Release of nanomaterials from consumer products and implications for consumer exposure assessment

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Preface

This PhD thesis presents research in the field of nanotechnology, with a specific focus on consumer exposure to nanomaterials from consumer products. The PhD study was conducted in the period from October 2013 to October 2016 at the Department of Environmental Engineering, Technical University of Denmark (DTU) under supervision of Associate professor Steffen Foss Hansen, PhD and co-supervision of Analytical chemist Mikael Emil Olsson, PhD.

The thesis is organized in two parts: the first part puts into context the findings of the PhD in an introductive review; the second part consists of the papers listed below. These will be referred to in the thesis by their paper number written with the Roman numerals **I-VII**.

I Hansen, S.F., Heggelund, L.R., Besora, P.R., **Mackevica, A.**, Boldrin, A. and Baun, A., 2016. Nanoproducts–what is actually available to European consumers? *Environmental Science: Nano*, 3(1), 169-180.

II **Mackevica, A.** and Hansen, S.F., 2016. Release of nanomaterials from solid nanocomposites and consumer exposure assessment–a forward-looking review. *Nanotoxicology*, 10(6), 641-653.

III **Mackevica, A.**, Besora, P.R., Brinch, A., and Hansen, S.F., 2016a. Current uses of nanomaterials in biocidal products and treated articles in the EU. *Environmental Science: Nano. In press.*

IV **Mackevica, A.,** Olsson, M.E. and Hansen, S.F., 2016b. Silver nanoparticle release from commercially available plastic food containers into food simulants. *Journal of Nanoparticle Research*, 18(1), 1-11.

V **Mackevica, A.,** Olsson, M.E. and Hansen, S.F., 2016c. The release of silver nanoparticles from commercial toothbrushes. *Journal of Hazardous Materials. In press.*

VI **Mackevica, A.,** Olsson, M.E. and Hansen, S.F., 2016d. Quantitative Characterization of Nano-TiO_2 Release from Fabrics by Single Particle ICP-MS. *Manuscript.*

VII **Mackevica, A.,** Olsson, M.E., Mines, P.D., Heggelund, L.R. and Hansen, S.F., 2016e. Estimation of dermal transfer of nanoparticles from consumer articles by wipe sampling. *Manuscript.*

In this online version of the thesis, paper **I-VII** are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from DTU Environment, Technical University of Denmark, Bygningstorvet, Building 115, 2800 Kgs. Lyngby, Denmark, info@env.dtu.dk.

In addition, the following publications, not included in this thesis, were also conducted during this PhD study:

Mackevica, A., Skjolding, L.M., Gergs, A., Palmqvist, A. and Baun, A., 2015. Chronic toxicity of silver nanoparticles to *Daphnia magna* under different feeding conditions. *Aquatic Toxicology*, 161, 10-16.

Nowack, B., Boldrin, A., Caballero, A., Hansen, S.F., Gottschalk, F., Heggelund, L., Hennig, M., **Mackevica, A.**, Maes, H., Navratilova, J. Neubauer, N., et al., 2016. Meeting the Needs for Released Nanomaterials Required for Further Testing- The SUN Approach. *Environmental science & technology*, 50(6), 2747-2753.

Sakka, Y., Skjolding, L.M., **Mackevica, A.**, Filser, J., Baun, A., 2016. Behavior and chronic toxicity of two differently stabilized silver nanoparticles to *Daphnia magna. Aquatic Toxicology. In press.*

Neubauer, N., Scifo, L., Navratilova, J., Gondikas, A., **Mackevica, A.**, Borschneck, D., Chaurand, P., Vidal, V., Rose, J., von der Kammer, F., Wohlleben, W., 2016. Use of polymers containing nanoscale coloristic pigments: Upper limits on releases by leaching, in food contact and from aged materials. *Manuscript.*

Hansen, S.F. and **Mackevica, A.**, 2016. Chapter 11: Methods and Tools for Assessing Nanomaterials and Uses and Regulation of Nanosilver in Europe. Book: Silver Nanoparticles for Antibacterial Devices: Biocompatibility and Toxicity. CRC Press. ISBN:9781498725323. *In press*.

Acknowledgements

First and foremost I would like to thank my supervisor Associate professor Steffen Foss Hansen, for the continuous encouragement, and for providing guidance and advice throughout the three years of this PhD study. Thanks also to my co-supervisor Analytical chemist Mikael E. Olsson for all the fun times in the lab, and for his endless patience and support during the times that were more frustrating. Additionally, very sincere gratitude goes to the always positive lab technicians Susanne Kruse and Sinh Nguyen, who were always there to help and provide practical advice.

I would also like to thank my colleagues at DTU Environment who have made this such a nicer workplace and who have given me joy to come to work every day. Firstly, thanks to the Nanorisk-group and the Environmental Chemistry section - it has been great to be a part of this team. And a special thanks to Odell and Paul for providing the escape from the work routine when needed.

Much gratitude goes to Otto Mønsted Fonden for financial support for the trips to conferences during this PhD study.

Thanks to my friends who were always there for me and provided much needed support – Monica, Krista, Robin, Piotr, Pablo, Michele, Lorenzo, Laura, Bibi, and Antonio, and many, many more. Finally, I would like to thank my family and relatives for their encouragement and support, even from a distance.

Summary

During the past decade the number of consumer products that contain nanomaterials (NMs) has been rapidly increasing. Materials manufactured at the nanoscale exhibit unique physicochemical properties and have greater reactivity in comparison to the bulk material. Because of this, NMs are being utilized in a wide variety of products, ranging from food and personal care products to electronics and large appliances.

Over the course of the last four years, the number of products claiming to contain NMs has increased from 1,200 in 2012 to more than 2,300 in 2016. The increasing use of nanoproducts and the uncertainties associated with the risks they may pose is raising concerns about consumer safety. During the use of nano-enabled products there is a potential for NM release, which can consequently lead to consumer and/or environmental exposure. Consumer exposure testing has only recently started to receive some attention, and the data currently available in the literature is scarce. Most studies are addressing only a narrow range of product categories and a few NM types, having experimental setups that are rarely comparable from study to study. Moreover, the analytical techniques applied for release testing are rarely suitable for reporting NM release with particle number concentration, size distribution or surface area concentration, which are known to be of toxicological importance.

The work presented in this thesis addresses the lack of data on consumer exposure to NMs from various consumer products. First, data from literature and online databases was used to obtain an overview of what nanoproducts are available on the EU market, and which nanoproducts have been experimentally tested for their potential NM release. Specific focus was placed on evaluating suitable analytical methods for NM quantification and characterization. The findings showed that single particle inductively coupled plasma mass spectrometry (spICP-MS) in combination with other methods is a well suited analytical technique that can provide extensive NM characterization, such as mass and number concentration, and size distribution of NMs. Then, several nano-enabled products were selected for experimental testing of NM release, namely four types of food contact materials (Ag) and two types of toothbrushes (Ag) for potential oral exposure, as well as five types of textiles (TiO_2) and five different surface

coatings (Ag and CuO) for potential dermal exposure. The NM release was characterized by using spICP-MS and transmission electron microscopy (TEM), together providing data for NM mass and number concentration, size distribution, and morphology. In most cases, it was found that NM release from the consumer products was in the ng/g (or ng/cm^2 where applicable) range. Ag release from food contact materials and toothbrushes was tested in food simulants (deionized (DI) water, ethanol, acetic acid) and tap water, respectively. The results showed that there is a potential for Ag exposure both in dissolved and nano-particulate form (up to around 6 µg/L and 40,000 particles/mL), but the amounts were magnitudes below the permitted Ag exposure limits set by European Food Safety Authority (EFSA) and World Health Organization (WHO). The TiO_2 release was tested for five types of textiles that did not openly disclose TiO_2 content. The fabrics were immersed in DI water, and the resulting amounts of potential Ti exposure were found to be up to around 8,000 particles/cm^2 corresponding to around 24 ng/cm^2. These amounts may be considered negligible compared to the reported Ti amounts in a wide range of products available on the market that claim to contain nano-TiO_2 as an additive, especially when it comes to food products. Dermal exposure testing for Ag and CuO surface coatings was done by wiping tests and revealed particle release very close to background levels, unless the surface was subjected to abrasion before executing the wiping tests. In general, all the products that were tested released very low amounts of the initial NM content present in the product, indicating that throughout long-term use of the products there might be continuous NM release, or most of the NMs would end up in solid waste.

The NM release data obtained both from the literature and from the experimental studies presented in this thesis were subsequently used for consumer exposure estimation. Several consumer exposure assessment tools were identified and their applicability for NM exposure assessment is discussed in this thesis. It was concluded that current consumer exposure assessment models have not been designed for estimating NM-relevant exposures, as they are mainly dealing with mass as a dose metric, without taking NM properties into consideration. This highlights the need of developing tools that are specifically designed for NM exposure assessment, taking into account not only potential exposure in terms of total NM mass, but also number concentration and size distribution.

All in all, the work presented in this thesis underlined various important issues that need to be considered and addressed when completing nanoproduct release testing, NM quantification and characterization, data reporting, and consumer exposure assessment. Firstly, there is an urgent need to apply a combination of characterization methods to gain a better understanding about the potential NM exposure. Secondly, standardization of NM release testing and data reporting is of key relevance, to ensure that the data generated is comparable among studies and can be extrapolated to other nanoproducts with similar properties. Finally, standardized data reporting and exposure assessment is of utmost importance to move towards harmonization of NM exposure and hazard characterization that could further aid NM-relevant risk assessment.

Dansk sammenfatning

Igennem det seneste årti er antallet af forbrugerprodukter, der indeholder nanomaterialer (NM), steget kraftigt. Materialer produceret i nanoskala besidder unikke fysisk-kemiske egenskaber og har større reaktivitet sammenlignet med materialet i andre størrelser. På grund af dette, bliver NM benyttet i en lang række produkter lige fra madvarer og produkter til personlig pleje, til elektronik og større husholdningsapparater.

I løbet af de sidste fire år er antallet af produkter, der hævdes at indeholde NM, steget fra 1,200 produkter i 2012 til mere end 2,300 produkter i 2016. Det stigende forbrug af nanoprodukter samt usikkerhederne omkring hvilke risici de udgør, giver anledning til bekymringer vedrørende forbrugernes sikkerhed. Ved brug af nanoprodukter kan NM potentielt frigives, hvorved forbrugere og/eller miljøet kan eksponeres. Kun for nyligt er eksponering af forbrugere kommet i fokus, og der er på nuværende tidspunkt få data tilgængelige i litteraturen. I de fleste studier er kun et begrænset udvalg af produktkategorier og få typer NM adresseret, og opsætningen af deres eksperimenter kan sjældent sammenlignes. Desuden er de analytiske teknikker, der anvendes til at teste frigivelsen af NM, sjældent velegnede i forhold til at rapportere antal partikler og deres overfladeareal per volumen eller partikelstørrelsesfordelingen, selvom det vides, at disse har en toksikologisk betydning.

Arbejdet i denne afhandling adresserer manglen på data vedrørende forbrugeres eksponering for NM i forskellige forbrugerprodukter. Først blev data fra litteraturen og online databaser benyttet for at danne overblik over hvilke nanoprodukter, der er tilgængelige på det Europæiske marked, samt hvilke nanoprodukter, hvis potentielle frigivelse af NM, allerede er blevet undersøgt. Specifikt er der lagt vægt på at evaluere hvilke analytiske metoder, der er passende for at kvantificere og karakterisere NM. Resultaterne viste at metoden single particle inductively coupled plasma mass spectrometry (spICP-MS) i kombination med andre metoder er en velegnet analytisk teknik, der kan tilvejebringe omfattende karakterisering af NM, såsom koncentrationen af partikler som en masse og som antallet af partikler per volumen samt NMs størrelsesfordeling. Derefter blev flere nanoprodukter udvalgt og deres frigivelse af NM blev undersøgt eksperimentelt. Fire typer af materialer i kontakt med fødevarer (Ag) samt to typer tandbørster (Ag) blev undersøgt for potentiel oral eksponering, og fem type tekstiler (TiO_2) samt fem forskellige overfladebelægninger (Ag og CuO) for potentiel

hudrelateret eksponering. Frigivelsen af NM blev karakteriseret ved brug af spICP-MS og transmissionselektronmikroskopi (TEM). Disse leverer tilsammen data om massen af partikler og antal partikler per volumen, partikelstørrelsesfordelingen samt morfologi. I de fleste tilfælde var frigivelsen af NM fra forbrugerprodukterne i størrelsesordenen ng/g (eller ng/cm^2). Frigivelsen af Ag fra materialer i kontakt med fødevarer samt fra tandbørster blev undersøgt i henholdsvis fødevare-lignende væske (deioniseret vand (DI vand), ethanol og eddikesyre) og postevand. Resultaterne viste at eksponering for Ag i både opløst og nanopartikel form (op til omkring 6 µg/L og 40,000 partikler/mL) potentielt kan finde sted, men koncentrationerne var dog langt under grænserne for tilladt Ag eksponering, som sættes af den Europæiske Fødevaresikkerhedsautoritet (EFSA) og Verdenssundhedsorganisationen (WHO). Frigivelsen af TiO_2 blev undersøgt i fire typer tekstiler, som ikke, ifølge produktinformationerne, skulle indeholde TiO_2. Tekstilerne blev nedsænket i DI vand og dette resulterede i en potentiel Ti eksponering på op til 8,000 partikler/cm^2 svarende til cirka 24 ng/cm^2. Disse mængder kan betragtes som ubetydelige sammenlignet med de mængder af Ti, der kan findes i en lang række produkter på markedet, der hævder at benytte nano-TiO_2 som tilsætningsstof, særligt når det gælder fødevarer. Hudrelateret eksponering fra overfladebelægninger med Ag of CuO blev undersøgt ved hjælp af overtørrings-tests (wiping tests) og resulterede i en frigivelse af partikler tæt på baggrundsniveauet, medmindre overfladen først blev udsat for slitage. Alle de produkter, der blev testet, frigav generelt meget lave mængder af NM i forhold til det oprindelige indhold af NM. Dette indikerer, at der gennem langvarig brug af produkterne kan være en kontinuerlig frigivelse af NM eller at størstedelen af NM vil havne i fast affald.

Frigivelsesdata fra litteraturen og de eksperimentelle forsøg blev efterfølgende benyttet til at estimere forbrugerens eksponering. Adskillige forbruger-eksponeringsværktøjer blev identificeret og i denne afhandling diskuteres deres anvendelighed i forbindelse med at evaluere eksponering for NM. Det konkluderes at de nuværende modeller til at vurdere forbruger-eksponering ikke er designet til at estimere eksponering for NM, eftersom de primært arbejder med dosis som en masse uden at tage hensyn til NMs særlige egenskaber. Herved fremhæves behovet for at udvikle redskaber specifikt designet til at vurdere eksponering for NM, som netop kan udtrykke den potentielle eksponering som antal partikler per volumen og deres størrelsesfordeling, og ikke kun den totale masse af NM.

Alt i alt understregede arbejdet i denne afhandling forskellige vigtige problemstillinger, som skal overvejes og adresseres ved udarbejdelse af frigivelsestests, NM kvantificering og karakterisering, datarapportering samt vurdering af forbrugereksponering. For det første er der et akut behov for at anvende en kombination af karakteriseringsmetoder, så der opnås en bedre forståelse af den potentielle eksponering for NM. Her virker spICP-MS og TEM som en stærk kombination. For det andet er det af største betydning at frigivelsestests og datarapportering bliver standardiseret, for at sikre at data er sammenlignelige og kan ekstrapoleres til andre nanoprodukter med samme egenskaber. Afslutningsvis er det yderst vigtigt at standardisere datarapportering og vurdering af eksponering, for at kunne bevæge sig hen imod en harmoniseret eksponeringsvurdering og farekarakterisering af NM, som yderligere vil kunne understøtte en nano-specifik risikovurdering.

Table of contents

1 Background and aims

Engineered nanomaterials (NMs) are usually referred to as materials that are found 1-100 nm in size in at least two external dimensions, and nanoparticles (NPs) specifically are known as particles in size range of 1-100 nm in all three dimensions (BSI, 2007). These materials exhibit unique properties due to the considerably higher surface to volume ratio, such as increased reactivity in comparison to the bulk material (Navarro et al., 2008). The amount of commercially available consumer products containing engineered nanomaterials (NMs) has been markedly increasing during the last decade, covering a broad spectrum of applications ranging from electronics and appliances to personal care products and food items (PEN, 2016; The Nanodatabase, 2016), thereby implying potential environmental and/or human exposures through various routes.

Both the increasing production amounts and the uncertainties regarding NM properties and toxicity are raising concerns about human and environmental safety (Klaine et al. 2012; Mitrano et al. 2015). Currently, there are a number of major knowledge gaps regarding the health and environmental risks posed by NMs (Godwin et al., 2009; Lynch, 2015; WHO, 2013). One of the major knowledge gaps is the release of NMs from various commercial products. Nm release data would assist in characterizing the actual exposure thus helping to provide a more realistic hazard assessment and consequently risk assessment (Ostertag and Hüsing, 2008). The main concern is uncontrolled release during the use and disposal of NM-containing products (Gottschalk and Nowack, 2011; Nowack and Bucheli, 2007), which is difficult to account for.

The overall aim of this PhD project is **to investigate release of NMs from nano-enabled consumer products** by applying suitable analytical methods for NM analysis with specific focus on the following tasks:

- Using the available data from the literature to identify the current knowledge gaps when it comes to pontential consumer exposure to NMs (Paper I, II and III)
- Identifying and applying suitable analytical methods for quantification and characterization of NM release, and test NM release from simulated use and anticipated consumer handling of selected consumer products (Paper IV, V, VI and VII)

- Assessing and evaluating to what extent NM release data from the literature can be used for consumer exposure assessment and discuss the research needs (Paper II).

The above was accomplished by reviewing the literature, thus making a summary of which consumer products have been experimentally investigated to obtain release rates, as well as describing various methods that have been used to describe the release. As the next step, a set of various consumer products (food containers, toothbrushes, textiles, keyboard covers, painted wood) was chosen for experimental determination of the potential release of NMs by using such characterization techniques as Electron Microscopy (EM) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The final step included discussing challenges regarding integration of the obtained data in consumer exposure assessment models and discussing further research needs. The flow of the thesis as well as overlapping topics discussed in the papers is depicted in Figure 1.

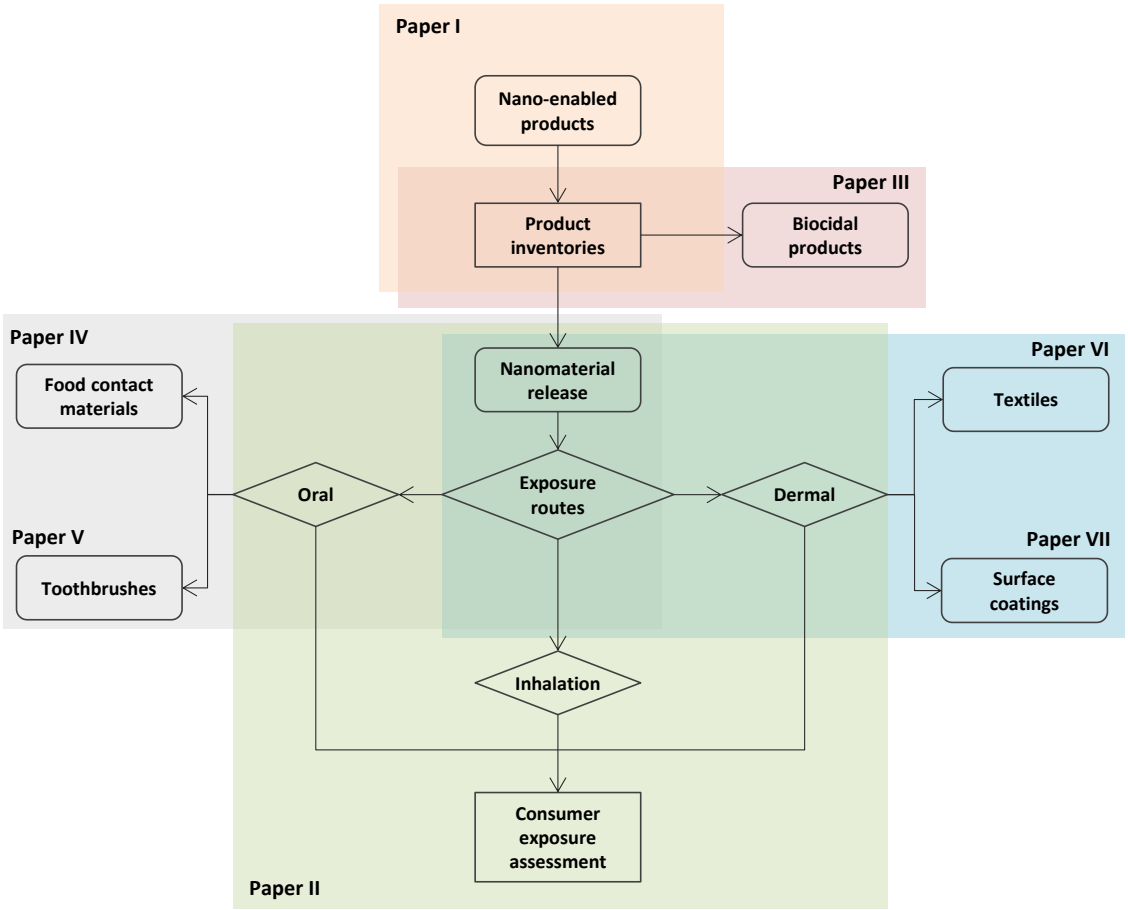

Figure 1: Flow chart reflecting the topics covered through the thesis and overlap of the themes addressed by the scientific papers included in the thesis.

