape stripping methods. A prerequisite to comparing studies at different laboratories is therefore a careful description of the procedure and equipment used to remove the stratum corneum.

5.1.7 Variability within the results and mass balance recovery

According to the Notes of Guidance (SCCS, 2015), 8 skin samples from at least 4 donors should be used to obtain a reliable dermal absorption study. The total recovery recommended is 85-115%. The results from the short-term exposure in the present study excluded two skin samples from the calculations of skin absorption because the recovery was out of the indicated range. Consequently only 3 donors (and 6 skin samples) remained valid, and the total skin absorption ranged from 0.60-6.14%. This implicates a higher degree of insecurity of the dermal absorption value obtained for the short-term exposure. However, a high degree of variability within the results is not necessarily an indication of poor experimental technique. Biological and physiological differences between donors may affect the dermal absorption, independent on the technique (OECD, 2011). When the basic requirements of the SCCS (2015) Notes of Guidance is fulfilled, 1SD is added to the mean absorption value. In cases of significant deviations from the requirements, 2SD should be added. Since the basic requirements were followed, 1SD was added to the total skin absorption used when calculating SED and MoS.

When the mass balance recovery from the long-term exposure was calculated, all 8 skin samples from the 4 different donors were within the recommended range and used for further calculations. The total skin absorption value for the long-term exposure was added 1SD, similarly to the short-term exposure.

5.1.8 Use of vehicle

The vehicle used in this study was propylene glycol, a common vehicle for soaps and shower gels (SCCS, 2015). It has been demonstrated by several studies that the absorption of chemicals is highly dependent on the vehicle (Karadzovska, Brooks, & Riviere, 2012; Limpongsa, Jaipakdee, & Pongjanyakul, 2015; SCCP, 2009). The solubility and physicochemical properties of the vehicle are crucial for the skin absorption. Some vehicles

may disrupt the skin surface by reducing the barrier function of stratum corneum, while others improve the solubility of skin lipids and consequently improves the penetration of the chemical (Karadzovska et al., 2012).

For deodorants, propylene glycol is often used as vehicle, but an equally common vehicle used for deodorants is alcohol containing solvents (Baki & Alexander, 2015). It has been demonstrated that ethanol is skin irritating and may contribute to penetration of dissolved substances through skin (OECD, 2011). Fang, Vanlandingham, da Costa, and Beland (2016) compared absorption of triclosan by application of 10 mg/kg bw triclosan on mice using three different vehicles; 95% ethanol, propylene glycol and a generic cosmetic cream. Their findings showed that the total amount of triclosan absorbed in mice was lower when using propylene glycol as vehicle compared to 95% ethanol and generic cosmetic cream, respectively 3.865 mg/kg bw, 5.747 mg/kg bw and 5.827 mg/kg bw. Another study investigated the effect of propylene glycol, ethanol and sodium lauryl sulfate on the *in vitro* dermal deposition and permeation in pig ear using finasteride as test item (Limpongsa et al., 2015). Their findings showed that ethanol as vehicle increased the solubility and penetration of finasteride compared to the use of propylene glycol and sodium lauryl sulfate as vehicle. Finasteride is a lipophilic drug with low solubility in water similar to triclosan, belonging to the same chemical class. Thus, the use of an ethanol containing vehicle, may have increased the absorption values obtained in the present study.

Dermal delivery of chemically active molecules in products applied on the skin is limited by their penetration through the stratum corneum. In order to achieve an increased dermal delivery of the active ingredients from the product, skin penetration enhancers may be used (Pathan & Setty, 2009). The use of skin penetration enhancer were not included in the experiments in the present study, however it is important to consider the presence of such ingredients in personal care products, as it may increase the absorption of all chemical substances present in the product. Today, skin penetration enhancers are added to most dermatological and cosmetic products (Lane, 2013). The main goal of adding skin enhancers is mainly to reduce the barrier function of stratum corneum and to achieve penetration of a specific ingredient. As a consequence, interactions among the chemical, vehicle and skin are more likely to occur and according to Karadzovska and Riviere (2013) the chemical`s capacity to penetrate through the skin barrier increases.