Papers I, II and III are based on the data found in the literature or online resources. Papers I and III are more specifically dealing with nanoproduct availability on the EU market. Paper II is a review over the literature that provides experimental data with release from NM-containing items, and this data is being used for consumer exposure assessment by using various models. Papers IV, V, VI and VII are experimental papers assessing the NM release from selected consumer products (food contact materials, toothbrushes, textiles, and surface coatings).

2 Nanomaterials in consumer products

Nanotechnology in general represents a field of technology that studies and utilizes various materials in sizes at the scale of 1 – 100 nm, called nanomaterials. Working at the nanoscale allows manipulation of the material to acquire properties that may differ from those present at the molecular level or in the bulk form of the material. Nanoscale therefore provides opportunity for novel applications, some of which can benefit from increased reactivity and functionality of the material in the nanoscale because of the greater surface-to-volume ratio, whereas for other applications it may be beneficial to utilize the optical properties at the nanoscale, or to obtain improved dispersions. This provides the basis for development of countless applications covering various fields such as medicine and drug delivery, water purification, electronics, catalysts, food additives, and personal care products to name a few (WHO, 2013).

A number of scientific publications and inventories have illustrated that the use of nanotechnology in consumer products has been considerably increasing in the last decade (e.g. PEN, 2016; The Nanodatabase, 2016; Vance et al., 2015). Several inventories have been established to compile the available information regarding nano-enabled products on the market. For instance, a few publically available databases are the Consumer Product Inventory (CPI) by the Project of Emerging Nanotechnologies (PEN), and The Nanodatabase established by DTU Environment, The Danish Ecological Council and The Danish Consumer Council (Hansen et al. 2016 - Paper I). The CPI was the first nanoproduct inventory and it arguably tends to have a focus on the North American market and is updated annually it was originally launched in 2006 (PEN, 2016; Vance et al., 2015). The Nanodatabase is an online inventory of NM-containing products, where the presence of NMs in the product is claimed by manufacturers or others (e.g. retailers, product reviews). The focus is set specifically on the products that are available on the European market. Along with a description of the product, The Nanodatabase provides the available exposure and hazard information (The Nanodatabase 2016; Hansen et al. 2016 - Paper I).

One major advantage for The Nanodatabase is the analysis function, which allows users to conduct their own independent analysis using the data available. By analyzing the ca.2300 products currently listed in the database, it becomes apparent that a large fraction (about one half) of all products contains nanomaterials that cannot be identified and are thus labelled

"unknown". Apart from that, the most popular nanomaterials found in the products are silver, titanium dioxide, carbon-based NMs and silicon dioxide, which is a similar pattern to what is also observed in the CPI (see Figure 2).

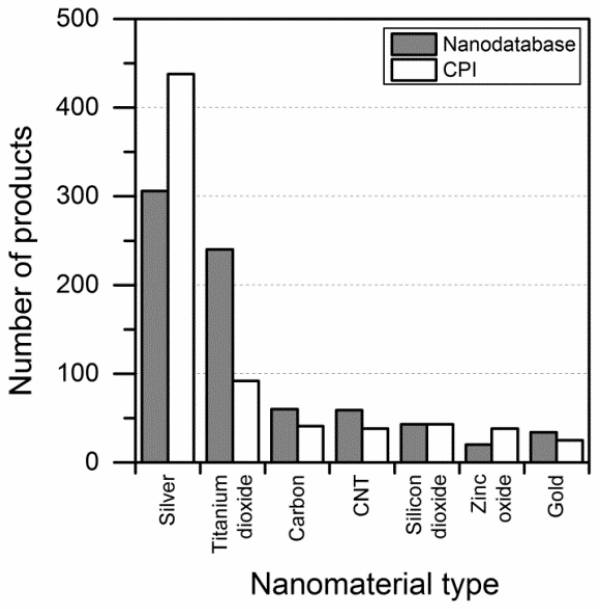

Figure 2: Nanomaterials claimed to being used in different commercial products in The Nanodatabase and in the CPI (Hansen et al. 2016 – Paper I).

Silver nanoparticles are used as additives for various products mainly due to their antimicrobial properties and low production cost (Kaegi et al., 2010). Some of the most popular uses involve applying Ag as an additive in fabrics, due to its odor fighting functions and for sanitary reasons, as well as for inner coatings of various food contact materials to keep the food fresh for longer periods of time (PEN, 2016). Titanium dioxide nanoparticles offer a broad range of applications due to their antibacterial, optical, UV-protective and photocatalytical properties (Bogdan et al., 2015; Radetić, 2013). Large amounts are produced and added as a pigment, as TiO_2 nanoparticles are white and can help make the product matrix more opaque, which is especially useful for coatings and paints (Varner, 2010), but is also widely used in food products (Peters et al., 2014c). TiO_2 is also widely applied as a photocatalyst which provides a self-cleaning effect for various surfaces (Bogdan et al., 2015). Another relatively popular nanomaterial, namely SiO_2, is used for coatings to provide hydrophobic properties, improve hardness and scratch resistance (Zhou and Gu, 2004), as well as in various food products as a thickening and anticaking agent (Peters et al., 2012).

When it comes to looking at NM use across different consumer product categories in The Nanodatabase, it becomes apparent that most products fall

under “Health and Fitness” category (55%), followed by “Home and Garden” (21%) and “Automotive” (12%) (see Figure 3). Silver nanoparticles constitute a considerably large fraction of products for most product categories, and are found in a wide range of items, such as food supplements and food contact materials, personal care products, textiles, household appliances, cleaning products, and many more. Advertising nano-Ag as a product ingredient usually comes hand in hand with an antibacterial claim. Nano TiO_2 content, on the other hand, is not advertised as much, although it is found mainly in sunscreens and other personal care products, and plenty of food items (The Nanodatabase, 2016).

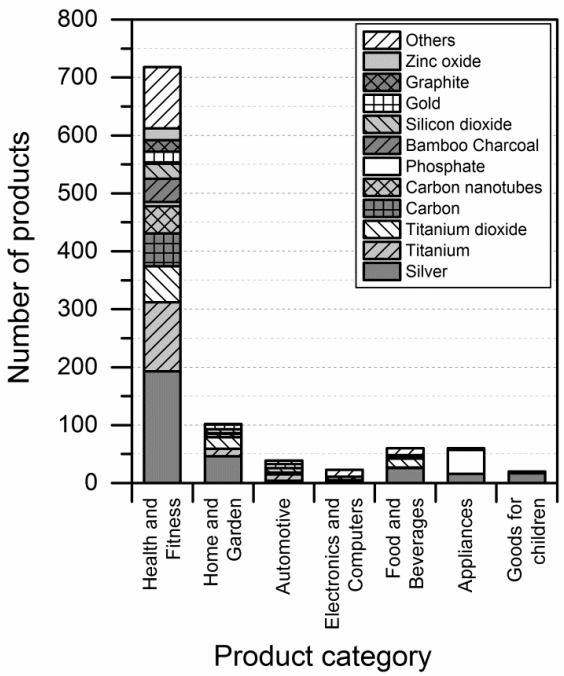

Figure 3: Identity of nanomaterials across different product categories, excluding the products where the nanomaterial identity is “unknown”. Individual products may include more than one type of nanomaterial (Hansen et al. 2016 – Paper I).

In this PhD thesis, the focus is set on the use of such NMs as silver, titanium dioxide, and copper oxide, which are present in consumer products and may lead to human exposure during use. The aforementioned NMs and their potential for consumer exposure will be discussed in greater detail later in the text.

3 Detection, characterization and quantification of nanomaterials

As noted above, engineered NMs have unique properties that are determined by their composition, size, shape and surface functionalization, which make them different from the bulk materials composed of the same elements. These nano-specific properties greatly influence the fate and possible transformation of NMs both in the environment and in the biological systems (Nowack et al., 2012). As nanotechnology is a relatively new and rapidly emerging field of technology, adequate characterization of NMs has become a fundamental need for industry, scientific research and risk governance. Working at the nanoscale brings a whole set of new challenges when it comes to sample preparation and characterization. NM-containing sample analysis require a wide range of considerations that may have to cover plenty of factors, such as concentration, size distribution, dissolution, aggregation, agglomeration, surface transformations, all of which can be quite challenging for current characterization methods (Montaño et al., 2014; von der Kammer et al., 2012). In this section the available analytical methods will be discussed and put into perspective when it comes to analyzing NMs in consumer products and the release of NMs from these products during their use.

Up until recent years, the analytical methods for characterization of NMs have been very limited and almost solely focused on analyzing NMs in pristine conditions. It is much more complicated to acquire data regarding the behavior of NMs in more complex matrices and media at realistic concentrations and conditions (Klaine et al., 2012). The behavior of NMs in the matrix and in the environment will be affected by a wide range of factors, such as particle number and mass concentration, surface properties, reactivity, size distribution, state of aggregation, chemical composition, as well as structure and shape. Therefore, the analysis of NM is different from traditional chemical analysis because both chemical and physical aspects must be considered.

3.1 Chemical analysis and characterization of released NMs

Ideally the analytical methods should provide information on the chemical identity of NM, mass and number concentration of the substance, aggregation and agglomeration, as well as size and shape of the NM present in the

sample. A wide range of analytical tools is available for characterizing NM-containing matrices to address the aforementioned questions, but they have various limitations when it comes to analyzing complex samples containing nanomaterials (von der Kammer et al., 2012).

Depending on what the sample of interest is – solid, liquid, or aerosol – there are different set of methods that can be applied to find and characterize NMs in a sample. As the work in this thesis has been mainly focusing on characterizing NMs suspended in liquids (i.e. NMs released from products into liquid media), this section will discuss various analytical tools and methods that are applicable for liquid sample characterization.

Several literature reviews have been published in the past decade to provide an overview of the available and developing analytical techniques for NM analysis (Hassellöv et al., 2008; Howard, 2010; Pérez et al., 2009; Tiede et al., 2008, to name a few). The most recent review by Laborda et al. (2016) has provided a very solid description of state-of-the-art techniques that have been applied in experimental work for detecting and characterizing NMs in various matrices, including consumer products, natural waters, wastewater, biological samples, as well as tools for the characterization of release of NMs from consumer products, at the same time highlighting a variety of sample preparation methods (Laborda et al., 2016). Some of the techniques that are commonly applied for detection and analysis of NMs include e.g. electron microscopy (SEM and TEM), dynamic light scattering (DLS), atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), size exclusion chromatography (SEC), asymmetric flow field flow fractionation (AF4), and ultraviolet-visible (UV-vis) spectroscopy techniques, among others.

For the detection and characterization of released fragments or aged surfaces, electron microscopy techniques such as TEM and SEM can be used in combination with electron dispersive X-ray spectroscopy (EDS) to identify the composition of the released materials. TEM and SEM are useful for characterizing NMs to provide information regarding size and shape of NMs, as well as providing an indication about agglomeration and aggregation of NMs in a sample. However, one must keep in mind that sample preparation can often result in introduction of artefacts, due to e.g. centrifugation or drying of the sample before analysis (Tiede et al., 2008). TEM and SEM are powerful tools for imaging and sizing NMs, but not for rapid quantitative analysis of particles in the media in which the particles may be released (e.g.

water, air, food simulants), mostly because of the low particle number concentrations in the media. It may become very time consuming and ineffective when attempting to work with environmentally relevant concentrations in the μg/L or ng/L range. However, for qualitative analysis of "unknown" samples (i.e. samples that are not homogenously distributed standard suspensions of NMs in deionized water with known chemical identity) it can be very useful to do EM imaging to acquire information regarding NM shape and size, and to verify whether the particles are occurring as single particles in suspension, or as aggregates/agglomerates, and whether or not they are attached to some other molecules, complexes, or solids (Mackevica et al. 2016b,c,d,e – Paper IV, V, VI, VII). Information regarding physical properties can be particularly useful for verification of measurements using other techniques that do particle sizing based on assumptions of particles being spherical, such as spICP-MS, AF4-ICP-MS or DLS.

A promising technique that has seen a rapid development in the past decade for detection and characterization of NMs is spICP-MS. It has the capability to address many of the current analytical challenges, since by using spICP-MS it is possible to report particle number concentration, particle size, particle mass concentration and dissolved fraction in both simple and complex matrices. The basis for spICP-MS particle size calculations is the information on presumed particle shape, density and composition. The basics of the spICP-MS technique were first described by Degueldre & Favarger (2003) for analysis of natural metal oxide colloids, and since then it has been further developed for different nanoparticle detection in various matrices, as described in a recent review over spICP-MS applications and recent development and perspectives (Laborda et al. 2013). In brief, the way spICP-MS operates is that each time an individual particle enters the plasma, the particle is ionized and subsequently detected as a pulse. The intensity of the pulse is proportional to the number of the detected ions, and therefore also the original size of the particle. By collecting what is called time-resolved data, it becomes possible to calculate both the number concentration and the size distribution of the particles in a sample.

An inter-laboratory study by Peters et al. (2014b) attempted to analyze metallic nanoparticles by applying various methods based on completely different physical principles, such as TEM, DLS, Differential centrifugal sedimentation (DCS), spICP-MS and Micro-proton induced X-ray emission (PIXE). The aim of the study was to estimate whether these techniques can

produce similar results for pure and homogenous suspensions of NMs. TEM, SEM and spICP-MS were found to be more accurate for the determination of particle size compared with NTA, DLS and DCS. However, it was shown that spICP-MS and PIXE were most accurate for the determination of mass concentrations of nanoparticles in pure suspensions.

Single particle ICP-MS has low detection limits when it comes to both nanoparticle mass and particle number concentrations. Usually it is recommended to have particle number concentrations in suspensions below 10^8 L^{-1} in order to avoid occurrence of multiple particle events and assure single particle events to better represent the conditions of the sample itself (Laborda et al., 2011). The lowest measurable particle number concentration is limited by the relative frequency of background effects or "false positives". This can be improved by acquisition of more data points and choosing a proper detection threshold to avoid including false positives as particle events (Tuoriniemi et al. 2012). When working with more concentrated samples, dilution might be necessary, but other than that spICP-MS allows rapid nanoparticle analysis with little or no sample preparation. By using various types of spICP-MS instruments, it has been experimentally determined that the optimal concentration range is from 10^3 to 10^5 mL^{-1}, and, depending on the element, experimentally determined lower size detection limits for metal and metal oxide particles usually range from 15 to 60 nm, and upper detectable size limits are around 200 nm (Laborda et al. 2013; Lee et al. 2014; Liu et al. 2014). The study by Lee et al. (2014) has presented lower size detection limits for 40 different elements and calculated that some elements (e.g. Ta, U, Ce) could be detected even in sizes below 10 nm. The issue of distinguishing smaller particle signals from background or from the dissolved analyte signal has been discussed by many, highlighting the importance of detecting smaller particles that might be of higher concern when it comes to hazard evaluation to NMs. A study by Cornelis & Hasselöv (2014) presented a signal deconvolution method to enable discrimination of smaller nanoparticles from the background signal. With this approach it was possible to acquire accurate particle number and size measurements for Au particles as small as 10 and 15 nm and successfully distinguish between overlapping dissolved and NP signals. This method could be of particular importance for NPs that tend to dissolve, such as Ag, ZnO or CuO NPs.

For majority of experimental data reported in the literature, the "window" for spICP-MS particle detection capabilities that allows accurate sizing lies somewhere between 20 and 250 nm (depending on the NM), which means

that there is still a high uncertainty associated with measuring particles below and above the size detection limit. However, it must be noted that the upper and lower size detection limits are dependent on the density of the particle. For instance, when it comes to upper detection limit, particles of lower density, low molecular weight of the target analyte, and low boiling point tend to vaporize more efficiently in the plasma and thus make it possible to detect and accurately measure the diameter of larger nanoparticles, even in sizes above 250 nm (Lee and Chan, 2015).

Most studies using spICP-MS have mostly been focusing on measuring homogenously distributed NP suspensions to evaluate the capabilities of the spICP-MS analysis. In the cases of heterogeneous samples in matrices other than DI water, there are several considerations that need to be taken into account. For more complex samples, the spICP-MS measurements would probably not represent the "whole picture", as the total NM content is composed of a wide size range of particles, and particles that are too small, too big, or aggregated/agglomerated, or attached to other molecules pieces of a matrix they have been embedded in, will not be detected (see Figure 4).

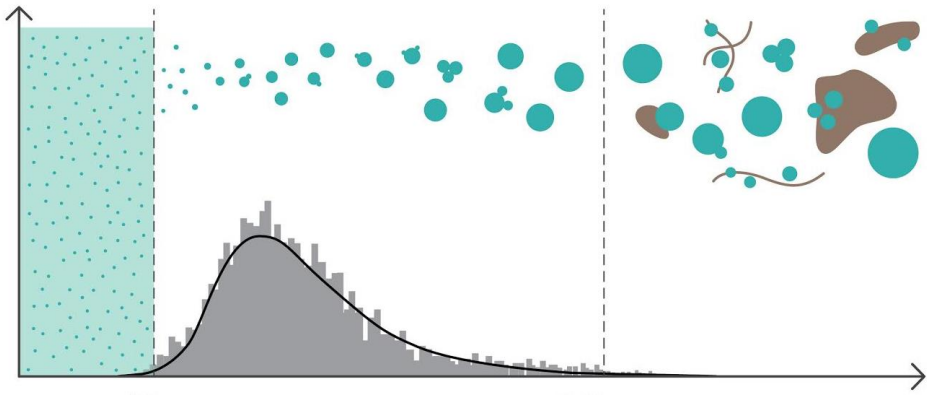

Figure 4: Illustrative schematic representation of single particle ICP-MS analysis and the challenges and limitations associated with it when assessing the release of nanoparticles from consumer products. Dashed lines represent lower and upper size detection limits.

This is especially important when working with "unknown" samples, like in cases of analyzing release of NMs from consumer products. In those cases, the sample may contain not only particles and dissolved material, but also nanoparticles attached to the pieces of the matrix of the product, which will not reach the plasma and will not be analyzed as particles. Several publications have illustrated this issue by providing electron microscopy images of released fragments, which have nanoparticles attached to them or embedded inside (e.g. Mackevica et al. 2016b,c – Paper IV and V). This is

why spICP-MS measurements should usually be complemented with EM imaging and/or the measurements of the total mass content of the NM.

As spICP-MS is an emerging analytical technique for nanomaterial characterization and is still under development to be established as a routine procedure for NM analysis, the information available in the literature is very limited when it comes to specific applications. To date, there are 56 publications available on ISI Web of Science™ (search term "single particle ICP-MS"), and most are focusing on analysis of gold or silver nanoparticles. The number of publications has been rapidly increasing in the past few years, and the focus is shifting towards analyzing nanoparticles in more complex matrices and more environmentally relevant conditions. It has been reported in a handful of experimental studies that spICP-MS has been used for the analysis of nanoparticle release from various consumer products, such as silver and titanium dioxide release from textiles (Wagener et al. 2016, Mackevica et al. 2016d – Paper VI), and silver release from washing machines (Farkas et al., 2011) and food contact materials (Echegoyen and Nerín, 2013; von Goetz et al., 2013a, Mackevica et al. 2016b – Paper IV), and toothbrushes (Mackevica et al. 2016c – Paper V). Single particle ICP-MS measurements of the released particles, together with other types of characterization methods, such as electron microscopy and conventional ICP-MS analysis are working towards providing an indication of the "whole picture" when it comes to assessing what consumers might be exposed to while using different products.

3.2 Identification of NMs in consumer products

As noted above, there is a large amount of consumer products available on the market that claim to be containing NMs. There are many products that contain NMs and do not properly label the NM content, even though several product groups, such as cosmetics, biocidal products and food items, are required to note "nano" in the list of ingredients (Mackevica et al. 2016a – Paper III). The verification of the claim that the products in fact contain NMs can become quite complicated, depending on what matrix the product represents – whether it is a spray (e.g. disinfectants and cleaning products), liquid (e.g. dietary supplements, personal care products), or solid (e.g. appliances, food contact materials, textiles). Various methods exist to characterize the NMs in the product or to extract particles from their product matrix that would allow analyzing the NMs that are actually present in the product.

For example, when it comes to characterization of NMs in liquid samples such as sunscreens or other personal care products, suitable analysis techniques include electron microscopy in combination with electron dispersive X-ray spectroscopy (EDS) to find and identify the nanoparticles (Lewicka et al., 2011). Alternatively, spICP-MS may also be utilized as a tool for identification and quantification of NM content in such samples simultaneously providing size distribution, as described by (Dan et al., 2015) for nano-TiO_2 analysis in commercial sunscreens. The same method may also be applicable for many other personal care products with comparable matrices. Similar output can be achieved with other tools such as AF4 coupled with ICP-MS or UV-Vis (Contado and Pagnoni, 2008; Nischwitz and Goenaga-Infante, 2012). Other examples include Ag and ZnO nanoparticle characterization by TEM from disinfectant sprays (Hagendorfer et al., 2010; Lorenz et al., 2011) and characterization of various dietary supplements for Ag, Au, Cu, Si, Zn and other nanoparticle content by both spICP-MS and TEM-EDS (Reed et al., 2014). Testing of several food additives in food items has also been addressed in the literature, for instance quantification of nano-TiO_2 in commercial food items by AF4-ICP-MS and spICP-MS following a digestion procedure with hydrogen peroxide H_2O_2 (Peters et al., 2014a). Another approach is spiking of various food items with nanoparticles in laboratory setting, such as e.g. chicken meat spiked with Ag nanoparticles, which was subsequently enzymatically digested and analyzed by AF4-ICP-MS or spICP-MS (Loeschner et al., 2015, 2013; Peters et al., 2014a).

All in all, even when it comes to analyzing NMs in consumer products with complex matrices, there are various methods available to tackle the NM characterization and quantification issues. However, many of the methods require extensive sample preparation for e.g. NM extraction, which may result in particle dissolution or aggregation, and the digestion medium might include even more uncertainties during analysis of the samples, which may hinder accurate nanoparticle characterization.

3.3 Current challenges for NM analysis

Even though there have been great advancements for NM analysis in the recent decade, there is still a long way to go until there will be standardized procedures for NM characterization and quantification that would be routine in the specialized labs working with nanomaterials. To reach this stage, there is a great need for additional development of standard methods both for

sample preparation and analysis in simple and complex matrices, as well as for reference materials.

Considering current situation regarding NM analysis, there are several well established techniques for obtaining physicochemical information about inorganic NMs. For quantitative measurements, analytical techniques are emerging and existing techniques are rapidly improving and becoming more ubiquitously used. Specifically the use of spICP-MS and AF4-ICP-MS are becoming more extensively used and new applications are being developed for quantitative NM characterization in different matrices (Laborda et al., 2016).

Another important issue when it comes to NM analysis is the question of how to report the data, i.e. which metrics to use for reporting of NM content and properties. It has been reportedly pointed out that even if mass concentration is generally regarded as the most robust metric for concentration reporting, it might not be the most significant metric when it comes to NM exposure or hazard assessment. It is usually recommended to report at least one other metric alongside with mass content (WHO, 2013). Experimental research papers dealing with NM and consumer products (excl. airborne materials and sprays) are generally reporting NM content as mass percentage or mass per unit volume in case for liquids. As for the release of NMs from products, mass concentration is most often the only metric reported, with some papers reporting qualitative characterization by electron microscopy (Mackevica & Hansen, 2016 – Paper II). In the past couple of years, however, quantitative characterization of size distributions and particle number concentration are also becoming increasingly popular for reporting in papers, especially when applying such experimental techniques as spICP-MS and AF4-ICP-MS for particle analysis.

4 Nanomaterial release from consumer products

With an increasing variety of consumer products, there is a growing opportunity for humans and environment to be exposed to NMs and their residuals. Generally, research has been focusing primarily on hazard identification, i.e. toxicological impacts of NMs, and exposure assessment has received much less attention (WHO, 2013). Environmental or human exposure to NMs can occur in various steps, it might be during NM production, product formulation, product use and disposal. When it comes to NM-enabled consumer products, the end user may be intentionally or unintentionally exposed to the NMs. During common use of the products, they can be subjected to various controllable and uncontrollable conditions, which may increase the potential for the release and transformations of NMs. Released NMs are often different from the pristine parent material in terms of size, aggregation and agglomeration state, and they can be released either free or together with fragments of the product matrix.

Over the past decade, the use and diversity of NM applications has expanded rapidly, leading to more and more questions about uncertainties regarding potential human and environmental exposure and toxicity. Therefore, it is of utmost importance to investigate and quantify the release of NMs from consumer products, to move closer towards understanding NM fate and transformations, as well as risks associated with exposure to NMs.

This section will focus on release of NMs from consumer products, discussing such issues as the importance of understanding exposure scenarios from a consumer perspective, as well as discussing current guidelines and methods that are used for potential consumer exposure measurements, highlighting the issues that arise from inapplicability of the current guidelines to nano-specific release measurements.

4.1 Life cycle considerations for nanomaterial release

There are multiple ways in which nanomaterials can be released from consumer products along the life-cycle of a given product. As stated before, currently there is a high uncertainty over effects of NMs to human health and the environment, which is why a comprehensive risk assessment is needed, taking into account all the possible exposure scenarios throughout the life-

cycle of the product that may lead to NM release (Ostertag and Hüsing, 2008). The life-cycle is generally understood as the life span of a certain product, covering production of pristine NM and then manufacturing the NM-containing products, product use, disposal and possibly recycling (see Figure 5). The product life cycle determines in what phase and what environmental compartment NMs may be released and where they might end up (Som et al. 2010).

There is general consensus in the scientific community and regulatory agencies that ideally, the potential health and environmental risks of NMs should be evaluated over their entire life-cycle (Seager & Linkov 2008; Grieger et al. 2012; SCENIHR 2009). The reason behind this is that investigating these risks should consider the various life-cycle stages which span from extracting the raw materials for producing NM through their end-of-life stages as a disposed product or material. This would help acquiring a more comprehensive and complete overview over all potential risks to human health and the environment (Grieger et al., 2012).

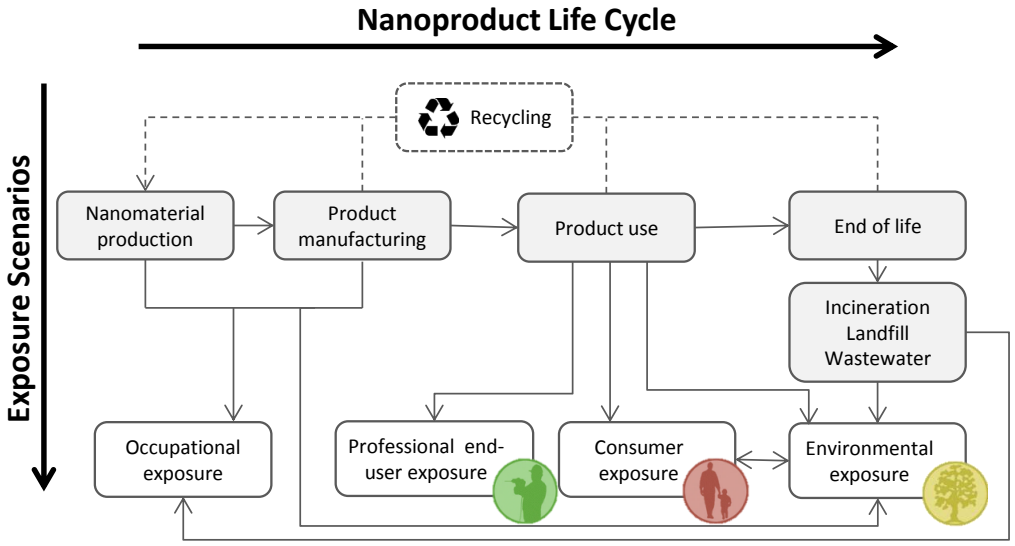

Figure 5: Simplified stages of the nano-enabled product life cycle and the fate of the released NMs.

Generally, the release of NMs to the external environment can happen at any point of a product's life cycle (production, use, disposal, Figure 5), but most often NMs will enter the environment when they are released from products through product use, disposal or weathering (Gottschalk and Nowack, 2011). The NM emissions in the production stage are usually easier to characterize, as the working environment is more controlled and the working conditions have to be regulated. Therefore, uncontrolled and/or unintentional release

during use and disposal of NM-containing products is raising more concerns (Gottschalk and Nowack, 2011; Nowack and Bucheli, 2007). Occupational exposure has been evaluated relatively extensively, while there is distinct lack of understanding for what happens during use and disposal of NMs. In this thesis the focus is placed on NM release from consumer products during the use phase.

When it comes to assessing consumer exposure to NMs, the most important part of the life-cycle is the use phase, which is basically when a "finished" product is available on the market and the consumer is free to use it. As the products are different, and the users may have their own interpretation about how the products should be used, it is difficult to identify one unifying scenario of how a product could be utilized and under what conditions the NM release might occur (ECHA, 2012).

Most research related to NM release from consumer products and consumer exposure so far has mainly been focusing on illustrating NM release rates in relatively short time frames (short relative to the real-life use of the product) and imitating only few scenarios of product use that can represent possible NM emissions. The experimental setups that attempt to mimic product use are often far from the real-life conditions, which makes it difficult to interpret the data in the context of environmental and consumer risk of exposure. As an example, washing of textiles without detergent can hardly provide characterization of real-life emissions of NMs, but it makes it easier to characterize the NM release, as it is a simpler matrix. As it is very time consuming and expensive to conduct experimental investigations of the whole use phase of the life-cycle of a certain product, it is necessary to find a method that would be representative enough for describing the NM release rates over a longer period of time. This could eventually make it possible to extrapolate the NM release over the whole use phase of the consumer product (Mackevica & Hansen, 2016 – Paper II).

4.2 Nanoproducts and the potential for consumer exposure

Human exposure to NMs from consumer products may occur in various different ways. The most obvious one is direct exposure, during consumer use of the product, as for instance, application of sunscreen on the skin, or ingestion of dietary supplements. Another option is indirect exposure, when NMs are released from the products e.g. in the air from disinfectant sprays, or

in food items from food contact materials. The way product is used will contribute to the extent of exposure, possible exposure routes being inhalation, dermal, oral, or a combination (Wijnhoven, 2007).

The location of NM in the product will have a high influence on the NM properties and the potential for NM release that subsequently could lead to consumer or environmental exposure. The categorization framework presented by Hansen et al. (2007), groups the products based on the location of NM in the product, the options being: dispersed in solids, suspended in liquids, surface bound, or free airborne particles (Hansen et al., 2007). For instance, such products as dietary supplements or cosmetics would fall into "suspended in liquids" category and products like coated food contact materials would have "surface bound" NMs. The products that have NMs "suspended in solids" are e.g. plastics with CNTs or silica fillers to have stronger, more durable and abrasion-resistant material, which is useful for such products as tennis rackets and tires (The Nanodatabase, 2016). The overall use of NM-containing products and potential human exposure is schematically depicted in Figure 6. Briefly, the potential consumer exposure is dependent on the way a product is being used and the way NM is present in the product. For example, spray products are present as liquids to begin with, but they will be aerosolized into droplets when the spray is being used and therefore it may result in inhalation exposure. In case of disinfectants and spray-on coatings, when the spraying is done, the NMs can be present on the surface the spray was applied to, which consequently may result in dermal exposure, if this surface is being e.g. touched by hands. It must be noted that all the exposure scenarios that lead to dermal exposure can also lead to inadvertent oral exposure, and all the scenarios that involve formation of aerosol droplets or airborne NMs can lead to deposition on the skin, i.e. dermal exposure.

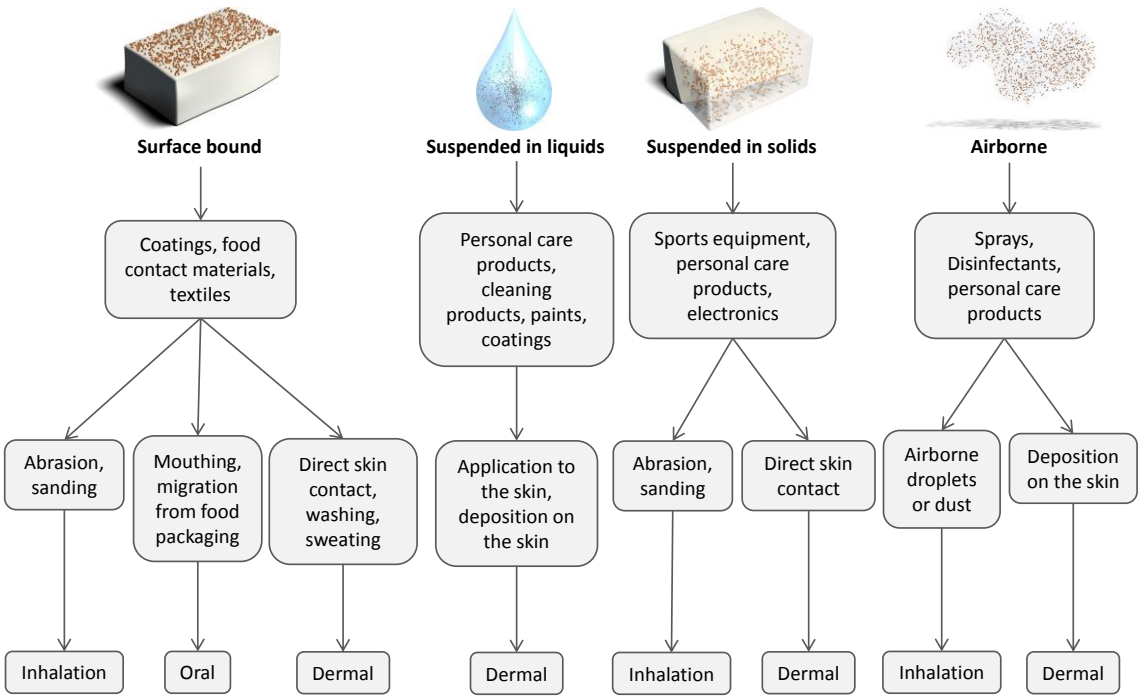

Figure 6: Nanoproduct categories, examples of representative consumer products, the potential human exposure scenarios during intended product use, and the resulting potential exposure routes.

According to The Nanodatabase, around half of the registered products fall into the "suspended in liquids" category (49%), the next two main categories being products where NMs are "surface bound" (29%) or "suspended in solids" (12%). Most products where NM location is "suspended in liquid" are personal care products, cosmetics and sunscreens, and the exposure of such products is self-evident, given that these products are intentionally applied, such as sunscreens applied on the skin as an example. Exposure to NMs from solid articles is a bit more complicated to assess, given that the exposure scenarios may vary. Health and fitness products have the highest number of NM-enabled products, which is why this category should be carefully evaluated when it comes to consumer exposure. Apart from the already mentioned personal care products, cosmetics and sunscreens, which also fall under "Health and Fitness" products, there are a number of additional articles where NMs are surface bound or suspended in solids. These articles represent such sub-categories as clothing, sporting goods and personal care products, which include various items such as hairbrushes, curling irons and other small home appliances, toothbrushes, and sports equipment (The Nanodatabase, 2016).

As already described in Figure 6, most nano-enabled consumer products may lead to human exposure in one or more of the exposure routes, namely dermal, oral or inhalation. Dermal exposure was found to be the most prominent route of exposure for most product categories, apart from "food and beverage", which obviously has the potential for oral exposure for most products (see Figure 7). It must be noted though that the fact that there is potential for dermal, inhalation or oral exposure, does not necessarily mean that exposure will take place while using the product. The likelihood of these exposures to occur is dependent both on product properties and on product use.

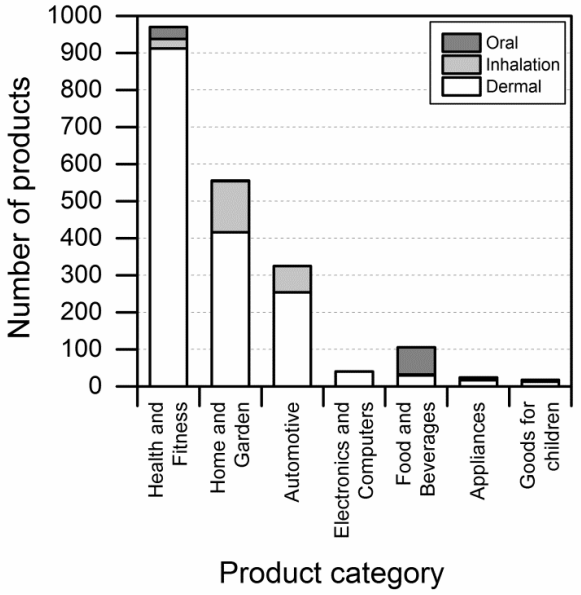

Figure 7: Potential route of exposure for individual product categories. Individual products may have more than one route of exposure (Hansen et al. 2016 – Paper I).

According to the NanoRiskCat framework (introduced by Hansen et al. (2014)), exposure potential can be categorized as high, medium, low or unknown for individual products. For consumer products and consumer exposure, the most important product types are containing surface bound NMs, and NMs suspended in liquids. The exposure potential is dependent on the way NM is present in a certain product. The exposure potential is assumed to be higher for nanoparticles that are airborne or suspended in liquids, medium for nanoparticles that are surface bound, the lowest for nanoparticles suspended in solids. The different functions of consumer products would determine where NMs are present in the product, influencing the potential consumer exposure pathways. However, it is not only the location of the product influencing the likelihood and extent for human

exposure, other factors come in to play as well, such as NM concentration, shape, size and chemical composition.

More than half of the products (64%) found in The Nanodatabase are assumed to have a high potential for consumer or environmental exposure. However, it must be noted that the exposure evaluation is based merely on the product information provided by the manufacturer and evaluation of the location of the NMs in the product. Usually there is not much information provided by the manufacturer that would allow a more detailed evaluation of potential exposure, which is why the issue of the need for experimental data has been indicated by several research papers in the past few years (e.g. Froggett et al. 2014; Larsen et al. 2015; Koivisto et al. (*submitted*), Mackevica & Hansen 2016 - Paper II).

In order to better illustrate and investigate the potential human exposure to NMs from consumer products, we have chosen to work with a few case studies representative of some of the already existing uses of popular NMs. As some of the most commonly used NMs are Ag and TiO_2, this work will primarily focus on these materials as case studies to discuss the potential consumer exposures from articles that contain NMs that are “surface bound” or “suspended in solids”.

5 Experimental determination of nanomaterial release from consumer products

When it comes to possible consumer exposure scenarios, the experimental testing of NM release is posing a series of significant challenges in terms of choosing the right experimental setups, as well as NM detection and characterization methods. The understanding of NM physicochemical characteristics and particle transformations is essential for assessing potential human exposure through such routes as inhalation, ingestion or skin absorption (WHO, 2013).

The release of NM from consumer products depends, to a large extent, on the stresses and environmental factors that the product is exposed to, as well as NM characteristics and the method for NM embedding in the product. These factors will influence both the quantity of the NMs released and the form that NMs are released in (i.e. free NMs, embedded or attached to product matrix, dissolved material, aggregated or agglomerated NMs) (Vílchez et al., 2015).