5.1.9 Amount of dose applied and calculation in percentage

The applied dose on the skin should mimic the amount used in the product, and the recommended amount for *in vitro* studies is up to 10 µl/cm^2 according to OECD (2004a). The volume of test solution applied in the present study was 30 µl and the application area of the skin surface was 1.76 cm^2 (17 µl/cm^2). Finite doses are doses applied to mimic realistic human exposure to a certain chemical. When investigate infinite doses (dose applied to achieve and maintain maximum absorption rate), there are some other factor that has to be considered (OECD, 2004a). It has been shown that absorption from an infinite dose is higher than from a finite dose (Karadzovska et al., 2012).

The amounts of skin absorption in the present study were calculated in percentage. According to Desmedt et al. (2016), there may be an inverse relationship between the amount applied and the percentage absorbed. If the skin is saturated, dependent on the applied dose, the calculation of percentage may lead to an underestimation of the skin absorption. However, Buist et al. (2009) investigated in their review the relationship between dermal loading and relative absorption of chemical substances. They illustrated no inverse relationship of dermal loading and absorption of triclosan. It can therefore be assumed that the use of percentage in the present study did not result in underestimation of the skin absorption.

5.2 Dermal absorption of triclosan

The second aim in the present study was to investigate and quantify the absorption of triclosan using *ex vivo* human skin in the established *in vitro* model. Triclosan was applied to the skin surface for 20 minutes, a realistic duration for hand soap and shower-gel, and 24 hours, which is a realistic duration for leave-on products like deodorants. The total absorbed dose (including receptor fluid, epidermis and dermis) for short-term exposure (20 minutes) and long-term exposure (24 hours) was 4.08% and 25.9% (mean +1SD), respectively.

SCCP (2009) are referring to human *in vitro* studies in their opinion on triclosan showing skin absorption of triclosan (short-term exposure) of 7.2% (soap formulation) and 12% (dishwashing liquid) skin absorption after 10 and 30 minutes exposure, respectively. Furthermore, the long-term exposure studies (24 hours exposure) showed a skin absorption of 7.7% (deodorant formulation), 11.3% (water/oil emulsion) and 30% (ethanol/water). Except for the study by Moss et al. (2000) that reported on 30% skin absorption, the original study

reports cited in SCCP (2009) were not published. Because the information on details that might affect the results is inaccessible, direct comparisons of the different studies were not possible. The applied concentration of triclosan in the studies referred to by SCCP (2009) was 0.02 - 0.2% contrary to the concentration of 0.3% used in the present study. Another difference, was that the number of tape strips used to remove stratum corneum was not specified further than described as using 1-20 tape strips. If the total number of tapes used to remove stratum corneum was less than in the present study (five), it could be a reason for the higher absorption values obtained for the short-term exposure. If the number of tape strips used was larger, it could be an argument for lower absorption values in the long-term exposure. The vehicles used are also a factor that might influence the skin absorption, however, the vehicles were described as soap formulation, dishwashing formulation, deodorant formulation and water/oil emulsion with no further sepcifications. Taken together, the discrepancies in the absorption values presented by SCCP compared to the absorption values obtained for both short- and long-term exposure in the present study, may be explained by the use of different concentrations, the number of tape strips used to remove stratum corneum and the type of vehicle used.

Moss et al. (2000), using 24 hours exposure to triclosan, showed a total skin absorption of 30%, with an amount of 23.7 % of applied dose remaining in the skin (epidermis and dermis). The amount of triclosan found in epidermis and dermis after long-term exposure in the present study (12.5 %), is less than Moss et al. (2000) findings. In contrast to the present study, Moss et al. (2000) used an ethanol-water containing vehicle. Since it has been demonstrated that ethanol as vehicle increases the skin absorption (Limpongsa et al., 2015; Wang & Tian, 2015), the discrepancy in absorption values for 24 hour exposure may be caused by the use of different vehicles.

Queckenberg et al. (2010) calculated percutaneous absorption based on urinary levels after dermal application of 2% triclosan containing cream on human volunteers (n=6). Urine were collected up to 7 days. Their findings showed an absorbed dose of 5.9% ± 2.1% (mean ± SD). Queckenberg et al. (2010) used a concentration of 2% and an exposure duration of 12 hours, whereas the present study used a concentration of 0.3% for both 20 minutes and 24 hours exposure. The latter used an *in vivo* method, thus these results are not directly comparable with the results obtained in the present study. Although triclosan is predominantly excreted via urine in humans, it cannot be excluded that the pharmacokinetic results of the study by

Queckenberg et al. (2010) may have been confounded by incomplete renal excretion of triclosan, thus leading to an underestimation of the absorbed dose.