As pointed out by several literature reviews (e.g. Froggett et al. 2014, Koivisto et al. (*submitted*), Mackevica & Hansen, 2016 – Paper II), the experimental studies addressing NM release for the most part use very different test setups, rarely following any standardized guidelines for testing. The review by Mackevica & Hansen (2016 – Paper II) attempted to extract data regarding quantitative NM release and characterization from 76 experimental papers on nano-release to gain a better understanding about consumer exposure to NMs. Additionally, a critical review by Koivisto et al. (*submitted*) identified a total of 89 scientific publications that addressed quantitative NM release from various nano-enabled items, and in those publications they found a total of 320 different scenarios that were used for quantitative release measurements. This illustrates how difficult it may be to select representative and inter-study comparable test setups for NM release investigations. In general, there is a high need for harmonization of the release testing methods. Harmonization of testing as well as characterization methods would also largely aid nano-specific exposure assessment and consequently also support risk assessment.

For the assessment of potential inhalation exposure, most studies have used setups that involve various types of abrasion of NM-enabled items. For

example, one of the most commonly addressed NM when it comes to assessing potential inhalation exposure is TiO_2, which is widely used in paints as a white pigment. Several studies have measured the emissions of airborne particles during sanding of surfaces coated with TiO_2 (e.g. Koponen et al. 2011; Gomez et al. 2014; Shandilya et al. 2014). However, as with most other studies dealing with mechanical treatment (abrasion, sanding, drilling, sawing, cutting, crushing) of coated materials or nanocomposites, the measurement methods can only go as far as to measure the sizes of the particles released, not providing sufficient information regarding chemical composition of the airborne particulates (Mackevica & Hansen, 2016 – Paper II). Still, these types of measurements provide insight into the extent to which a person might be exposed to NM-containing dust, and what fraction of that might deposit into their lungs. Most of these studies also do SEM or TEM imaging to obtain information on the sizes and shapes of the particles, as well as to provide an indication on whether NMs can be found as single entities or they are attached to or embedded into the matrix. However, it is usually reported that NMs are for the most part released embedded into pieces of product matrix after mechanical treatment rather than being released as free NMs. To name a few examples, studies by Wohlleben et al. (2011), Hirth et al. (2013) and Gomez et al. (2014) investigated the CNT release from CNT-polymer nanocomposites by sanding, and their SEM-EDS analysis revealed that CNTs are released only as integrated parts of the nanocomposite debris.

Experimental setups that are targeting potential dermal exposure measurements most commonly deal with leaching from NM-containing textiles. The leaching is usually done either in deionized water (e.g. Benn & Westerhoff 2008; Benn et al. 2010; Pasricha et al. 2012), wash water (e.g. Geranio et al. 2009; Lorenz et al. 2012; Impellitteri et al. 2009; Mitrano et al. 2014), or various types of artificial sweat (e.g. Geranio et al., 2009; von Goetz et al., 2013b), and the most commonly addressed NM is Ag, with only a few cases where the targeted NM is TiO_2.

Consumer products which have a potential for oral exposure are mostly food items and food contact materials, and such products as food storage articles and ceramic water filters have been addressed in the experimental studies. The NMs include Ag, TiO_2 and CuO, though the most popular NM is again nano-Ag, which is not surprising as it is the one which is more commonly present in the products relevant for oral exposure.

The experimental work conducted within this PhD project has been dealing mostly with experimentally investigating releases from articles that have the potential for dermal or oral exposure, so the following two sections will be focused on the findings through these experimental investigations (Mackevica et al. 2016b,c,d,e – Paper IV, V, VI and VII).

5.1 NM release from consumer products with potential for oral exposure

Nano-silver is used for a broad range of products that are relevant for both direct and indirect oral exposure. These products include food and beverages, cosmetics and personal care products and nanocomposites used as food contact materials. For children, direct oral exposure can result from many more product groups, such as textiles, toys, or any kind of surfaces. Report by Larsen et al. (2015) investigated a total of 12 oral exposure scenarios from various consumer products, and their findings showed that food items and cosmetics represent the sources with the highest oral exposure potential, and products as textiles, air purifiers, dental fillings or composite materials likely result in low or negligible exposures.

The use of food supplements and food items would result in self-evident dosing, but understanding the dynamics of NM release from various solid nanocomposites that might lead to oral exposure is more complex. Unlike with food items, it is not enough to know the NM concentration in the product and that would be directly related to the dose (i.e. what a person ingests is the resulting NM dose). When it comes to solid articles that contain NMs, it is important to assess how much of the NM can actually migrate from the article and lead to oral exposure.

According to The Nanodatabase, from the 137 products that have the potential for oral exposure, most of them are food items or food contact materials, with a few examples of personal care products. More than a third (35%) of the products claim to contain nano-silver (The Nanodatabase, 2016).

5.1.1 Release from food contact materials

Nanosilver is applied as an inner coating for various food storage containers, mainly due to the antimicrobial activity and the purpose of keeping the food fresh for a longer time. Several studies have addressed silver release from various lab-made and commercially available food contact materials (von Goetz et al. 2013a; Echegoyen & Nerín 2013; Huang et al. 2011; Hauri &

Niece 2011; Song et al. 2011; Smirnova et al. 2012; Cushen et al. 2014; Artiaga et al. 2015). Most release studies use a European Commission directive (10/2011) specifically designed for the purpose of quantifying the release of chemicals from plastic food contact materials into food simulants (European Commission, 2011). Food simulants usually include acetic acid, ethanol and deionized water, to represent various types of food that might come in contact with the material. Food contact material is then incubated with the food simulant for 10 days at 40°C as a "worst case scenario", but there are different variations of the test setup regarding duration and temperature, as well as choice of food simulants.

A study by Huang et al. (2011) was amongst the first to address Ag release from nano-enabled food storage containers. In this study, polyethylene bags were incubated with four different food simulants (DI water, 4% acetic acid, 95% ethanol, and hexane) for 15 days, and SEM-EDS analysis confirmed nano-Ag release from the products. A similar setup was used by Hauri and Niece (2011) to investigate Ag release from commercial nano-enabled food containers, but the characterization of nano-release was lacking and the study was limited to reporting only the total Ag release. Other experimental studies, e.g. von Goetz et al. (2013a), Artiaga et al. (2015) and Mackevica et al. (2016b – Paper IV), have also experimentally tested the migration of Ag NPs from commercially available food storage containers. The study by von Goetz et al. (2013a) exposed pieces of cut up food containers to food simulants and found that the release rates per available surface area were considerably lower as compared to intact food containers from the same brand. They also noted that distribution of Ag in different parts of containers was inhomogeneous, which was shown by total Ag concentrations at different areas. Furthermore, it was found that total Ag release dropped up to 10-fold between the first and subsequent uses of the food containers. It is an important finding as more often than not food containers are re-used multiple times. The same trend was observed in the study by Echegoyen and Nerin (2013) with multiple uses of food storage containers.

There have been only a handful of studies quantifying the particulate Ag release rather than just total Ag migration from the food contact material. The study by von Goetz et al. (2013a) was characterizing the Ag nanoparticle release by spICP-MS and their results showed that around 12% of the total Ag migration was in nanoparticulate form for one of the food storage box samples. However, the detected particle size estimation was particles between 100 – 350 nm in diameter, which means there is still high uncertainty

regarding the fraction of particles that are smaller than 100 nm and aggregates that are bigger than 350 nm. The TEM and SEM images revealed that released particles can occur as bigger agglomerates with primary particle sizes of 20-100 nm, so it is highly plausible that the fraction of particulate Ag released could be higher than 12% if particles below 100 nm are taken into account. The study by Echegoyen and Nerin (2013) was also using spICP-MS to quantify particulate Ag release, and found that particulate Ag was around 1 to 20% of the total Ag migrated from the food containers, and in all three food containers they examined, the migration in acetic acid showed less nanoparticle release in comparison to particle migration in ethanol. However, this study did not report the sizes of particles that were detected by spICP-MS, so it is unclear what exactly is meant by "particulate fraction". Moreover, based on the raw data of obtained nanoparticle signals from spICP-MS, it is obvious that during the 300s measurements the signal has been drifting quite a lot, raising a question about the robustness and reliability of the spICP-MS measurements. Their SEM-EDS imaging revealed that the sizes of the released silver particles can range from 10-200 nm, depending on the type of the food container, and some particles were also found still attached to or embedded in to the polymer matrix. It has also been reported in Mackevica et al. (2016b – Paper IV) that the released silver particles can occur not only as agglomerates or embedded in product matrix pieces, but also as free single nanosized released particles (see Figure 8). It must be noted though that the electron microscopy investigations can provide merely qualitative information about the particle sizes and forms in the sample, as in most cases only a small number of particles can be found on the TEM grid. Sample preparation for TEM analysis is also prone to introduction of artefacts that might lead to misconceptions about the NM behavior in the samples.

Measurements by spICP-MS, as stated earlier in the text, are limited to measuring and counting particles somewhere between 20 – 250 nm in size, depending on the particle type and sensitivity of the equipment. When it comes to particles migrating from food contact materials (and other articles), there is a chance that a considerable fraction of released particles will not be detected by spICP-MS. For instance, as shown in Figure 8 F and several electron microscopy images reported by Echegoyen and Nerin (2013), silver particles may be released together with larger pieces of polymers, which may decrease the chances that these silver particles will be detected by spICP-MS, as the pieces can be too big to reach the plasma during spICP-MS analysis.

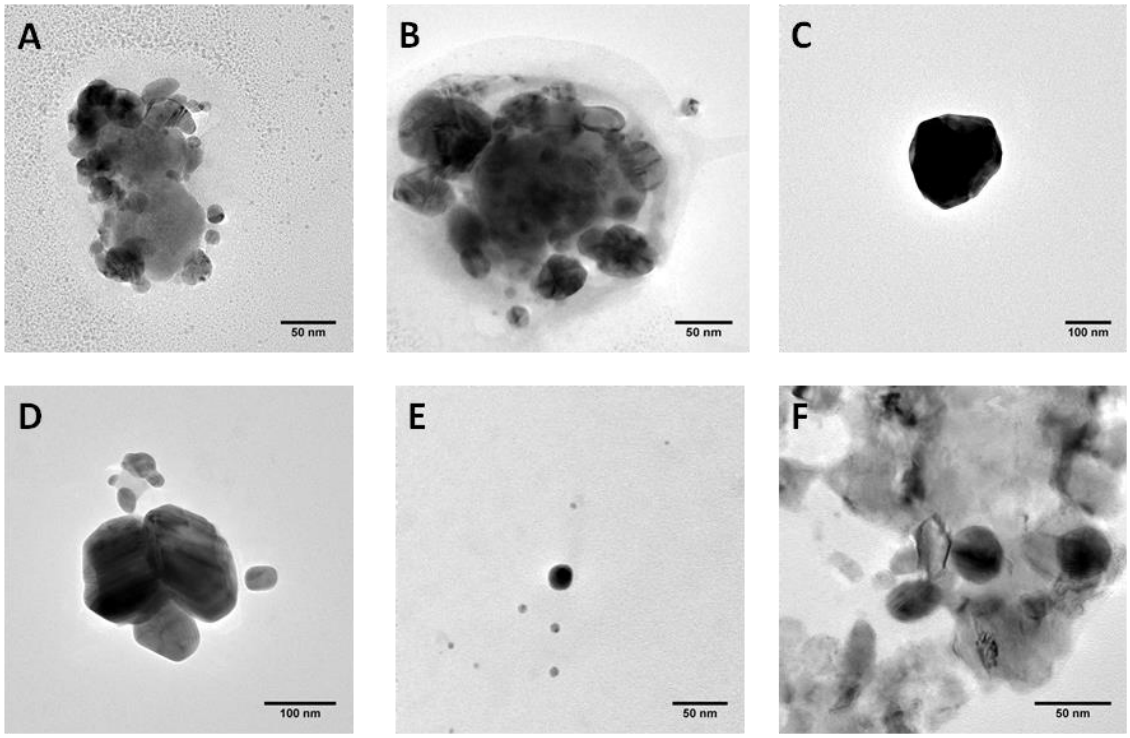

Figure 8: TEM images for particles released from food storage containers after 10 days in 3 % acetic acid. A: Fresher Longer™ Miracle Food Storage™ bags, B: Special Nanosilver Mother's milk pack, C, D: The Original Always Fresh Containers™, E, F: Kinetic Go Green™ Premium Food Storage Containers. All the presented images were analyzed by EDS and confirmed the presence of silver (Mackevica et al. 2016b – Paper IV).

To fully understand exposure to NMs, spICP-MS measurements can be very useful, as they can provide information not only about the particle count, but also the size distribution of particles released as well as the dissolved ionic fraction which all together can lead to oral exposure. It has been reported that size distributions of the released silver nanoparticles can vary depending on both the type of food simulant and the type of the food contact material. For more acidic substances, such as acetic acid as a food simulant, a larger fraction of total Ag may be present as dissolved Ag in comparison to other food simulants, such as ethanol or deionized water (Mackevica et al. 2016b – Paper IV). The size distributions of released silver particles were quite similar for deionized water and 10% ethanol, but the diameter of particles released into acetic acid was considerably higher for three out of four food container brands investigated, one of which only had dissolved Ag by the end of the 10-day test (see Figure 9). Acetic acid clearly is facilitating the Ag NP release, dissolution and aggregation/agglomeration, however the possibility of formation of particles by released Ag ions cannot be excluded. It is known that Ag ion reduction can be facilitated by the hydroxide ion addition (Chou

et al., 2005), and it has also previously been shown that Ag ions can be reduced and hence form Ag NPs when such substances as fulvic (Sal'nikov et al., 2009) or humic acids (Akaighe et al., 2011) are present in the solution.

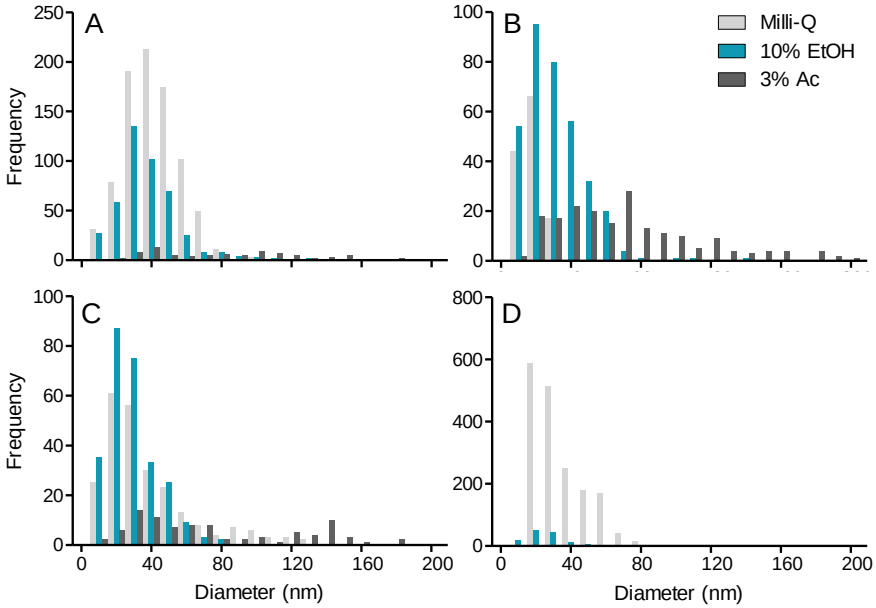

Figure 9: Silver nanoparticle size distributions for food containers incubated with food simulants after 10 days, measured by spICP-MS (modified from Mackevica et al. 2016b – Paper IV). A: The Original Always Fresh Containers™, B: Kinetic Go Green™ Premium Food Storage Container, C: Special Nanosilver Mother's milk pack, D: Fresher Longer™ Miracle Food Storage™ bags. The cut-off for the particle diameters is set at 200 nm, excluding the larger aggregates from the graphs, frequency represents NP event counts.

The higher amounts of total Ag released into acetic acid in comparison to ethanol or deionized water as observed by several studies (Echegoyen and Nerín, 2013; von Goetz et al., 2013a; Mackevica et al. 2016b – Paper IV) were expected, knowing that Ag ion release rates tend to increase with decreasing pH values (Liu and Hurt, 2010). When comparing the total Ag content in the material and total Ag release both on weight/weight basis and weight/area basis, no apparent relationship was found between total Ag content and release for the various food container types investigated. This might be an indication that the manufacturing methods differ and some container types would have more loosely bound particles on the inner surface. Type of plastic and different sizes and/or coatings of particles could also influence the silver ion or NP release. As reported by the atomic force microscopy (AFM) analysis in von Goetz et al. (2013a), the inner surface of food containers can be relatively rough and have up to 10 µm variations in height, which in turn can affect the actual surface area, making it markedly different from the measured surface area assuming a smooth surface.

5.1.2 Release from toothbrushes

Other consumer items relevant for potential oral exposure to Ag nanoparticles addressed in the literature include ceramic water filters (Bielefeldt et al., 2013; Ren and Smith, 2013), baby toys and textiles (Quadros et al., 2013) and toothbrushes (Mackevica et al. 2016c – Paper V). In the latter case, two types of toothbrushes (Ag-infused adult and baby toothbrushes) were tested for their whole intended usage period and analyzed for both nanoparticulate release and total Ag release. The experimental setup included taking sub-samples at representative time points, such as 2 min intervals which is corresponding to one brushing event. The release was tested in tap water to have a closer resemblance to a real life use scenario. Since including the toothpaste to the release medium would introduce another degree of complexity, it was omitted. From the two toothbrush types that were tested, the adult toothbrushes showed a slightly higher Ag release both in terms of total Ag release and nano-Ag release. Particle release substantially declined after the first 6 minutes of testing for most samples, and after 16 h of testing the total Ag release reached a plateau. It was found that around 1-3% of all released Ag was found in particulate form, having median particle sizes of around 45 nm measured by the spICP-MS (see Figure 10). The total Ag release was corresponding to <0.1% of the total Ag content measured in the toothbrush bristles before use, which hence indicates that only a relatively negligible fraction of Ag is released and most of the Ag is remaining embedded in the polymer matrix (Mackevica et al. 2016c – Paper V). Consequently, there is concern about the rest of Ag ending up in solid waste. A study by Lee and Kim (2015) addressed this issue by setting up a landfill leaching batch test with two types of nano-Ag containing toothbrushes with different pH (4.0, 7.3 and 10.0) for 100 days. With continuous leaching over time, a maximum of 2.1% from the initial Ag content had been released after 100 days of testing, and it was found that both ionic and nano Ag were present in the leachate solution. Extrapolating these results to the population of South Korea and assuming only 5% nano-Ag toothbrush usage ratio within the population and use of 3 toothbrushes per person per year, the amounts of leached silver in landfills could reach 17 tons over 20 years (Lee and Kim, 2015). However, there is a great need for more “End of Life”-phase studies for nano-enabled consumer products that would provide data for NM leaching over longer time periods.

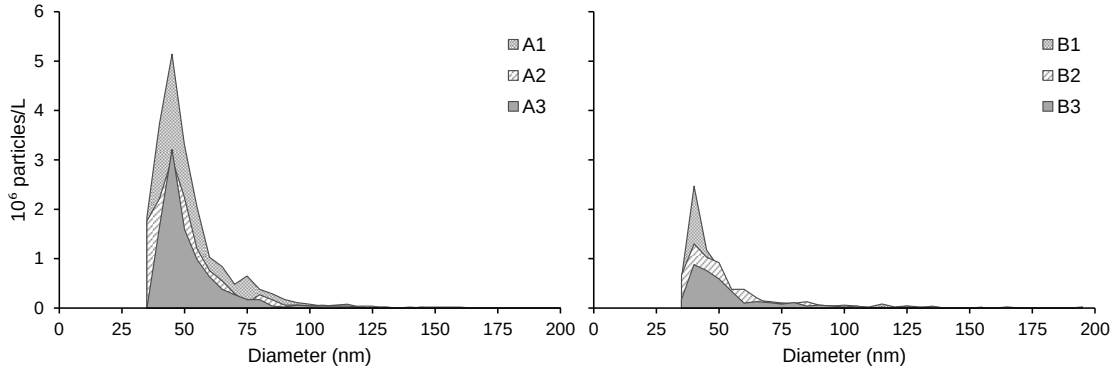

Figure 10: Cumulated released silver nanoparticle size distribution after 24 hours of testing. A1-3: three adult toothbrushes, B1-3: three baby toothbrushes (Mackevica et al. 2016c – Paper V).