5.3 Calculation of total skin absorption

The calculation of the total skin absorption in the present study included receptor fluid, dermis and the viable epidermis, excluding five tape strips to remove stratum corneum. This is according to the guidance documents which argues that the uppermost layer, stratum corneum, is not a part of the total dermal absorption (SCCS, 2015).

Different approaches are described in the literature in terms of estimating the total dermal absorption. The OECD (2004a) guidelines consider the viable epidermis, dermis and receptor fluid as part of the total absorption, similar to the present study, but estimates 15-25 tape strips necessary to remove stratum corneum from the viable epidermis. The guidance of dermal absorption by the European Food Safety Authority (EFSA, 2012) argues that the two first tape strips should be excluded when calculating the total skin absorption. Other researchers has excluded the two first tape strips, of total eight, when calculating the total amount absorbed (Desmedt et al., 2016). While some regard all of the test item as present in epidermis, including stratum corneum, stratum granulosum, stratum spinosum and stratum basale, as not available for systemic distribution (Abdallah et al., 2015). These different approaches is justified by the fact that the chemicals within these layers are eliminated by desquamation and not counted as systemically available. A different approach to calculate the total dermal absorption than used in the present study may had resulted in higher or lower absorption values dependent on the total tape strips used to remove stratum corneum. There is a need to standardize the inclusion criteria for calculating total skin absorption more strictly to obtain comparable studies on dermal absorption.

5.4 Estimation of systemic exposure doses (SED)

The third aim of this thesis was to estimate the systemic exposure dose (SED) of triclosan from dermal exposure and calculate the margin of safety (MoS). Based on the skin absorption value from the short-term exposure (20 minutes exposure), the estimated SED for triclosan from hand soap and shower-gel was 0.00041 mg/kg bw/day and 0.00034 mg/kg bw/day, respectively. The wash of the skin surface was performed after 20 minutes for the short-term

exposure, to mimic the realistic rinse-off procedure similar to the use of hand soap and shower-gel. The estimated SED for deodorant based on the skin absorption value from the long-term exposure in the present study was 0.01716 mg/kg bw/day.

In SCCS (2011), the estimated SED values for hand soap and shower-gel were 0.0066 mg/kg bw/day and 0.0192 mg/kg bw/day, respectively, whereas the SED value for deodorant was 0.003 mg/kg bw/day. Thus, the estimated SED values in SCCS (2011) were higher for all the three product types compared to the ones in the present study. The discrepancy between the SED values estimated in the present study and by SCCS may be due to several reasons. First, the skin absorption values used for SED calculations in the present study and by SCCS differs. Secondly, the SCCS used the amount of absorbed triclosan in µg/cm^2, not as percentage as in the present study. According to SCCS (2015) Notes of Guidance, two different equations has to be used depending on whether the skin absorption is reported as percentage or µg/cm^2. The equation used when the amount absorbed is reported in µg/cm^2 includes the skin surface area expected to be treated with the finished cosmetic product, the estimated frequency of application of the finished product and a default human body weight of 60 kg as three separate default values (SCCS, 2015, p. 75) . In comparison, the equation used when the amount is reported in percentage include one default value for estimated daily exposure to a cosmetic product per kg body weight that is based upon the amount applied, and the frequency of application (SCCS, 2015, pp. 76-77). Since the two equations are based on different default values, this may add to the discrepancy between the SED values. Thirdly, when calculating SED based on the percentage of absorbed triclosan, the concentration of triclosan in the product is included in the equation in contrast to the equation used when the amount is reported in µg/cm^2.

SED for aggregated exposure in the present study were 0.01719 mg/kg bw/day. In comparison, the SED for aggregated exposure in SCCS (2011) were 0.0357 mg/kg bw/day. Since SCCS included more product types in their calculation (common-use products: toothpaste, hand soap, body soap/shower-gel and deodorant stick), and considering the arguments described above, the SED value for aggregated exposure cannot be compared.

5.5 Risk assessment and implications for public health

The MoS calculations values was based on the NOAEL (12 mg/kg bw/day) estimated based on long-term toxicity studies in rats (SCCS, 2011). The calculated MoS values for hand soap, shower-gel and deodorant in the present study were 29 411, 35 140 and 699, respectively, whereas the total MoS for hand soap, shower-gel and deodorant was 670. Since MoS above 100 is generally considered safe, the conclusion in the present study is that the use of triclosan at a concentration of 0.3% in hand soaps, shower-gels and deodorants is considered to be safe. The SCCS (2011) reached the same conclusion in the risk assessment from 2011. Their conclusion was based on the calculated MoS value of 336 for common-use products containing 0.3% triclosan (including toothpaste, hand soap, body soap/shower-gel and deodorant stick).