The overall results from most of the nano-Ag release experiments done so far conclude that oral exposure during nanoproduct use is negligible, as most often the leached amounts are very low (in magnitudes of ng/L). Also, most of the studies dealing with potential oral exposure to NMs from consumer products are presenting release as total Ag rarely using other metrics for nano release representation. More studies with extensive NM characterization are needed to understand to what extent consumers may be exposed to NMs from nano-enabled consumer products, as the information about the total Ag exposure is not sufficient to assess the actual risks associated with nano-enabled product use. It is well known that silver nanoparticles have different toxicological properties compared to ionic and bulk Ag. However, it has been reported that toxic effects of Ag NPs are exerted only in mg/kg bw concentrations (e.g. Chang et al. 2006; Kim et al. 2010; Kim et al. 2008). For this reason, using Ag NP-enabled products would most likely have negligible risk of causing adverse health effects, but it cannot be completely excluded as long-term study data is lacking.

5.2 NM release from consumer products with potential for dermal exposure

As shown before in Figure 6, dermal exposure is the most prevalent exposure route when it comes to using nano-enabled products. It is especially relevant for products from the health and fitness category, as well as personal care products. As personal care products have a somewhat self-evident and in most cases also intentional dosing (e.g. sunscreens, lotions, cosmetics), it is more important to focus on the use of products that may lead to unintentional consumer exposure through dermal contact. Most of such products include nanocoatings and nano-enabled textiles, the latter of which has also been

relatively widely addressed in the literature in terms of nanoparticle release through simulated product use that may lead to dermal exposure (i.e. wearing the clothing items or washing them).

It has been a focus of a number studies to investigate to what extent NMs can be taken up by the skin and what sizes are able to penetrate deeper layers of skin to reach the lymphatic system. Particularly nano-TiO_2 and ZnO have been the NMs causing concern, as they are commonly used in sunscreens and other personal care products. Most experimental and theoretical evidence suggests that insoluble NPs are not able to penetrate into or through healthy or damaged skin. In the case of TiO_2 specifically, a review of the published experimental studies (Nohynek et al., 2007) concluded that TiO_2 particles do not penetrate further than upper layers of stratum corneum, even with particles in sizes as small as 20 nm (as reported in the study by Mavon et al. 2007). The same conclusion was also reached by a human safety review of nano TiO_2, where it was stated that, based on the current weight of evidence of all available toxicity and exposure data, the risk of using nano-TiO_2 in cosmetics and sunscreens is negligible (Schilling et al., 2010). Based on all this information, as of July 2016 the use nanoscale TiO_2 as a UV-filter in sunscreens is allowed within the EU at a concentration up to 25% w/w. It can be considered that TiO_2 is not posing any risk of toxic effects after application of the products on healthy, intact or sunburnt skin (European Commission, 2016).

Other NMs in different product groups, however, have not been studied that extensively. When it comes to dermal exposure to other commonly used NMs such as Ag, the studies are less abundant and there is no definite conclusion whether or not the use of the NMs is safe and poses no risk to human health (Wijnhoven et al., 2009). A few experimental studies have indicated that Ag NPs are able to penetrate through healthy skin. For instance, an *in vivo* study of dermal absorption using human skin discovered that Ag NPs in sizes of less than 100nm can penetrate through the skin layers and end up as deep as the lower layer of the dermis (George et al., 2014). Additionally, another study investigated a more realistic scenario using volunteers wearing Ag NP-infused textiles and subsequently assessing skin absorption by tape stripping method. The findings indicated that Ag NPs in sizes up to 1µm could be found in epidermis and dermis (Bianco et al., 2015).

Generally, information available in the literature suggests that there are still some uncertainties associated with understanding the NP behavior and

interactions with the skin barrier that lead to dermal absorption, as well as the degree of adverse short term and long term effects they may cause (Labouta and Schneider, 2013). Especially data is lacking when it comes to proper NP characterization and long term exposure to NPs released from nano-enabled products. In the following sections a few product groups that are relevant for dermal exposure are addressed, namely textiles and surface coatings that may come into contact with skin. Specifically the focus is set on understanding NP exposure through quantitative nano-measurements that can aid nano-specific dermal exposure assessment.

5.2.1 Release from textiles

Around 14% of the nanoproducts are textiles according to the information available in The Nanodatabase (2016), and most of them claim to contain nano-Ag. Until recently, NM release from textiles and dermal exposure had not been addressed extensively in the literature. In the past few years there have been an increasing number of studies investigating release from commercially available and lab-prepared textiles, mostly with Ag NPs and a handful of studies with TiO_2 NPs.

There are four main factors affecting the NP release from the textiles and their subsequent transformations, including: 1) NP incorporation into the fibers (surface-bound or composite, NP form, textile composition), 2) use of textile (sweat, abrasion, UV-light, temperature, and other external factors), 3) cleaning/washing (properties of the detergent or bleach), and 4) disposal (Mitrano et al., 2015a). Proper characterization of the released NPs is the key to understanding the possible consumer exposure during product use of various types of nano-enabled textiles.

Several experimental studies have investigated some of the real-life scenarios of nano-Ag textile use by exposing the textiles to artificial sweat at body temperature (Kulthong et al., 2010; Stefaniak et al., 2014; von Goetz et al., 2013b; Wagener et al., 2016; Yan et al., 2012). The released Ag quantities were found to be dependent on several factors, such as pH and composition of the sweat solution and amounts of Ag in the fabrics, as well as the method for incorporation of Ag in the fabrics. Mostly the total Ag release was quantified by such techniques as AAS or ICP-OES and ionic Ag fraction determined using ultracentrifugation, and having particle characterization done by SEM-EDS. These types of measurements provide a good indication regarding possible total Ag exposure, but have little indication regarding sizes and quantities of particles released. Study by Wagener et al. (2016)

addressed this issue by comparing differently functionalized textiles and their Ag release with spICP-MS and found released particle sizes of 40 to 60 nm, and higher particle number releases for surface-modified textiles (as opposed to composite materials). A similar trend was observed in a study by Geranio et al. (2009) with washing of different textiles, which led to higher amounts of Ag released for surface-modified fabrics. When it comes to speciation of Ag species released, it has been shown that Ag can be released both as ions and particles, and Ag^+ can undergo transformations to form Ag^0, AgCl or Ag_2S particles (Lombi et al., 2014; Mitrano et al., 2016b, 2014).

Nano-TiO_2 is used as an additive in textiles because of its photocatalytic properties, UV-protection, antibacterial and anti-odor effect, and hydrophilic surface functionalization (Kohler and Som, 2014). The TiO_2 NP release from textiles could lead to consumer and environmental exposure, and this issue has been addressed by a few studies (von Goetz et al., 2013b; Windler et al., 2012; Mackevica et al. 2016d - Paper VI). Windler et al. (2012) tested TiO_2 release from six different textiles (all of them synthetic blends) during washing and rinsing cycle. Electron microscopy and filtration (0.45 µm filter) data confirmed the release of NPs, however mostly in the form of agglomerates. The total release of titanium ranged between 0.01-0.06% of the initial content in the fabrics, indicating that TiO_2 was incorporated in and strongly bound to the fibers. Using the same methods, a similar conclusion was reached by von Goetz et al. (2013b), where the TiO_2 release was tested for the same six textiles, but this time in artificial sweat solutions. Only one of the textiles released measurable amounts of titanium, and most of it was found in particulate form. However, it must be noted that the detection limit for Ti in this study was 12.4 µg/g/L. Generally, when it comes to characterizing and quantifying TiO_2 release it becomes quite complex firstly because of the low amounts released, and secondly because 0.45 µm filtration only provides information regarding particle size of <450 nm, making it difficult to account for the actual particle size distributions, as electron microscopy provides merely qualitative information about released particles. Several studies using spICP-MS have succeeded to measure sizes of particles as well as quantify the mass of the particulate TiO_2 in several items, such as sunscreens (Dan et al., 2015), drinking water (Donovan et al., 2016), and food items (Peters et al., 2014a). When it comes to NP release from textiles, spICP-MS has been utilized in a few Ag NP release studies (e.g. Mitrano et al., 2015b; Wagener et al., 2016) and one TiO_2 NP release study (Mackevica et al. 2016d – Paper VI), revealing size distributions of released NPs. Nano-

TiO_2 release was investigated for five different commerically available textiles that were not having labels regarding TiO_2 content or UV-protection, and four of them were eco-labelled and one was hand-crafted. The main focus of the study was to compare the nanoparticulate fraction of released TiO_2 versus the total amount of released Ti (the experimental setup is depicted in Figure 11), discussing the challenges that arise when working with very low concentrations in ng/L range (Mackevica et al. 2016d – Paper VI). The experimental setup highlights the need for a multi-method approach when working with nano-exposure estimation, as several techniques have to be employed to fully understand the release from the products.

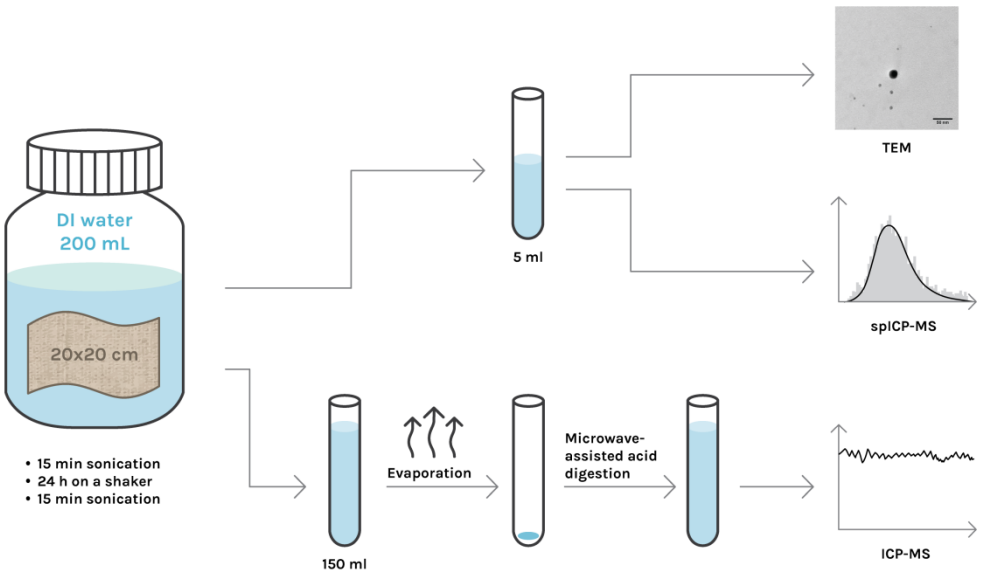

Figure 11: Schematic representation of the experimental setup (Mackevica et al. 2016d – Paper VI).

It was found by spICP-MS analysis that three out of five textiles released measurable amounts of nano-TiO_2 particles, with median sizes ranging from 49 to 77 nm. The nanoparticles (sizes from around 25 to 200 nm) represented 8 to 80% of the total titanium measured by ICP-MS after acid digestion. The total Ti release was estimated by using two methods – conventional ICP-MS measurements after microwave-assisted acid digestion and nanoparticle measurements by spICP-MS. In the latter case, the total Ti content was calculated by adding up the masses of all the particles released for a given sample, and calculating the corresponding mass concentration according to equations presented by Pace et al. (2011).

The acid-digested Ti measurements showed a high variation in between replicates of the same fabric type, while the spICP-MS measurements were more robust for analyzing the particles below 200 nm in size (see Figure 12).

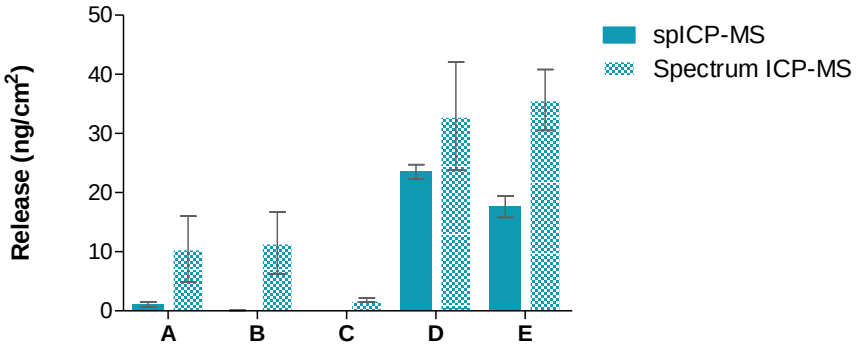

Figure 12: Total Ti release from all the samples (n=3) measured by spICP-MS and conventional ICP-MS after acid digestion. For A and E samples n=2. Error bars indicate standard error of mean (Mackevica et al. 2016d – Paper VI).

Generally, both nano-Ag and TiO_2 release from the textiles (especially commercial textiles) is rather low and would arguably cause negligible consumer exposure and have little effect on total NP content in the waste water treatment plants and the environment. As for nano- TiO_2, the consumer exposure assessment for TiO_2 textiles by von Goetz et al. (2013b) indicated that the release from textiles is negligible compared to the input from a wide range of food products that are widely available on the market and contain nano-TiO_2 as an additive. However, even if during the use phase of the textiles there is little or negligible NP release, there might be concerns when it comes to NP release during the end of life phase, i.e. disposal or recycling. This issue was addressed in a study by Mitrano et al. (2016a), which tested leaching of washed and unwashed Ag and Au NP-containing textiles in a simulated landfill leaching scenario. Their findings showed that while there was relatively high release from unwashed fabrics (up to around 35% of the initial content), the washed fabrics released very small amounts of Ag and Au, indicating that the NPs most likely stay intact in the textile fibers also when disposed of in a landfill. However, as also stated in the paper (Mitrano et al., 2016a), the release amounts and properties are highly dependent on the characteristics of the release medium, as well as the properties of the NPs and the functionalization of the textile.

5.2.2 Release from surface coatings

As already indicated above, nano-enabled surface coatings is one of the consumer product groups that has a relatively high potential for consumer exposure. One of the pathways for human exposure to NMs from surface coatings is direct dermal contact, e.g. hands touching a surface treated with nano-containing substance or surface where NMs are deposited. Testing of such products has mostly been done in association with environmental exposures, such as weathering of paint and leaching into environment. The release of other types of NPs, such as TiO_2 and Ag, from painted surfaces has been investigated by several studies (e.g. Olabarrieta et al. 2012; Kaegi et al. 2008; Kaegi et al. 2010), which have shown that weathering or abrasion are important factors for NP release in the environment.

When it comes to human exposure to NMs from treated surfaces, the information in the literature is scarce. Dermal transfer of NPs through simulated skin contact has been addressed by only a few of studies (Platten et al., 2016; Quadros et al., 2013). These studies have successfully used different wipes as a surrogate for human skin to mimic a real-life scenario of hands touching a surface. For example, Quadros et al. (2013) tested dermal transfer of Ag NPs from various baby products, such as plush toy, baby blanket, disinfecting spray and kitchen scrubber. The results from surface wiping experiments revealed that there is considerable Ag transfer from the products to the wipes, and therefore there is also a potential for skin exposure through the use of those products. More recently, a study by Platten et al. (2016) studied the dermal exposure potential of different types of copper particle pressure-treated lumber by also using wiping test setup to simulate skin contact. Both of these studies provided relevant data for potential dermal transfer from NM-containing products, however, the measurements were presented as total amount of the chemical that is transferred from the surface to the wipes.

As noted above, the nano-specific effects that might arise from NP exposure are not well known at this point, so characterization of the released NPs is an essential part for understanding the actual exposure to NPs. In the study conducted within this thesis (Mackevica et al. 2016e – Paper VII), it was attempted to design and perform a dermal transfer study that allows not only reporting the total mass exposure to NMs, but also particle number concentration and the sizes of the particles that may come into contact with skin. By utilizing spICP-MS, it was found that it is possible to extract most of the particles transferred to wipes by ultrasonication and gain quantitative

information regarding potential dermal transfer of Ag and CuO NPs (Figure 13). Each wiping event was conducted as follows: the wipe was wetted with artificial sweat, then the surface of the article was wiped three times, and the wipe was then immersed in DI water, ultrasonicated, and the resulting suspension was immediately analyzed by spICP-MS. This procedure was repeated three times on the same surface. This method was tested on several nano-enabled surfaces, such as Ag-containing keyboard covers and wooden blocks painted with CuO-containing paint (before and after accelerated wear and tear). With this setup the dermal wiping tests were able to provide information on total mass concentration, particle number concentration, as well as the released particle size distribution.

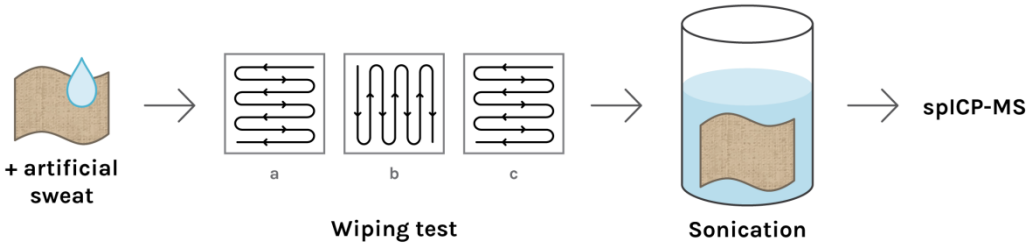

Figure 13: Schematic representation of execution of wiping tests, the procedure depicted here is referred to as one wiping event (modified from Mackevica et al. 2016e – Paper VII).

From the three types of commercial Ag-containing keyboard covers that were tested in this study, only one showed Ag NP release that was higher than the Ag-free control (Figure 14). However, it was concluded that Ag NP transfer can be considered negligible, as the total Ag release was in she sub-ng per cm^2 range. It was also observed that Ag NP transfer was slightly decreasing with each wiping event, indicating that most likely with increasing frequency of keyboard cover use the Ag NPs would gradually detach from the surface until no more transfer is possible (Mackevica et al. 2016e – Paper VII).

The maximum measured Ag nanoparticle transfer was 0.001 ng/cm^2, corresponding to around 2,000 particles per cm^2. For this sample, the mean particle size was 28 nm. The dermal loading from these Ag NP keyboard covers may be considered negligible, as the amounts of Ag released are very low, also in comparison with dermal transfer from other items that have been tested with a similar setup. For example, dermal wiping study by Quadros et al. (2013) investigated surface transfer from baby blanket, plush toy, dried disinfecting spray, dry surface wipes and kitchen scrubber. The highest Ag

release was 2.3 ng/cm^2, which was measured for the baby blanket sample. Even for the highest observed Ag transfer, the authors concluded that the amounts of Ag released are below the threshold that could cause damage to children.

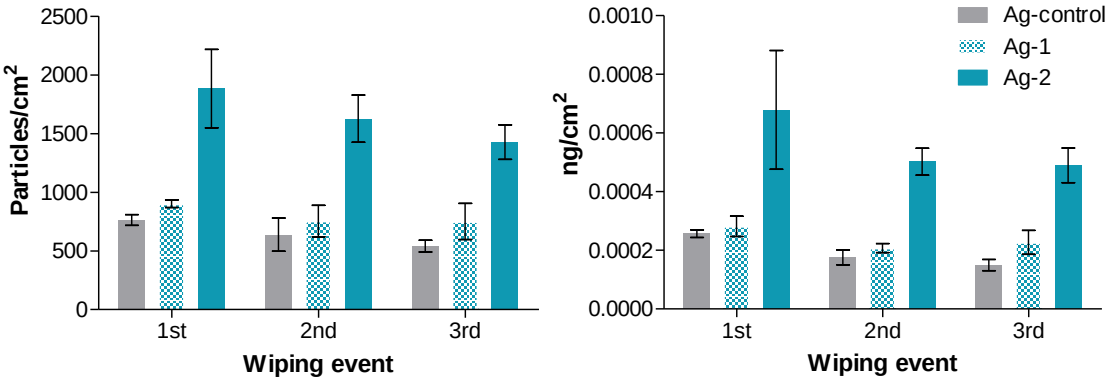

Figure 14: Ag NP transfer from keyboard covers to wipes. Left – transfer in number of particles per cm^2, Right – mass transfer in ng/cm^2. Error bars represent standard error of mean, n=3 (Mackevica et al. 2016e – Paper VII).

Dermal transfer testing for the CuO-painted wooden blocks showed that there is nearly no CuO NP release from the painted surface when the paint is fresh. However, after sanding of the paint surface to simulate accelerated weathering and wear and tear, the CuO release was increased considerably (Figure 15). The particle count was notably higher when the sanded wooden blocks were wiped, resulting in transfer of up to $5 \cdot 10^5$ CuO particles per cm^2, whereas for paint without sanding it was around $2 \cdot 10^5$ particles per cm^2 (observed in the 2nd wiping event). There were also some differences observed between the control samples (coated with paint with no CuO added) before and after sanding, indicating that there might be some background Cu levels in the paint matrix. The mean sizes of the released particles were around 84 nm and 79 nm for CuO-paint without and with sanding, respectively, and the mode sizes were 61 nm and 54 nm without and with sanding, respectively (Mackevica et al. 2016e – Paper VII).