Since consumers are exposed to triclosan from a variety of products, calculation of aggregate exposure is an important contribution to risk assessment. The most common products containing triclosan is personal care products, including skin care products. Some other products containing triclosan are cleaning supplies, toys, bedding, socks, textiles, plastics and kitchen utensils according to ECETOC (2016). To obtain data about habits and usage of the products is therefore crucial to estimate realistic exposure doses. Inclusion of more products than in the present study, would may have given a lower MoS value. In addition, the present study used the maximum allowed concentration (0.3%) for triclosan. While consumer products contains different concentrations of triclosan ranging from 0.02% to 0.3% (SCCS, 2011), it is important to consider the range of concentration when calculating exposure doses in risk assessment. Risk assessments based on concentrations not related to realistic exposure may result in inaccurate MoS values.

The urinary levels of triclosan have been showed to increase with the total use of triclosan containing products (Weiss et al., 2015). Calculation of exposure doses based on urinary levels can however give an underestimation of exposure doses due to the metabolism and excretion of triclosan (Soeborg, Frederiksen, & Andersson, 2014). Chemicals transported to the liver by the blood-stream, results in changed toxicity and an increased rate of excretion (Soeborg et al., 2014). This is important to consider when comparing oral absorption versus dermal absorption as chemicals entering the gastrointestinal tract will have a different metabolism than when being absorbed dermally (Buist et al., 2009). Studies estimating internal exposure to triclosan using the oral route may consequently not be representative when the absorption route is dermal.

Another consideration is that the available human data on the metabolism of chemicals is to a large extent based on studies on healthy adult volunteers, which may not reflect the situation for vulnerable groups (SCCS, 2015). It has been discussed whether MoS above 100 is safe for groups with reduced metabolism and skin barrier (SCCS, 2015) However, when performing risk assessment, vulnerable groups require extra considerations. According to several studies, individuals with compromised skin, such as psoriasis, atopic eczema, has higher TEWL and a less effective barrier, a factor that may have an impact on absorption of chemicals (Davies et al., 2017; Kielhorn et al., 2006; Pineau et al., 2012). In a previous study, it has been shown that compromised skin (reduced barrier of stratum corneum) gives a significantly higher amount of applied dose to reach the receptor fluid (25%) compared with normal skin (16%) (Davies et al., 2017). These findings suggest that humans with compromised skin might be a vulnerable group for dermal exposure of triclosan. Thus, risk assessment using dermal absorption values based on healthy skin may underestimate the exposure to humans with compromised skin barrier, like individuals with atopic dermatitis and other skin diseases.

6 Conclusions and implications

The present study aimed to establish an *in vitro* method using a Franz diffusion cell system to study dermal absorption using human skin. By using the absorption values obtained from the experiments conducted, systemic exposure doses and margin of safety was calculated for the use of triclosan in hand soap, shower-gel and deodorant. The results was then used in a risk assessment of triclosan.

The present study demonstrated that triclosan was absorbed through the human skin, but to a less extent for the short-term exposure compared to the long-term exposure (4.08% vs 25.9%). The calculated systemic exposure doses for hand soap, shower-gel and deodorant in the present study differs from the systemic exposure doses calculated by SCCS. However, the discrepancy between the SED values may be due to different skin absorption applied in the calculations and the equations used to calculate SED in the present study and by the SCCS. Due to vehicle used in the skin absorption experiments in the present study, only personal care products like soaps, body soaps/shower-gels and deodorant sticks were included in the risk assessment. In future risk assessments, other personal care products that may contain triclosan such as oral hygiene products, body lotion and make-up, should be included in the calculations of aggregated exposure.

Since the MoS values for both the separate product types and aggregated exposure were above 100, the results suggest that the use of triclosan up to 0.3% in these products can be considered safe. This is in agreement with the findings by the SCCS`s risk assessment of triclosan. As the present study was conducted using intact human skin, future experiments using compromised skin are needed to conclude on the safe use of triclosan-containing products in individuals with a disrupted or reduced skin barrier.

8 References

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Appendix I: Informed consent

Biobanken DermaTox

Vil du gi bort huden du fjerner til forskning?

Bakgrunn og hensikt

Huden er vårt største organ som daglig utsettes for mange kjemikalier fra blant annet kosmetikk og kroppspleie-produkter, husholdningsprodukter og tekstiler. Det er lite kunnskap om hva som skjer med de kjemiske stoffene som kommer i kontakt med huden, og hva det har å si for vår helse.

Enkelte kjemikalier trenger lett gjennom huden og andre ikke. Noen kjemikalier fraktes uendret gjennom huden før de kommer over i blodbanen og sirkuleres rundt i kroppen vår, mens andre stoffer kan endre karakter i huden og dermed oppføre seg annerledes enn det opprinnelige kjemiske stoffet.

Ved Folkehelseinstituttet ønsker vi å finne ut mer om hvordan ulike kjemikalier tas opp i huden vår og hva som skjer med stoffene etter at de er tatt opp i huden. Til vårt laboratoriearbeid trenger vi hud fra mennesker.

Vi ber om at du donerer huden som er planlagt fjernet ved ditt kirurgiske inngrep til vår biobank DermaTox.

Ved å undersøke hva som skjer med de kjemiske stoffene som kommer i kontakt med huden, vil vi få mer kunnskap om hvordan disse kjemikaliene kan påvirke vår helse. Dette er kunnskap som også vil være viktig for lovreguleringen av bruken av kjemikalier som kommer i kontakt med hud, slik at vi på sikt kan få produkter som er trygge å bruke.

Hva skjer med huden og informasjonen om deg?

Huden vil bli gitt anonymt, det vil si at klinikken kun gir informasjon til Folkehelseinstituttet om donorens kjønn og alder, samt hvilket område av kroppen huden er hentet fra.

Mulige fordeler og ulemper

Da vi ber om at du donerer hud fra et allerede planlagt kirurgisk inngrep, anser vi at det ikke er noen ytterligere risiko knyttet til det å donere hud til biobanken DermaTox.

Frivillig deltakelse

Det er frivillig å donere huden. Dersom du ønsker å donere hud, må du undertegne en samtykkeerklæring. Denne samtykkeerklæringen beholdes av klinikken slik at din anonymitet ved Folkehelseinstituttet blir opprettholdt.

Dersom du velger å donere hud, setter vi stor pris på ditt bidrag.

Personvern

Opplysninger vi ønsker å registrere er kun informasjon om kjønn, alder og hvor på kroppen den donerte huden er hentet fra. Forsøkene med huden vil bli utført av forskere ved Folkehelseinstituttet, og kjemiske analyser vil bli utført ved Folkehelseinstituttet eller hos internasjonale partnere. Folkehelseinstituttet ved administrerende direktør er databehandlingsansvarlig.

Biobank

Huden som er donert vil bli lagret i den generelle forskningsbiobanken DermaTox ved Folkehelseinstituttet. Seniorforsker Berit Granum er ansvarshavende for forskningsbiobanken. Det biologiske materialet kan bare brukes etter godkjenning fra Regional komité for medisinsk og helsefaglig forskningsetikk (REK).

Kontakter på Folkehelseinstituttet:

Ansvarshavende:
Berit Granum
Tlf.: 21 07 66 96
E-post: berit.granum@fhi.no

Prosjektmedarbeider:
Ellen Namork
Tlf.: 21 07 64 33
E-post: ellen.namork@fhi.no

folkehelseinstituttet

Folkehelseinstituttet, Postboks 4404 Nydalen, 0403 Oslo

Biobanken DermaTox

Samtykkeerklæring

SAMTYKKE til donasjon av hud til biobanken DermaTox:

Jeg har lest informasjonsskrivet om biobanken DermaTox og er villig til å donere hud.

Navn (blokkbokstaver):_____

Dato: _____ Underskrift: _____

Kontakter på Folkehelseinstituttet:

Ansvarshavende:
Berit Granum
Tlf.: 21 07 66 96
E-post: berit.granum@fhi.no

Prosjektmedarbeider:
Ellen Namork
Tlf.: 21 07 64 33
E-post: ellen.namork@fhi.no

folkehelseinstituttet

Folkehelseinstituttet, Postboks 4404 Nydalen, 0403 Oslo

Appendix II: American Radio-labelled Chemicals, Inc. St. Louis, USA

American
Radiolabeled
Chemicals, Inc.