The dermal transfer from painted surfaces has not been extensively addressed in the literature so far. There has been a recent publication regarding dermal transfer of Cu from pressure-treated lumber, which was focusing on micronized and ionic copper azole-treated wood (Platten et al., 2016). The total released Cu was extracted from the wipes by acid digestion, and showed that there were no significant differences in total Cu release for the timber

that was impregnated with micronized or ionized Cu. A total of 12 wiping events showed that there was higher initial release of Cu from micronized Cu-treated timber during the first three wiping events. Additionally, the wood that was previously weathered was showing considerably higher total Cu release quantities, going up to around 25 mg Cu/m^2 for the first wiping event after wood exposure to weathering. Generally, dermal exposure to copper compounds is not known to be of concern apart from potential allergic reactions. However, ingestion of copper has been shown to have toxic effects (Civardi et al., 2015), which is why hand-to-mouth exposure might be of concern when it comes to Cu-treated surfaces that come into contact with skin.

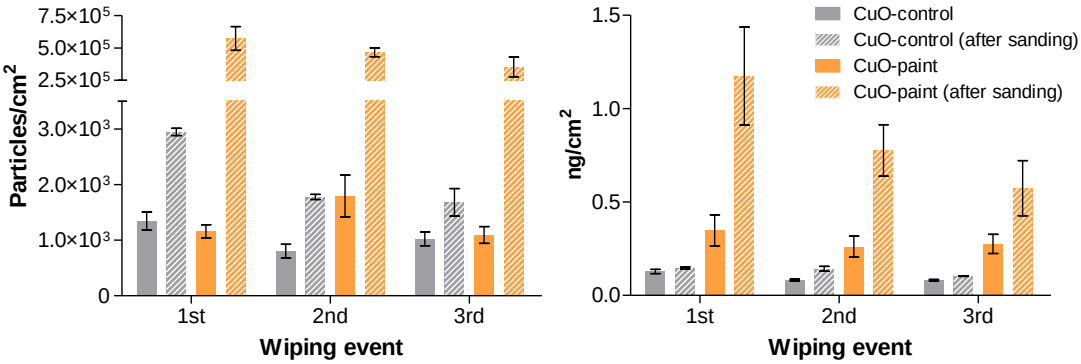

Figure 15: CuO NP transfer from painted wooden blocks to wipes. Left – transfer in number of particles per cm^2, Right – mass transfer in ng/cm^2. Error bars represent standard error of mean, n=3 (Mackevica et al. 2016e – Paper VII).

6 Consumer exposure assessment to nanomaterials

It has been well established that certain types of NM-containing products have the potential to lead to consumer exposure when being used. However, quantifying these exposures is quite challenging, as the exposure is dependent on many different factors, such as product properties, NM properties, as well as consumer behavior (frequency of use, use of personal protection equipment, misuse, etc.). The main question associated with pretty much any consumer product is whether or not it is safe to use. The presence of a certain substance in a product does not necessarily pose a health hazard in itself, it is only not safe for use if there is a potential for exposure and a potential hazard associated with the substance. Exposure assessment therefore plays a key role in understanding whether or not there are any risks of adverse health effects from a certain chemical substance or product (Thomas et al., 2006). Exposure assessment is therefore an essential and integrated part in risk assessment and management.

As already discussed before, for nano-enabled products the exposure is highly dependent on the location of the NM. If the NM is attached to or embedded in the product in a manner that aims to minimize the NM release, the exposure potential will also be negligible. However, in such cases where NMs do have the potential to be released during product use (or misuse), exposure might take place via dermal, oral or inhalation route. Consumer exposure assessment is therefore of utmost importance to quantify possible exposures for various nano-enabled products via likely routes of exposure during product use.

Consumer exposure estimation is often complex as it requires substantial understanding of the nature of the product and great detail about the circumstances of their foreseeable use and misuse, as well as the amount of a certain substance present in the product used per event and the frequency and duration of use. Exposures can range from short-term, as low as seconds of each event, or long-term going as far as life-time exposure, depending on the product. The means of controlling possible consumer exposure are very limited and cannot normally be monitored, and suggested product use guidelines cannot be enforced beyond the point of sale of the products (ECHA, 2012). Because of so many “uncontrollable” and uncertain parameters regarding both consumer behavior and the product properties,

there is a necessity to consider certain assumptions to obtain a quantitative estimate for consumer exposure. Very often the assumptions have to be based on already derived default values, to generalize the exposure parameters and perform a reasonable exposure assessment. In the following sections several models and tools intended to be used for consumer exposure assessment will be discussed, as well as their applicability for nano-specific consumer exposure assessment.

6.1 Guidelines and tools for consumer exposure assessment

Exposure assessment tools apply a specific set of parameters into equations or decision trees, which are further used to quantify or rank the exposure potential of a certain material. Qualitative tools are lower tier tools that are commonly used for exposure estimation based solely on assumptions or qualitative data. By using simpler equations or decision trees, the final output is usually categorization or ranking of exposure potential. Examples include several control banding tools and categorization tools, such as NanoRiskCat (Hansen et al., 2014) and Swiss Precautionary Matrix (Hock et al., 2013). These tools basically provide an indication whether or not there is a high likelihood for exposure and further actions needs to be taken. Quantitative tools on the other hand, are higher tier tools that use predictive models and are based on quantitative or semi-quantitative data. For consumer exposure assessment, several quantitative tools have been developed. For instance, the European Chemical Agency (ECHA) has developed a number of guidance documents to aid risk assessment and management, one of them being a guideline for consumer exposure estimation, providing step-wise and iterative models for quantifying potential consumer exposure to substances found in mixtures, articles, or to substances on their own (ECHA, 2012). A few other quantitative tools can be applied for consumer exposure estimation, such as ConsExpo (Delmaar et al., 2005) or ECETOC TRA (ECETOC, 2009), both of those having very similar properties as the consumer assessment model developed by ECHA. These tools provide conservative default values and assumptions for worst-case scenarios, for instance, in some cases an instantaneous release of substance from the product or release with no dilution (in air or liquid). Assuming the worst case scenario, however, provides a very crude estimate and does not necessarily reflect a likely real-life scenario.

Consumer exposure assessment according to ECHA provides models for various exposure routes, such as inhalation, ingestion and two separate scenarios for dermal exposure, namely cases where substance migrates from the article in contact with skin, and cases where the product is directly applied to skin or certain body parts are dipped in a mixture that contains the substance. Inhalation exposure to NMs is most relevant for products like sprays or powders, but, depending on product use and exposure scenario, it can also be relevant for solid articles that are subjected to sanding or other types of abrasion that may cause airborne particle release. In a quantitative manner, inhalation exposure is expressed as the average concentration of the substance in the breathing zone atmosphere (mg/m^3) over a reference time period (per day or per event). However, when it comes to exposure to airborne NMs, the ECHA guidance suggests to also note the number concentration and surface area concentration (i.e. n/m^3 and cm^2/m^3). The underlying worst-case assumptions are that 100% of the substance is released from the product in a confined room with no ventilation, and the event duration is 24h (ECHA, 2012). Oral exposure from consumer products is likely in cases where substance can migrate from the product due to sucking, chewing, licking, or unintentionally swallowing the article that contains the substance. These routes are especially relevant for children's oral exposure. Oral exposure can also result from substance migration from food contact materials, such as cutlery or food packaging. In the model oral exposure is expressed as the average amount of substance ingested per kg body weight per day ($mg/kg_{bw}/d$), and the worst case assumption is that all the substance is released from the article, or, where applicable, the whole article is accidentally ingested. In cases of dermal exposure, hand or body contact with the product is one of the most common exposure scenarios. Other likely exposure options are dipping hands or other body parts in a mixture that contains the substance, or directly applying a product on the skin, such as sunscreen. Deposition of aerosols on skin or splashes on the skin (e.g. during painting) is also considered as a pathway to possible dermal exposure. The resulting quantification of exposure is presented as the amount of substance per unit skin surface area (mg/cm^2) or as an external dose ($mg/kg_{bw}/d$).

Other quantitative consumer exposure assessment tools (ConsExpo and ECETOC TRA) provide similar outputs and are based on some of the same principles and algorithms as described in the ECHA guidelines. ECETOC is using established exposure prediction models called EASE (Estimation and Assessment of Substance Exposure), which includes considerations of

specific consumer article use scenarios with specific activities to make a more conservative exposure assessment. The outputs are in the same metrics as for ECHA equations, resulting in inhalation exposure in mg/m^3, but oral and dermal exposure in $mg/kg_{bw}/d$. The ECETOC tool requires the user to provide information on consumer product and consumer behavior, highlighting duration of use and frequency of events, for example. The tool includes default values, such as amount of substance used per application, exposure duration, surface area, body weight, and these values are consistent with the ones used by ConsExpo model. Even though the default values are provided, the user of the tool can still choose to insert a different set of values into the model (ECETOC, 2009). The same is true for ConsExpo model, which is considered to be more of an expert tool and is recommended and used by REACH for exposure assessment of industrial chemicals and biocides. ConsExpo is a computer program based on algorithms published by Delmaar et al. (2005). The program provides models for inhalation, oral and dermal exposure routes and consists of both simpler screening models and higher tier models for more quantitative and scenario-specific exposure estimation. For instance, for all exposure routes, there are models for both instantaneous release and constant release over longer periods of time. As an example, for dermal exposure, the model allows calculating exposure for scenarios where all the substance is directly applied to the skin at once, as well as scenarios where the product is applied at a constant rate. Dermal exposure can also be assessed to solid surfaces treated with a substance, and skin is either in direct contact with the surface or there is migration/diffusion of the substance from the material to the skin. The model is coupled to a database that contains so-called fact sheets of default values that enable obtaining product-specific information regarding circumstances and characteristics of possible exposure. However, the model can be considered to be quite complex and require a certain level of expertise to perform exposure assessments and use own data (as opposed to defaults). Using ConsExpo model the user can choose to use distributions rather than point values for a number of input parameters, if the mean and standard deviation is known. Consequently the program can perform probabilistic (Monte Carlo) calculations (Delmaar et al., 2005).

6.2 Nano-specific consumer exposure assessment tools

The available tools for quantitative consumer exposure assessment of chemicals are all using mass-based metrics for exposure assessment calculations, and the output is mass-based as well (i.e. mg/m^3, mg/cm^2 or $mg/kg_{bw}/d$). During the past few years, the issue of having the need for nano-specific consumer exposure assessment tools has been highlighted by many, and recently there has been some progress with regard to developing models and tools that allow computing exposure to NMs in a way that is more representative and elucidating for NM exposure characterization.

To date, the only model available specifically designed for consumer exposure to NMs is ConsExpo nano, developed by RIVM (RIVM, 2016). In its current version, it is designed specifically for consumer exposure assessment to NMs in spray products. It allows consumer exposure assessment based on various default scenarios for different product groups, such as cleaning and washing products, cosmetics, disinfectants, painting products, pest control products, and do-it-yourself products. To use the factsheets already available in the software, the only input for completing the calculations is aerosol particle density, weight fraction of the nanomaterial in the aerosol, nanomaterial density, shape and diameter. It is also possible to specify all the other parameters if the data is available and the factsheets for developed default scenarios are not necessary. The model output provides information on inhaled dose per event and the alveolar dose per event, and dose can be defined by mass, number of nanoparticles, surface area of nanoparticles, volume or nanoparticles, number of aerosol particles, surface area of aerosol particles or volume of aerosol particles (RIVM, 2016).

Apart from ConsExpo nano, RIVM have also developed a NanoCosmetics tool for risk assessment of nanomaterials in cosmetics, which aims to evaluate and manage consumer health risks that may be associated with use of nano-containing cosmetics (De Jong et al., 2015). The tool is containing the physicochemical characterization of the NMs, as well as the consumer exposure estimation, and possible hazards posed by NMs, which all together aid the risk assessment. However, even if the tool claims to be aimed at nano-specific risk assessment, the consumer exposure assessment is based on the existing ConsExpo dermal exposure models, which are mass-based.

6.3 Applicability of consumer exposure assessment tools to nanoproducts

There have been a handful of attempts to perform consumer exposure assessment to several nano-enabled products based on experimental data. Generally, when it comes to nano-enabled products, several challenges arise when conducting consumer exposure assessment. First of all, unless the product is a spray, the exposure assessment will be solely mass-based, and there is often very limited information available for using the consumer exposure assessment models described above.

A review study by Mackevica & Hansen (2016 – Paper II) identified 76 experimental studies that were providing data for release from nano-enabled articles, and attempted to apply the aforementioned consumer exposure assessment tools to quantify the possible consumer exposure by using these products. Out of those 76 studies, 33 provided necessary data for consumer exposure assessment using ECHA models. In total, 194 exposure scenarios were developed for consumer exposure calculations. The key input parameters were the concentration of the substance in product, and the fraction of substance that can migrate from the product. An instantaneous 100% release (worst case scenario) was assumed when other data were not provided. For inhalation exposure studies it was particularly difficult to obtain data for the fraction of substance released, as data was usually reported as particle number concentration per volume of the room. For inhalation exposure studies, the most commonly addressed substances were CNTs, TiO_2 and SiO_2 NMs by sanding or abrasion of various nanocomposites, surface coatings and paints. Based on the model presented by ECHA, the potential inhalation exposures could go up to 40 $mg/kg_{bw}/d$, for sanding of samples such as TiO_2-containing coatings and assuming the worst case scenario. When actual data of substance mass release is provided, the exposure estimation can be several orders of magnitude lower. Most studies relevant for potential oral exposure were dealing with the Ag release from food contact materials or ceramic water filters. For most cases, the estimated oral exposure was in magnitudes of $ng/kg_{bw}/d$, rarely reaching levels of $\mu g/kg_{bw}/d$, and generally the higher exposures were resulting from lab-made products with higher amounts of substance applied to the product. Dermal exposure was calculated as dose in $mg/kg_{bw}/d$ (rather than mg/cm^2), and for most products and exposure scenarios the resulting dermal exposure was negligible. Nearly all of the articles addressed in the reviewed literature were

textiles containing Ag NPs. Highest exposure potentials were observed for lab-prepared fabrics, and for fabrics that had NPs attached to the surface of the fibers rather than embedded in the textile matrix (Mackevica & Hansen, 2016 – Paper II).

It is cumbersome to group and rank the exposure potentials for different products or product groups, as the data reported in the literature may have very different exposure scenarios for selected products. Even within the same product groups the exposure dose can vary several orders of magnitude. A similar trend was observed by a recent critical review conducted by Koivisto et al. (*submitted*), where 89 scientific publications were identified which included measurements of release data from nano-enabled articles and products. Thirty-three out of the 89 studies were identified as viable for extraction of quantitative release data, describing 320 different exposure scenarios for release from nano-enabled products. The release rates were grouped according to the NM release scenarios, including weathering by UV irradiation, leaching, abrasion, and spraying. The aim of the study was to provide a more harmonized data library for release from nano-enabled articles that could serve as a valuable resource for both exposure scenarios and occupational, consumer and environmental exposure assessment. However, the authors of the aforementioned review also highlight the importance of having more nano-specific data, such as size distribution, which is crucial for more detailed exposure assessments of nanomaterials.

A report by the Danish EPA (Larsen et al., 2015) evaluated the existing methods for consumer exposure assessment and developed 20 exposure scenarios for various product groups that were further used for the actual exposure assessment. The product categories included in this assessment were food and beverages, cosmetics, cleaning products, coatings, and textiles, among others. They reviewed available exposure and risk assessment tools that could be applicable for consumer exposure assessment and concluded that nano-specific tools are able to provide mostly qualitative information, whereas non nano-specific tools were generally more quantitative (including, but not limited to, ConsExpo, ECETOC TRA). Then, based on the information about specific products available on the market, and the information about experimentally determined or modelled exposure assessments from products in the same product categories, it was attempted to perform a specific nanomaterial exposure assessment. It was also highlighted that the manufacturers of the nanoproducts rarely provide enough information about the NMs that are present in the product or specify how

they are incorporated in the product and at what quantities they have been applied. To build more accurate and relevant specific exposure routes, several crucial pieces of information is needed, such as NM identification, surface coating, particle size distribution, matrix properties, attachment to the matrix, and the product formulation. The report concluded that no single tool was suitable for adequate and harmonized exposure assessment for nano-enabled consumer products, as the tools are not specifically designed for estimating NM exposure. It was also pointed out that using mass-based metrics for NM exposure might not be the most relevant, however, it can provide a somewhat conservative and reliable estimate of NM exposures, especially when it comes to dermal and oral exposure (Larsen et al., 2015).

From the 20 examples addressed in this report, it was found that highest oral exposures were predicted for food items and cosmetics, with oral doses up to 5.5 $mg/kg_{bw}/d$. Cosmetics was also the product group that resulted in the highest dermal exposure potential, with dermal applications resulting in a dermal dose of up to 450 $mg/kg_{bw}/d$. As for inhalation exposure, sanding and spray painting presented the highest exposure potentials with exposure concentrations up to 109 mg/m^3 corresponding to an inhalation dose of 1.5 $mg/kg_{bw}/d$. Overall, the highest exposures were estimated for food items (oral), sunscreens (dermal and oral), surface coatings and paints (dermal and inhalation), as well as construction materials (dermal and inhalation) and wound dressings (dermal). Relatively very low exposure potentials were found for food contact materials, composite materials and textiles, among others (Larsen et al., 2015).

Several experimental papers presenting release from nanoproducts have attempted to assess potential consumer exposure. For example, when it comes to oral exposure to silver from food containers, von Goetz et al. (2013a) and Mackevica et al. (2016b – Paper IV) estimated potential consumer exposure that might result from using a nanosilver-containing food container by storing food for 10 days without prior washing or pre-treatment of the container. Based on experimental data for total Ag release, the resulting maximum exposures were calculated to be 4.2 µg Ag (for storing 100mL of food) and 2 µg Ag (for storing food corresponding to 250 mL of volume), respectively. For one time use of a toothbrush (2 min) the maximum observed release in 15 mL of volume was found to be around 2.7 ng Ag, containing 3.8 $x10^4$ Ag particles with median size of 46 nm (Mackevica et al. 2016c – Paper V). Arguably, the actual consumer exposure would be in even lower amounts,

given that only a negligible fraction would get ingested and most of the released Ag would end up in the wastewater.

To have an indication whether or not it is considered a high exposure or not, we can compare it to Ag ingestion limits provided by the authorities, e.g. European Food Safety Authority (EFSA) has set the limit for total permitted Ag migration from food contact materials to 0.05 mg per kg of food (EFSA, 2006). Based on this information, the Ag exposure from selected food containers tested in aforementioned studies may be regarded negligible, but it has to be noted that the Ag exposure limits are not taking NMs into consideration. There is still some degree of uncertainty when it comes to understanding potential health hazards that might be caused by nano-Ag, especially when it comes to long term exposures (Hansen and Baun, 2012; SCENIHR, 2009).

Taking all this into consideration, it is apparent that we do not only we need more studies that are suitable for nano-specific exposure estimation, but that we also need to make sure that we have suitable models addressing he specific NM properties. Along with the mass-based metrics there is also a need for NM characterization data, such as particle number concentration and size distribution as a minimum. Future studies should aim at reporting results that are not only scientifically relevant, but also regulatory relevant and can be used when it comes to consumer exposure assessment.

7 Discussion

Even though nanomaterial use in consumer products has become increasingly ubiquitous, there is still a lack of understanding whether the use of these products is safe for consumers and the environment. Additionally, there is currently no widely accepted systematic approach for assessing potential consumer risks that are associated with nano-enabled product use. Current methods for NM analysis are promising, but there is still some development needed to have a standardized set of analysis tools and methods for NM characterization and quantification. Even with extensive NM characterization, it is unclear in what manner this kind of data could be used for exposure assessment and risk evaluation. Moreover, understanding of exposure conditions and pathways are of utmost importance when it comes to developing realistic and relevant exposure scenarios that could be representative for a larger population of consumers.