101 ARC Dr.
St. Louis, MO 63146 U.S.A.
Ph. (314) 991-4545 or (800) 331-6661
Fax (314) 991-4692 or (800) 999-9925
Web: http://www.arc-inc.com
E-mail: arcinc@arc-inc.com

TECHNICAL DATA SHEET
ARC 3829 Triclosan [dichlorophenoxy ring-^{14}C(U)]

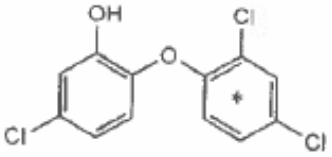
SPECIFIC LOT DATA:

Lot number: 150501

Specific activity: 77 mCi/mmol

Solvent: EtOH

Concentration: 0.1 mCi/ml



M.W. 289.54

PACKAGING INFORMATION:

ARC 3829 is packaged as a solution in ethanol in a screw cap vial. It is shipped in dry ice.

STABILITY AND STORAGE RECOMMENDATIONS:

The recommended storage temperature for ARC 3829 is -20°C. Its stability is currently under investigation.

RADIOCHEMICAL AND CHEMICAL PURITY:

It was determined on 5/01/15 and was found to be greater than 98 % by using the following conditions:

HPLC:

Column: Zorbax SB-C18 (250 x 4.6 mm), 5 µ
Mobile Phase A: 10 mM potassium phosphate
Mobile Phase B: Methanol
Isocratic: 72:28 B/A, v/v
Flow Rate: 1.0 ml/min
Detector A: UV at 280 nm
Detector B: β-RAM [^{14}C]

SYNTHESIS:

ARC 3829 was synthesized from 2,4-dichlorophenol [ring-14C(U)]. The product was purified by preparative HPLC.

Appendix III: Approval from Regional Committees for Medical and Health Research Ethics

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Gjøril Bergva	22845529	30.06.2015	2015/1032 REK sør-øst D
			Deres dato:	Deres referanse:
			12.05.2015	

Vår referanse må oppgis ved alle henvendelser

Berit Brunstad Granum
Nasjonalt folkehelseinstitutt

2015/1032 DermaTox

Vi viser til søknad om forhåndsgodkjenning av ovennevnte generelle forskningsbiobank. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst D) i møtet 10.06.2015. Vurderingen er gjort med hjemmel i helseforskningsloven § 25.

Forskningsansvarlig/databehandlingsansvarlig: Nasjonalt folkehelseinstitutt
Ansvarshavende: Berit Brunstad Granum

Prosjektleders beskrivelse av den generelle forskningsbiobanken
Hensikten med hudbiobanken er å kunne undersøke hvordan ulike kjemikalier absorberes i huden og hva som skjer med stoffene etter at de er tatt opp, samt undersøke fysiologiske og morfologiske endringer i huden. Vi vil dermed få mer kunnskap om hvordan disse kjemikaliene kan påvirke vår helse. Dette er kunnskap som vil være nyttig med tanke på risikovurdering og for lovreguleringen av bruken av kjemikalier som kommer i kontakt med huden vår, slik at vi på sikt kan få produkter som er trygge å bruke.

Vurdering
Komiteen understreker at forhåndsvurderingen knyttet til opprettelse av en generell forskningsbiobank er avgrenset til å gjelde tillatelse til å samle inn og lagre humant biologisk materiale. Selve bruken av materialet i konkrete forskningsprosjekter vil være underlagt krav om forhåndsgodkjenning av REK.

Det søkes om godkjenning til å opprette en generell forskningsbiobank for innsamlingsperioden 1/6-2015 til 1/6-2035. Biobanken skal oppbevare hud fra pasienter som gjennomfører plastisk kirurgi, fortrinnsvis mageplastikk, ved klinikker som utfører plastisk kirurgi. Hudprøvene vil avleveres anonymt, med opplysninger om donors kjønn og alder, samt hvilket område av kroppen huden er hentet fra. Det anslås at hudprøven vil forringes i løpet av 1-2 år, og at det til en hver til vil være om lag 30 hudprøver tilgjengelig i biobanken. Biobanken skal benyttes til forskning. Formålet med biobanken er å undersøke hvordan ulike kjemikalier absorberes i huden, hva som skjer med stoffene etter at de er tatt opp, samt undersøke fysiologiske og morfologiske endringer i huden. Donorene avgir samtykke. Det skal ikke innhentes hud fra mindreårige eller personer uten samtykkekompetanse.