The overall aim of this thesis was to investigate the release rates of NMs from consumer products to gain an understanding regarding various issues associated with proper characterization of NMs and consumer exposure assessment. The literature review of current state-of-the-art of NM release testing highlighted a number of knowledge gaps for both testing methods and exposure assessment tools. Moreover, until now research has been mostly focused on a narrow range of products (e.g. textiles, paints, coatings) and NMs (Ag, TiO_2, CNTs), and the data is difficult to extrapolate to other product groups or NM types. Prioritization of NMs and specific product groups is needed to assess risks that can be associated with various items. As suggested by Dekkers et al. (2016), focus should be set on NMs that are produced in large quantities, and that have the potential for hazardous effects (or when the toxic effects are not known or not sufficiently investigated). Moreover, there is a need for more quantitative and well characterized NM exposure data, addressing a larger variety of consumer product groups and NM types.

Additionally, research done so far has been mostly investigating NM release rates in relatively short time frames (relative to the real-life use of the product) and imitating only a small number of consumer use scenarios. The experimental setups for NM release testing are often far from real-life scenarios, which make it even more difficult to extrapolate the data in the context of possible environmental and consumer exposures (Mackevica & Hansen, 2016 – Paper II). Also quantification of NM release is a complex

issue considering that NM characterization usually requires a multimethod approach. As discussed earlier in the thesis, the available NM characterization methods are still in developing stage, and it will take time until there are standardized widely accepted and widely used set of methods for NM measurements. A few of the techniques for NM characterization have been applied in connection with the work done within this thesis (Mackevica et al. 2016b,c,d,e – Paper IV, V, VI, VII), namely electron microscopy and spICP-MS. The advantages and limitations of using spICP-MS have been increasingly discussed in literature, and this technique offers high throughput analysis of such parameters as particle mass, size distribution and particle number concentration. However, it must be noted that it is a relatively new analysis technique that has received a lot of attention in the past five years, which means that there is still a long way to go before it becomes a standardized analytical technique. The American Society of the International Association for Testing and Materials (ASTM) is currently working on developing a standard guideline for spICP-MS analysis of NMs. The aim for this guide is to "*cover information on the optimization, calibration, and operational guidance for Inductively Coupled Plasma Mass Spectrometers (ICP-MS) for the analysis of nanoparticles containing metallic elements in various matrices by the technique of Single Particle ICP-MS*" (ASTM, 2016). A standardized procedure for NM characterization would aid researchers to report data in a more uniform format, which could provide better inter-study comparisons and a more solid basis for consumer exposure estimations. As evidenced by the experimental research conducted for this thesis, there is still a high degree of uncertainty associated with NM characterization when it comes to NM release measurements.

By using the information from nanoproduct inventories and the nano-exposure research presented in the literature, as well as experimental data gathered from experimental studies presented in this thesis, several issues were identified when it comes to exposure assessment of NMs to consumer products. In the following sections, the aim is to discuss the issues related to exposure scenarios, consumer exposure assessment, and the exposure metrics for data reporting. In the following, knowledge gaps and future research needs will be discussed, reflecting on the findings presented throughout the thesis, putting it in the context of what the future research needs are.

7.1 Exposure scenarios

As shown throughout the thesis, NMs in general have a very broad range of applications in consumer products. During product life cycle, it is more specifically the use phase that may result in uncontrolled and, in many cases unintentional, NM releases. The release of NMs from consumer products is an inevitable outcome from consumer activities, and may lead to both environmental and human exposure. The assessment of exposure requires identification of relevant scenarios for NM release that are representative of intended product use and foreseeable consumer behavior. This is why it is essential to develop relevant exposure scenarios to determine possible NM releases.

Potential exposure is often application-specific and is highly dependent on NM physicochemical properties, their incorporation in the product and the way the product is being used. For instance, NMs in textiles may lead to dermal exposure through sweat, abrasion and skin contact, whereas nanoparticles suspended in spray products such as disinfectants could result in both inhalation and dermal exposure. The quantities and characteristics of NM release from consumer products highly depend on the external stresses and environmental factors that the product is exposed to. These factors will influence both the quantity of the NMs released and the properties of released NMs (i.e. free occurring NMs, embedded or attached to product matrix, dissolved, aggregated or agglomerated NMs) (Vílchez et al., 2015). For the consumer products addressed in this work, it was observed that the properties of media that are in contact with the product can highly influence the NM release – as evidenced in Ag NP leaching from food containers (Mackevica et al. 2016b – Paper IV). The release rates were also observed to change over time, when the whole intended use period of the product was taken into account, as shown when testing Ag NP release from toothbrushes over time that corresponds to regular use over 3 months (Mackevica et al. 2016c – Paper V). Repeated uses of nano-enabled consumer products have rarely been addressed in the literature, but the available studies have shown that surface-bound particles have a tendency to show high initial release and relatively lower subsequent releases from re-used products. A few examples include multiple use testing of food containers (Echegoyen and Nerín, 2013; von Goetz et al., 2013a) and sequential washing textiles (Reed et al., 2016; Windler et al., 2012). The release rates can, to some extent, be dependent on the amounts of NM present in the product. For lab-prepared articles, the relationship is usually more pronounced, as both the matrix effects and the

NM content can be controlled (Wagener et al., 2016). When it comes to commercial products, it is rarely possible to simply relate the initial NM content to NM release. As shown in the case of nano-TiO_2 release from commercial textiles, the release was dependent not only on the initial TiO_2 content, but also on product matrix – having higher observed releases from synthetic fabrics compared to natural ones (Mackevica et al. 2016d – Paper VI).

Both selecting a representative set of products and choosing relevant exposure scenarios, is posing a series of significant challenges. As the products are very different, and the users may have their own interpretation about how the products should be used, it is difficult to identify one unifying scenario for release testing. To date, most studies are relying on guidelines that have been developed for assessing release of conventional chemicals, or they are simply developing their own test setups that may represent relevant consumer use. Several literature reviews have pointed out that, all in all, the experimental studies addressing NM release use very different test setups that are rarely comparable, and they are seldom following any standardized guidelines for release testing (e.g. Froggett et al. 2014, Koivisto et al. (*submitted*), Mackevica & Hansen, 2016 – Paper II). The most recent review identified 89 experimental studies addressing release from NM-containing articles (Kooivisto et al. (*submitted*)), and inter-study comparison was concluded to be particularly cumbersome due to the large variety of experimental setups and analytical methods applied. The harmonization of release testing and adequate NM-relevant reporting of the results would be highly beneficial for more realistic and relevant consumer exposure assessment.

In this regard, several suggestions have been brought up by various reports in the past couple of years. For example, some suggestions can be found in a report from a workshop entitled Quantifying Exposure to Engineered Nanomaterials (QEEN, 2015), specifically from discussions dealing with consumer exposure studies. The concluding recommendations were, "*When dealing with consumer products the first step in quantifying exposure to ENMs should be to characterize the intact products to confirm that ENMs are indeed present, and to characterize the ENMs within the product in terms of the following: composition, size and shape, where they are located, how much ENM is present (mass and, if possible, number concentration), how they are dispersed or attached to the product matrix*". Thereafter, it is suggested not to assume the worst case scenario where all of the NM is

released from the product, but perform tests considering situations of foreseeable use and misuse, examples including UV-degradation of coatings and composites, leaching from food contact materials, temperature extremes in cookware, and mechanical stresses in sporting equipment. The key dimensions of exposure to be considered include material characteristics, expected duration and magnitude of exposure, and receptor characteristics (QEEN, 2015).

7.2 Exposure assessment

Performing an actual consumer exposure assessment to NMs is often associated with a lot of uncertainties. Very often instead of using real measurement data, one has to rely on assumptions and default values provided by various exposure assessment models. At this point, NM-relevant exposure assessment tools are scarce, and most are developed for occupational exposure specifically. Both the lack of nano-specific modelling tools and the lack of guidelines for exposure data reporting hinder the consumer exposure assessment to NMs.

Several suggestions have been provided in the literature when it comes to working towards a NM-relevant consumer exposure assessment. As an example, a more extensive set of data that should be provided to aid reasonable nano-specific consumer exposure assessment was given in a report by Larsen et al. (2015), which clearly illustrates the magnitude of different parameters that would facilitate the understanding of nano-exposures. Quantitative parameters that would ideally provide data for exposure assessment of nanoproducts were identified to be:

- Size distribution of particles and fraction in nano-size
- Concentration of nanomaterial in the product
- Volume used per use event
- Retention rate of product (e.g. dermal exposure or fraction ingested)
- Degree of liberation/ migration of nanomaterial from a matrix (dermal exposure, oral exposure)
- Body surface area exposed (dermal exposure)
- Article surface area in contact (dermal exposure, oral exposure)
- Volume released to air (inhalation)
- Concentration in air (inhalation)
- Duration of exposure
- Frequency of exposure.

According to these recommendations, proper consumer exposure assessment would require a very detailed characterization of the NM in the product, as well as development of the likely exposure scenarios and the parameters influencing NM release and magnitude of exposure. This extent of information is difficult to come by, and at this point it is unclear how this data could be incorporated in consumer exposure assessment with the models that are available at this point. For the proper use of the existing models, it was concluded that the minimum set of key data needed would be weight of the product, concentration of substance in the product, and released amount (or fraction released) (Mackevica & Hansen, 2016 – Paper II). This set of data in combination with default values from the models for specific exposure scenarios can provide somewhat quantitative exposure assessment from different products, though it would not take NM-specific properties into consideration. There is still research needed for understanding which metrics are the most suitable and practical to use to characterize and quantify the exposure to NMs, and new models should be developed for nano-specific consumer exposure assessment. The harmonization of nano-specific hazard and exposure assessment models for integration into risk assessment and management is essential for understanding the real risks associated with the use of NMs.

7.3 Exposure metrics

To date, when it comes to reporting experimentally determined potential exposures to NM-containing items, it has been mostly mass-based reporting of a substance from the product (Mackevica & Hansen, 2016 – Paper II, Koivisto et al. (*submitted*)). Mass-based exposures provide a good indication with regard to quantities of a certain chemical that consumers might be exposed to, but they cannot provide necessary characterization of NM-specific exposures. In the past few years, there has been increased abundance of experimental studies reporting more than just mass-based releases, going beyond the well-established paradigm of measuring the potential exposures to chemicals. Parameters such as particle size, shape, speciation, number concentration, surface area, agglomeration, and surface properties are being recognized as relevant factors and are consequently reported in an increasing number of experimental studies. The “base set” of NM characterization has been discussed much in the past and is being increasingly discussed also in current scientific publications, raising the same issues. As an example from more than a decade ago, from a hazard assessment perspective, Oberdorster et al. (2005) suggested that toxicity studies should measure at least three

primary metrics for nanoparticles, namely mass, surface area, and particle number, to have a well characterized material which can facilitate quantitative interpretation of data. When it comes to human exposure measurements, the recommendations for characterizations that are to be considered "essential" were size distribution, shape, composition, physicochemical structure, agglomeration state, and concentration (Oberdörster et al., 2005). In more recent papers (e.g. Larsen et al. 2015; Mackevica & Hansen, 2016 – Paper II) similar recommendations were presented, highlighting the importance to report more than just the mass-based release, and also including particle number concentration and surface area measurements to provide more nano-relevant specifications.

The development of new characterization methods for NMs allows reporting more relevant NM characterization than before. As illustrated by the experimental work presented in this thesis (Mackevica et al. 2016b,c,d,e – Paper IV, V, VI, VII) as well as other scientific publications, utilizing state-of-the-art methods for NM characterization can aid in understanding consumer exposures to NMs from consumer products. For example, knowing the size distributions of the particles allows estimating whether there is a high likelihood of NMs to penetrate human skin or cells, where they can potentially dislocate within the body and cause damage. Knowing particle number concentrations and the fractions of dissolved vs. particulate material also provides an understanding about what a consumer can potentially be exposed to. This, in turn, helps characterizing the overall risks that may arise from using different consumer products. Well-characterized exposure can enable quantitative interpretation of data when it comes to risk assessment, provided that hazard data can be interpreted by using the same metrics. The nano-toxicology studies are therefore urged to transition to facilitate an improved understanding of how such parameters as particle size, particle number concentration, and surface area affect the hazard potential (Hull et al., 2012). Harmonization in terms of metrics across the exposure and hazard characterization is essential for risk management, as working with the same metrics will allow understanding the overall risks associated with NM exposure.

Both exposure potential and hazard assessment are essential parts for performing appropriate risk assessment. When it comes to NMs, hazard assessment is usually done by using pristine NMs, i.e. NMs in an early stage of their life cycle. It is well known that NMs are likely to undergo various transformations through their life cycle, especially if NMs are incorporated in

consumer products and then are released through various processes, such as weathering, abrasion or just simply normal/intended use of a consumer product, as discussed in a few examples during this thesis. These transformations are rarely taken into account when doing hazard testing, which leaves a major gap between exposure assessment and hazard assessment of NMs.

8 Conclusion and outlook

The focus of this thesis was to investigate release of NMs from consumer products by applying different methods and setups. Subsequently, reflecting on both data found in the literature and data generated in this thesis, it was discussed how the data fits into current paradigm of consumer exposure assessment, and what are the current issues and knowledge gaps regarding nano-relevant exposure assessment.

Different experimental setups and methods for characterization of NM release from various consumer products were applied in order to better understand strengths and limitations of the current paradigm of consumer exposure assessment to NMs. Through both theoretical and experimental investigations, issues and challenges associated with determining the release of NMs from nano-enabled products currently available on the market and potential consumer exposures that may be a result of their normal use was investigated.

The consumer products that were investigated in this thesis were Ag NP-containing food containers, toothbrushes, keyboard covers, TiO_2 NP-containing textiles, and CuO NP-containing paint. It was found that commercially available items rarely have high NP release, in most cases having rather low potential for consumer exposure. However, due to the high degree of uncertainty associated with nano-specific hazardous effects it still remains unclear whether or not these nanoproducts are “safe” to use.

However, the work done in this thesis highlights the necessity to apply a combination of methods for NM characterization to gain a better understanding about exposure to NMs. Specifically the focus was set to evaluating strengths and limitations, and perspectives of the use of spICP-MS in combination with other characterization techniques. The findings showed that spICP-MS is a powerful technique that can provide extensive NM characterization, such as mass and number concentration, and size distribution of NMs. However, there is still a need for standardization of spICP-MS analysis and data reporting, as well as the need for supporting the obtained data with measurements by other techniques.

Most existing consumer exposure assessment tools are mainly based on mass-metrics, rarely taking NM-specific properties into account. The models available at this point have limited applicability to estimate exposures to NMs. There is currently a lack of standardized guidelines targeting NMs for

consumer exposure testing and exposure assessment. Future research should be directed to testing NMs that are produced in high production volumes and/or may exert risk to human and environmental health. The exposure testing should also benefit from development of exposure scenarios for selected groups of articles that can be extrapolated to a larger set of products covering important parts of their life-cycle. Standardization of experimental setups, measuring methods and data reporting, as well as exposure modelling, is of utmost importance to move towards harmonization of NM exposure and hazard characterization that could further aid NM-relevant risk assessment.

9 References

Akaighe, N., MacCuspie, R., Navarro, D., 2011. Humic acid-induced silver nanoparticle formation under environmentally relevant conditions. Environ. Sci. Technol. 45, pp 3895–3901.

Artiaga, G., Ramos, K., Ramos, L., Cámara, C., Gómez-Gómez, M., 2015. Migration and characterisation of nanosilver from food containers by AF4-ICP-MS. Food Chem. 166, 76–85.

ASTM, 2016. WK54613 New Guide for Standard Guide for the Analysis of Nanoparticles by Single Particle Inductively Coupled Plasma Mass Spectrometry (SP-ICP-MS) [WWW Document]. URL https://www.astm.org/DATABASE.CART/WORKITEMS/WK54613.htm (accessed 9.18.16).

Benn, T., Cavanagh, B., Hristovski, K., Posner, J.D., Westerhoff, P., 2010. The Release of Nanosilver from Consumer Products Used in the Home. J. Environ. Qual. 39, 1875.

Benn, T.M., Westerhoff, P., 2008. Nanoparticle Silver Released into Water from Commercially Available Sock Fabrics. Environ. Sci. Technol. 42, 4133–4139.

Bianco, C., Kezic, S., Visser, M.J., Pluut, O., Adami, G., Krystek, P., 2015. Pilot study on the identification of silver in skin layers and urine after dermal exposure to a functionalized textile. Talanta 136, 23–28.

Bielefeldt, A.R., Stewart, M.W., Mansfield, E., Scott Summers, R., Ryan, J.N., 2013. Effects of chlorine and other water quality parameters on the release of silver nanoparticles from a ceramic surface. Water Res. 47, 4032–4039.

Bogdan, J., Jackowska-Tracz, A., Zarzyńska, J., Pławińska-Czarnak, J., 2015. Chances and limitations of nanosized titanium dioxide practical application in view of its physicochemical properties. Nanoscale Res. Lett. 10, 57.

BSI, 2007. Terminology for nanomaterials, PAS 136:2007. BSI Br. Stand.

Chang, A.L.S., Khosravi, V., Egbert, B., 2006. A case of argyria after colloidal silver ingestion. J. Cutan. Pathol. 33, 809–811.

Chou, K., Lu, Y., Lee, H., 2005. Effect of alkaline ion on the mechanism and kinetics of chemical reduction of silver. Mater. Chem. Phys. 94, 429–433.

Civardi, C., Schwarze, F.W.M.R., Wick, P., 2015. Micronized copper wood preservatives: An efficiency and potential health risk assessment for copper-based nanoparticles. Environ. Pollut. 200, 126–132.

Contado, C., Pagnoni, A., 2008. TiO2 in Commercial Sunscreen Lotion: Flow Field-Flow Fractionation and ICP-AES Together for Size Analysis. Anal. Chem. 80, 7594–7608.

Cornelis, G., Hasselöv, M., 2014. A signal deconvolution method to discriminate smaller nanoparticles in single particle ICP-MS. J. Anal. At. Spectrom. 29, 134-144.

Cushen, M., Kerry, J., Morris, M., Cruz-Romero, M., Cummins, E., 2014. Silver migration from nanosilver and a commercially available zeolite filler polyethylene composites to food simulants. Food Addit. Contam. Part A 31.

Dan, Y., Shi, H., Stephan, C., Liang, X., 2015. Rapid analysis of titanium dioxide nanoparticles in sunscreens using single particle inductively coupled plasma-mass spectrometry. Microchem. J. 122, 119–126.

De Jong, W.H., Delmaar, C., Gosens, I., Nijkamp, M., Quik, J., Vandebriel, R., Van Kesteren, P., Visser, M., Park, M., Wijnhoven, S., 2015. Description of a NanoCosmetics Tool for Risk Assessment. RIVM Briefrapport 2015-0157. National Institute for Public Health and the Environment, Bilthoven, The Netherlands.

Degueldre, C., Favarger, P.-Y.Y., 2003. Colloid analysis by single particle inductively coupled plasma-mass spectroscopy: A feasibility study. Colloids Surfaces A Physicochem. Eng. Asp. 217, 137–142.

Dekkers, S., Oomen, A.G., Bleeker, E.A.J., Vandebriel, R.J., Micheletti, C., Cabellos, J., Janer, G., Fuentes, N., Vázquez-Campos, S., Borges, T., Silva, M.J., Prina-Mello, A., Movia, D., Nesslany, F., Ribeiro, A.R., Leite, P.E., Groenewold, M., Cassee, F.R., Sips, A.J.A.M., Dijkzeul, A., van Teunenbroek, T., Wijnhoven, S.W.P., 2016. Towards a nanospecific approach for risk assessment. Regul. Toxicol. Pharmacol. 80, 46–59.

Delmaar, J., Park, M., Van Engelen, J., 2005. Cons Expo 4.0 – consumer exposure and uptake models - program manual. RIVM report 320104004/2005 [WWW Document]. Natl. Inst. Public Heal. Environ. Bilthoven, Netherlands. URL http://www.rivm.nl/ (accessed 9.7.16).

Donovan, A., Adams, C., Ma, Y., Stephan, C., Eichholz, T., Shi, H., 2016. Single particle ICP-MS characterization of titanium dioxide, silver, and gold nanoparticles during drinking water treatment. Chemosphere 144, 148–153.

ECETOC, 2009. Technical Report No. 107: Addendum to ECETOC Targeted Risk Assessment Technical Report No. 93. Brussels, Belgium.

ECHA, 2012. Guidance on information requirements and chemical safety assessment, Chapter R. 15: Consumer exposure estimation. European Chemicals Agency, Helsinki, Finland.

Echegoyen, Y., Nerín, C., 2013. Nanoparticle release from nano-silver antimicrobial food containers. Food Chem. Toxicol. 62, 16–22.

EFSA, 2006. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request related to a 12th list of substances for food contact materials. European Food Safety Authority, EFSA J 395–401:1–21.