Komiteen har ingen innvendinger til at det opprettes en generell forskningsbiobank. Etter komiteens syn er temaområdet avgrenset på en tilfredsstillende måte.

<u>Bruk av materiale fra forskningsbiobanken</u>
Komiteen forutsetter at Nasjonalt folkehelseinstitutt, ved ansvarshavende for forskningsbiobanken, i sitt arbeid følger lover og forskrifter vedrørende bruk av det humant biologiske materialet som inngår i den

generelle forskningsbiobanken. Dette innebærer blant annet at forskningsprosjekter som skal benytte humant biologisk materiale fra forskningsbiobanken må være forhåndsgodkjent av REK.

Når det gjelder opplysninger som er avledet fra det biologiske materialet, det vil si opplysninger som er generert ved analyser og prøver, vil oppbevaring, bruk og behandling av disse følge de vilkår som REK oppstiller i vurderingen av de konkrete forskningsprosjektene. Det påhviler ansvarshavende å påse at de vilkår som oppstilles av REK blir etterfulgt.

Hvis den generelle biobanken skal utvides til å omfatte annet enn beskrevet i søknad, må det søkes REK om utvidelse.

Informasjonsskriv og samtykkeerklæring
Komiteen har følgende kommentarer til informasjonsskrivet:
• Det bør komme tydeligere frem i første avsnitt at dette er en forespørsel om avgivelse av biologisk materiale til en generell forskningsbiobank som skal benyttes til fremtidig forskning.
• Overskriften i tredje avsnitt «Hva innebærer studien for deg?» og siste setning i tredje avsnitt «Da huden doneres anonymt, vil det ikke være mulig å identifisere deg i resultatene av studien når disse publiseres» må omskrives. Det dreier seg her om avgivelse av biologisk materiale til en generell forskningsbiobank, ikke deltagelse i en konkret studie.
• Det må tas inn informasjon om at donorene kan bli kontaktet på et senere tidspunkt med forespørsel om deltakelse i forskningsprosjekter, som ikke dekkes av allerede avgitt samtykke.
• Under avsnittet om Biobank står det: "Hvis du sier ja til å donere hud, gir du også samtykke til at huden og analyseresultater inngår i biobanken.". Komiteen gjør oppmerksom på at oppbevaring, bruk og behandling av analyseresultater vil følge de vilkår som REK oppstiller i vurderingen av de konkrete forskningsprosjektene. Det er altså kun det biologiske materialet som inngår i biobanken, ikke analyseresultatene. Det bes om at skrivet revideres i henhold til dette.
• Det oppgis i søknaden at det kan være aktuelt å utføre biologisk materiale anonymt til utlandet, uten at dette nevnes til deltagerne. Dette må deltagerne få informasjon om.

På denne bakgrunn setter komiteen følgende vilkår for godkjenning av den generelle forskningsbiobanken:
- Informasjonsskrivet skal revideres i tråd med komiteens kommentarer. Skrivet skal sendes komiteen til orientering.

Vedtak
Med hjemmel i helseforskningsloven § 25, tredje ledd, godkjenner komiteen opprettelse av en generell forskningsbiobank.

Godkjenningen er gitt under forutsetning av at vilkårene som er anført ovenfor blir etterfulgt.

Godkjenningen omfatter kun innsamling og oppbevaring av humant biologisk materiale. Bruk av humant biologisk materiale fra den generelle forskningsbiobanken må godkjennes av REK gjennom en konkret søknad om gjennomføring av et medisinsk og helsefaglig forskningsprosjekt.

Ansvarshavende skal sende søknad om biobankendring til REK dersom det skal gjøres vesentlige endringer i forhold til de opplysninger som er gitt i søknaden, jf. helseforskningsloven § 11.

Dersom forskningsbiobanken opphører, nedlegges eller overtas av andre, skal det søkes REK om tillatelse, jfr. helseforskningsloven § 30.

Melding om godkjenningen blir sendt Biobankregisteret.

Klageadgang
REKs vedtak kan påklages, jf. forvaltningslovens § 28 flg. Klagen sendes til REK sør-øst D. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK sør-øst D, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Vi ber om at alle henvendelser sendes inn på korrekt skjema via vår saksportal: http://helseforskning.etikkom.no. Dersom det ikke finnes passende skjema kan henvendelsen rettes på e-post til: post@helseforskning.etikkom.no.

Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen

Finn Wisløff
Professor em. dr. med.
Leder

Gjøril Bergva
Rådgiver

Kopi til: toril.attramadal@fhi.no
Biobankregisteret ved Nina Hovland: nina.hovland@fhi.no

Appendix IV: Approval from Regional Committees for Medical and Health Research Ethics

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Anette Solli Karlsen	22845522	02.10.2015	2015/1522/REK sør-øst A
			Deres dato:	Deres referanse:
			18.08.2015	

Vår referanse må oppgis ved alle henvendelser

Monica Andreassen
Divisjon for miljømedisin, Nasjonalt folkehelseinstitutt

2015/1522 Opptak og metabolisme av triklosan via hud

Forskningsansvarlig: Nasjonalt folkehelseinstitutt
Prosjektleder: Monica Andreassen

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst) i møtet 17.09.2015. Vurderingen er gjort med hjemmel i helseforskningsloven (hfl.) § 10, jf. forskningsetikkloven § 4.

Prosjektbeskrivelse (revidert av REK)

Formålet med dette prosjektet er å etablere en metode for å undersøke absorbsjon av kjemikalier via hud, samt å undersøke opptak og metabolisme av triklosan over hud.

Risikovurdering av kjemiske stoffer er på generelt grunnlag basert på oralt inntak. Da man også eksponeres for mange kjemiske stoffer via huden, vil ikke slike risikovurderinger nødvendigvis gi et bilde på den totale eksponeringen. Årsaken til at eksponering via hud ikke alltid tas med i risikovurderinger skyldes mangel på kunnskap om absorpsjon og metabolisme gjennom hud. I denne studien skal det etableres en metode for å studere absorpsjon og metabolisme av kjemikalier via hud, noe som potensielt kan bidra til at det kan genereres mer kunnskap om hvordan kjemiske stoffer tas opp og metaboliseres denne veien.

Det planlegges å benytte hudprøver fra 12 donorer i prosjektet. Hudprøvene er tidligere innsamlet til generell forskningsbiobank «DermaTox» (REK sør-øst saksnummer 2015/1032).

Etter etablering av metoden skal hudprøver eksponeres for triklosan. Deretter skal grad av absorbsjon og metabolisering måles.

Vurdering

Etter komiteens syn er dette en nyttig studie, der det skal etableres en metodikk for undersøkelse av i hvilke grad kjemikalier tas opp og metaboliseres i hud.

Søknaden omfatter bruk av biologisk materiale innsamlet i tidligere godkjent generell forskningsbiobank, «DermaTox» (REK sør-øst saksnummer 2015/1032). Etter komiteens syn er det foreliggende samtykket dekkende for de undersøkelser som planlegges gjennomført på materialet, samt for utførsel av materialet til Tyskland.

Vedtak

Prosjektet godkjennes med hjemmel i helseforskningsloven §§ 9 og 33.

Godkjenningen er gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknaden og protokollen, og de bestemmelser som følger av helseforskningsloven med forskrifter.

Godkjenningen gjelder til 01.01.2020.

Av dokumentasjonshensyn skal opplysningene oppbevares i 5 år etter prosjektslutt. Opplysningene skal oppbevares avidentifisert, dvs. atskilt i en nøkkel- og en datafil. Opplysningene skal deretter slettes eller anonymiseres, senest innen et halvt år fra denne dato.

Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kapittel 2, og Helsedirektoratets veileder for «Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse- og omsorgssektoren».

Prosjektet skal sende sluttmelding på eget skjema, jf. helseforskningsloven § 12, senest et halvt år etter prosjektslutt.

Dersom det skal gjøres endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende endringsmelding til REK, jf. helseforskningsloven § 11.

Komiteens vedtak kan påklages til Den nasjonale forskningsetiske komité for medisin og helsefag, jf. helseforskningsloven § 10 tredje ledd og forvaltningsloven § 28. En eventuell klage sendes til REK sør-øst A. Klagefristen er tre uker fra mottak av dette brevet, jf. forvaltningsloven § 29.

Med vennlig hilsen

Knut Engedal
Professor dr. med.
Leder

Anette Solli Karlsen
Komitesekretær

Kopi til:toril.attramadal@fhi.no; folkehelseinstituttet@fhi.no

Norges miljø- og biovitenskapelig universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

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NO-1432 Ås
Norway