European Commission, 2016. Commission Regulation (EU) 2016/1143 of 13 July 2016 amending Annex VI to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products. Off. J. Eur. Union L 189/40.

European Commission, 2011. Commission Regulation (EU) No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. Off. J. Eur. Union 50, 1–89.

Farkas, J., Peter, H., Christian, P., Gallego Urrea, J.A., Hassellöv, M., Tuoriniemi, J., Gustafsson, S., Olsson, E., Hylland, K., Thomas, K.V., 2011. Characterization of the effluent from a nanosilver producing washing machine. Environ. Int. 37, 1057–1062.

Froggett, S.J., Clancy, S.F., Boverhof, D.R., Canady, R.A., 2014. A review and perspective of existing research on the release of nanomaterials from solid nanocomposites. Part. Fibre Toxicol. 11, 17.

George, R., Merten, S., Wang, T.T., Kennedy, P., Maitz, P., 2014. In vivo analysis of dermal and systemic absorption of silver nanoparticles through healthy human skin. Australas. J. Dermatol. 55, 185–190.

Geranio, L., Heuberger, M., Nowack, B., 2009. The behavior of silver nanotextiles during washing. Environ. Sci. Technol. 43, 8113–8118.

Godwin, H.A., Chopra, K., Bradley, K.A., Cohen, Y., Harthorn, B.H., Hoek, E.M.V., Holden, P., Keller, A.A., Lenihan, H.S., Nisbet, R.M., Nel, A.E., 2009. Decreasing Uncertainties in Asessing Environmental Exposure, Risk, and Ecological Implications of Nanomaterials. Environ. Sci. Technol. 43, 6453–6457.

Gomez, V., Levin, M., Saber, A.T., Irusta, S., Dal Maso, M., Hanoi, R., Santamaria, J., Jensen, K.A., Wallin, H., Koponen, I.K., 2014. Comparison of dust release from epoxy and paint nanocomposites and conventional products during sanding and sawing. Ann. Occup. Hyg. 58, 983–94.

Gottschalk, F., Nowack, B., 2011. The release of engineered nanomaterials to the environment. J. Environ. Monit. 13, 1145–1155.

Grieger, K.D., Laurent, A., Miseljic, M., Christensen, F., Baun, A., Olsen, S.I., 2012. Analysis of current research addressing complementary use of life-cycle assessment and risk assessment for engineered nanomaterials: have lessons been learned from previous experience with chemicals? J. Nanoparticle Res. 14, 958.

Hagendorfer, H., Lorenz, C., Kaegi, R., Sinnet, B., Gehrig, R., Goetz, N. V., Scheringer, M., Ludwig, C., Ulrich, A., 2010. Size-fractionated characterization and quantification of nanoparticle release rates from a consumer spray product containing engineered nanoparticles. J. Nanoparticle Res. 12, 2481–2494.

Hansen, S.F., Baun, A., 2012. When enough is enough. Nat Nano 7, 409–411.

Hansen, S.F., Jensen, K.A., Baun, A., 2014. NanoRiskCat: a conceptual tool for categorization and communication of exposure potentials and hazards of nanomaterials in consumer products. J. Nanoparticle Res. 16, 2195.

Hansen, S.F., Larsen, B.H., Olsen, S.I., Baun, A., 2007. Categorization framework to aid hazard identification of nanomaterials. Nanotoxicology 1, 243–250.

Hassellöv, M., Readman, J.W., Ranville, J.F., Tiede, K., 2008. Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles. Ecotoxicology 17, 344–361.

Hauri, J.F., Niece, B.K., 2011. Leaching of Silver from Silver-Impregnated Food Storage Containers. J. Chem. Educ. 88, 1407–1409.

Hirth, S., Cena, L., Cox, G., Tomović, Ž., Peters, T., Wohlleben, W., 2013. Scenarios and methods that induce protruding or released CNTs after degradation of nanocomposite materials. J. Nanoparticle Res. 15, 1504.

Hock, J., Epprecht, T., Furrer, E., Gautschi, M., Hofmann, H., Höhener, K., Al, E., 2013. Guidelines on the Precautionary Matrix for Synthetic Nanomaterials. Version 3.0. Federal Office of Public Health and Federal Office for the Environment, Berne, Switzerland.

Howard, A.G., 2010. On the challenge of quantifying man-made nanoparticles in the aquatic environment. J. Environ. Monit. 12, 135–142.

Huang, Y., Chen, S., Bing, X., Gao, C., Wang, T., Yuan, B., 2011. Nanosilver Migrated into Food-Simulating Solutions from Commercially Available Food Fresh Containers. Packag. Technol. Sci. 24, 291–297.

Hull, M., Kennedy, A.J., Detzel, C., Vikesland, P., Chappell, M.A., 2012. Moving beyond Mass: The Unmet Need to Consider Dose Metrics in Environmental Nanotoxicology Studies. Environ. Sci. Technol. 46, 10881–10882.

Impellitteri, C.A., Tolaymat, T.M., Scheckel, K.G., 2009. The Speciation of Silver Nanoparticles in Antimicrobial Fabric Before and After Exposure to a Hypochlorite/Detergent Solution. J. Environ. Qual. 38, 1528.

Kaegi, R., Sinnet, B., Zuleeg, S., Hagendorfer, H., Mueller, E., Vonbank, R., Boller, M., Burkhardt, M., 2010. Release of silver nanoparticles from outdoor facades. Environ. Pollut. 158, 2900–2905.

Kaegi, R., Ulrich, A., Sinnet, B., Vonbank, R., Wichser, A., Zuleeg, S., Simmler, H., Brunner, S., Vonmont, H., Burkhardt, M., Boller, M., 2008. Synthetic TiO2 nanoparticle emission from exterior facades into the aquatic environment. Environ. Pollut. 156, 233–239.

Kim, Y.S., Kim, J.S., Cho, H.S., Rha, D.S., Kim, J.M., Park, J.D., Choi, B.S., Lim, R., Chang, H.K., Chung, Y.H., Kwon, I.H., Jeong, J., Han, B.S., Yu, I.J., 2008. Twenty-Eight-Day Oral Toxicity, Genotoxicity, and Gender-Related Tissue Distribution of Silver Nanoparticles in Sprague-Dawley Rats. Inhal. Toxicol. 20, 575–583.

Kim, Y.S., Song, M.Y., Park, J.D., Song, K.S., Ryu, H.R., Chung, Y.H., Chang, H.K., Lee, J.H., Oh, K.H., Kelman, B.J., Hwang, I.K., Yu, I.J., 2010. Subchronic oral toxicity of silver nanoparticles. Part. Fibre Toxicol. 7, 20.

Klaine, S.J., Koelmans, A.A., Horne, N., Carley, S., Handy, R.D., Kapustka, L., Nowack, B., von der Kammer, F., 2012. Paradigms to assess the environmental impact of manufactured nanomaterials. Environ. Toxicol. Chem. 31, 3–14.

Kohler, A.R., Som, C., 2014. Risk preventive innovation strategies for emerging technologies the cases of nano-textiles and smart textiles. Technovation 34, 420–430.

Koponen, I.K., Jensen, K.A., Schneider, T., 2011. Comparison of dust released from sanding conventional and nanoparticle-doped wall and wood coatings. J. Expo. Sci. Environ. Epidemiol. 21, 408–418.

Koivisto, A.J., Jensen, A.C.Ø., Kling, K.I., Nørgaard, A., Brinch, A., Christensen, F., Jensen, K.A. (submitted). Quantitative material releases from articles containing manufactured nanomaterials: A critical review.

Kulthong, K., Srisung, S., Boonpavanitchakul, K., Kangwansupamonkon, W., Maniratanachote, R., 2010. Determination of silver nanoparticle release from antibacterial fabrics into artificial sweat. Part. Fibre Toxicol. 7, 8.

Laborda, F., Bolea, E., Cepriá, G., Gómez, M.T., Jiménez, M.S., Pérez-Arantegui, J., Castillo, J.R., 2016. Detection, characterization and quantification of inorganic engineered nanomaterials: A review of techniques and methodological approaches for the analysis of complex samples. Anal. Chim. Acta 904, 10–32.

Laborda, F., Bolea, E., Jimenez-Lamana, J., 2014. Single Particle Inductively Coupled Plasma Mass Spectrometry: A Powerful Tool for Nanoanalysis. Anal. Chem. 86, 2270–2278.

Laborda, F., Jiménez-Lamana, J., Bolea, E., Castillo, J.R., 2013. Critical considerations for the determination of nanoparticle number concentrations, size and number size distributions by single particle ICP-MS. J. Anal. At. Spectrom. 28, 1220–1232.

Laborda, F., Jiménez-Lamana, J., Bolea, E., Castillo, J.R., 2011. Selective identification, characterization and determination of dissolved silver(i) and silver nanoparticles based on single particle detection by inductively coupled plasma mass spectrometry. J. Anal. At. Spectrom. 26, 1362.

Labouta, H.I., Schneider, M., 2013. Interaction of inorganic nanoparticles with the skin barrier: current status and critical review. Nanomedicine Nanotechnology, Biol. Med. 9, 39–54.

Larsen, P.B., Christensen, F., Keld, C., Jensen, A., Brinch, A., Mikkelsen, S.H., 2015. Exposure assessment of nanomaterials in consumer products. Danish Environ. Prot. Agency, Environ. Proj. No. 1636, 2015.

Lee, S., Bi, X., Reed, R.B., Ranville, J.F., Herckes, P., Westerhoff, P., 2014. Nanoparticle Size Detection Limits by Single Particle ICP-MS for 40 Elements. Environ. Sci. Technol. 48, 10291–10300.

Lee, W.-W., Chan, W.-T., 2015. Calibration of single-particle inductively coupled plasma-mass spectrometry (SP-ICP-MS). J. Anal. At. Spectrom. 30, 1245–1254.

Lee, Y., Kim, J., 2015. Leaching of silver from commercial toothbrush products containing silver nanoparticles into pH controlled solution, in: 10thInternational Conference on the Environmental Effects of Nanoparticles and Nanomaterials. p. Poster.

Lewicka, Z.A., Benedetto, A.F., Benoit, D.N., Yu, W.W., Fortner, J.D., Colvin, V.L., 2011. The structure, composition, and dimensions of TiO2 and ZnO nanomaterials in commercial sunscreens. J. Nanoparticle Res. 13, 3607–3617.

Liu, J., Hurt, R.H., 2010. Ion Release Kinetics and Particle Persistence in Aqueous Nano-Silver Colloids. Environ. Sci. Technol. 44, 2169–2175.

Liu, J., Murphy, K.E., MacCuspie, R.I., Winchester, M.R., 2014. Capabilities of Single Particle Inductively Coupled Plasma Mass Spectrometry for the Size Measurement of Nanoparticles: A Case Study on Gold Nanoparticles. Anal. Chem. 86, 3405–3414.

Loeschner, K., Navratilova, J., Grombe, R., Linsinger, T.P.J., Købler, C., Mølhave, K., Larsen, E.H., 2015. In-house validation of a method for determination of silver nanoparticles in chicken meat based on asymmetric flow field-flow fractionation and inductively coupled plasma mass spectrometric detection. Food Chem. 181, 78–84.

Loeschner, K., Navratilova, J., Købler, C., Mølhave, K., Wagner, S., von der Kammer, F., Larsen, E.H., 2013. Detection and characterization of silver nanoparticles in chicken meat by asymmetric flow field flow fractionation with detection by conventional or single particle ICP-MS. Anal. Bioanal. Chem. 405, 8185–8195.

Lombi, E., Donner, E., Scheckel, K.G., Sekine, R., Lorenz, C., Goetz, N. Von, Nowack, B., 2014. Silver speciation and release in commercial antimicrobial textiles as influenced by washing. Chemosphere 111, 352–358.

Lorenz, C., Hagendorfer, H., von Goetz, N., Kaegi, R., Gehrig, R., Ulrich, A., Scheringer, M., Hungerbühler, K., 2011. Nanosized aerosols from consumer sprays: experimental analysis and exposure modeling for four commercial products. J. Nanoparticle Res. 13, 3377–3391.

Lorenz, C., Windler, L., Goetz, N. Von, Lehmann, R., 2012. Characterization of silver release from commercially available functional (nano) textiles. Chemosphere 89(7), 817-824.

Lynch, I., 2015. Compendium of Projects in the European NanoSafety Cluster. European Commission 2015 Edition.

Mavon, A., Miquel, C., Lejeune, O., Payre, B., Moretto, P., 2007. In vitro Percutaneous Absorption and in vivo Stratum Corneum Distribution of an Organic and a Mineral Sunscreen. Skin Pharmacol. Physiol. 20, 10–20.

Mitrano, D., Limpiteeprakan, P., Babel, S., Nowack, B., 2016a. Durability of nano-enhanced textiles through the life cycle: releases from landfilling after washing. Environ. Sci. Nano 3, 375–387.

Mitrano, D., Lombi, E., Dasilva, Y.A.R., Nowack, B., 2016b. Unraveling the Complexity in the Aging of Nanoenhanced Textiles: A Comprehensive Sequential Study on the Effects of Sunlight and Washing on Silver Nanoparticles. Environ. Sci. Technol. 50, 5790–5799.

Mitrano, D., Motellier, S., Clavaguera, S., Nowack, B., 2015a. Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products. Environ. Int. 77, 132–147.

Mitrano, D., Rimmele, E., Wichser, A., Erni, R., Height, M., Nowack, B., 2014. Presence of nanoparticles in wash water from conventional silver and nano-silver textiles. ACS Nano 8, 7208–7219.

Mitrano, D., Arroyo Rojas Dasilva, Y., Nowack, B., 2015b. Effect of Variations of Washing Solution Chemistry on Nanomaterial Physicochemical Changes in the Laundry Cycle. Environ. Sci. Technol. 49, 9665–9673.

Montaño, M.D., Lowry, G. V, von der Kammer, F., Blue, J., Ranville, J.F., 2014. Current status and future direction for examining engineered nanoparticles in natural systems. Environ. Chem. 11, 351–366.

Navarro, E., Baun, A., Behra, R., Hartmann, N.B., Filser, J., Miao, A.-J., Quigg, A., Santschi, P.H., Sigg, L., 2008. Environmental behavior and ecotoxicity of enginieered nanoparticles to algae, plants and fungi. Ecotoxicology 17, 372–386.

Nischwitz, V., Goenaga-Infante, H., 2012. Improved sample preparation and quality control for the characterisation of titanium dioxide nanoparticles in sunscreens using flow field flow fractionation on-line with inductively coupled plasma mass spectrometry. J. Anal. At. Spectrom. 27, 1084.

Nohynek, G.J., Lademann, J., Ribaud, C., Roberts, M.S., 2007. Grey Goo on the Skin? Nanotechnology, Cosmetic and Sunscreen Safety. Crit. Rev. Toxicol. 37, 251–277.

Nowack, B., Bucheli, T.D., 2007. Occurrence, behavior and effects of nanoparticles in the environment. Environ. Pollut. 150(1), 5-22.

Nowack, B., Ranville, J., Diamond, S., 2012. Potential scenarios for nanomaterial release and subsequent alteration in the environment. Environmental Toxicology and Chemistry 31(1), 50-59.

Oberdörster, G., Maynard, A., Donaldson, K., Castranova, V., Fitzpatrick, J., Ausman, K., Carter, J., Karn, B., Kreyling, W., Lai, D., Olin, S., Monteiro-Riviere, N., Warheit, D., Yang, H., 2005. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part. Fibre Toxicol. 2, 8.

Olabarrieta, J., Zorita, S., Peña, I., Rioja, N., Monzón, O., Benguria, P., Scifo, L., 2012. Aging of photocatalytic coatings under a water flow: Long run performance and TiO2 nanoparticles release. Appl. Catal. B Environ. 123, 182–192.

Ostertag, K., Hüsing, B., 2008. Identification of starting points for exposure assessment in the post-use phase of nanomaterial-containing products. J. Clean. Prod. 16, 938–948.

Pace, H.E., Rogers, N.J., Jarolimek, C., Coleman, V.A., Higgins, C.P., Ranville, J.F., 2011. Determining Transport Efficiency for the Purpose of Counting and Sizing Nanoparticles via Single Particle Inductively Coupled Plasma Mass Spectrometry. Anal. Chem. 83, 9361–9369.

Pasricha, A., Jangra, S.L., Singh, N., Dilbaghi, N., Sood, K.N., Arora, K., Pasricha, R., 2012. Comparative study of leaching of silver nanoparticles from fabric and effective effluent treatment. J. Environ. Sci. 24, 852–859.

PEN, 2016. The Project on Emerging Nanotechnologies: Consumer Products Inventory [WWW Document]. URL http://www.nanotechproject.org/cpi (accessed 7.4.16).

Pérez, S., Farré, M. la, Barceló, D., 2009. Analysis, behavior and ecotoxicity of carbon-based nanomaterials in the aquatic environment. TrAC - Trends Anal. Chem. 28, 820–832.

Peters, R., Kramer, E., Oomen, A., Rivera, Z.H., 2012. Presence of nano-sized silica during in vitro digestion of foods containing silica as a food additive. ACS Nano 6, 2441–2451.

Peters, R.J., Herrera Rivera, Z., van Bemmel, G., P Marvin, H.J., Weigel, S., Bouwmeester, H., 2014a. Development and validation of single particle ICP-MS for sizing and quantitative determination of nano-silver in chicken meat. Anal Bioanal Chem 406, 3875–3885.

Peters, R.J., Rivera, Z.H., Bouwmeester, H., Weigel, S., Marvin, H.J.P., 2014b. Advanced analytical techniques for the measurement of nanomaterials in complex samples: a comparison. Qual. Assur. Saf. Crop. Foods 6, 281–290.

Peters, R.J., Van Bemmel, G., Herrera-Rivera, Z., Helsper, H.P.F.G., Marvin, H.J.P., Weigel, S., Tromp, P.C., Oomen, A.G., Rietveld, A.G., Bouwmeester, H., 2014c. Characterization of titanium dioxide nanoparticles in food products: Analytical methods to define nanoparticles. J. Agric. Food Chem. 62, 6285–6293.

Platten, W.E., Sylvest, N., Warren, C., Arambewela, M., Harmon, S., Bradham, K., Rogers, K., Thomas, T., Luxton, T.P., 2016. Estimating dermal transfer of copper particles from the surfaces of pressure-treated lumber and implications for exposure. Sci. Total Environ. 548, 441–449.

QEEN, 2015. Quantifying Exposure to Engineered Nanomaterials (QEEN) from Manufactured Products: Addressing Environmental , Health , and Safety Implications. Workshop Proceedings. Consumer Product Safety Commission in collaboration with the National Nanotechnology Initiative. Arlington, VA, USA.

Quadros, M.E., Pierson, R., Tulve, N.S., Willis, R., Rogers, K., Thomas, T.A., Marr, L.C., 2013. Release of Silver from Nanotechnology-Based Consumer Products for Children. Environ. Sci. Technol. 47, 8894–8901.

Radetić, M., 2013. Functionalization of textile materials with TiO2 nanoparticles. J. Photochem. Photobiol. C Photochem. Rev. 16, 62–76.

Reed, R.B., Faust, J.J., Yang, Y., Doudrick, K., Capco, D.G., Hristovski, K., Westerhoff, P., 2014. Characterization of Nanomaterials in Metal Colloid-Containing Dietary Supplement Drinks and Assessment of Their Potential Interactions after Ingestion. ACS Sustain. Chem. Eng. 2, 1616–1624.

Reed, R.B., Zaikova, T., Barber, A., Simonich, M., Lankone, R., Marco, M., Hristovski, K., Herckes, P., Passantino, L., Fairbrother, D.H., Tanguay, R., Ranville, J.F., Hutchison, J.E., Westerhoff, P.K., 2016. Potential Environmental Impacts and Antimicrobial Efficacy of Silver- and Nanosilver-Containing Textiles. Environ. Sci. Technol. 50, 4018–4026.

Ren, D., Smith, J.A., 2013. Retention and Transport of Silver Nanoparticles in a Ceramic Porous Medium Used for Point-of-Use Water Treatment. Environ. Sci. Technol. 47, 3825–3832.

RIVM, 2016. ConsExpo nano [WWW Document]. URL https://www.consexponano.nl/ (accessed 9.8.16).

Sal'nikov, D., Pogorelova, A., Makarov, S., 2009. Silver ion reduction with peat fulvic acids. Russ. J. Appl. Chem. 82, 545–548.

Schilling, K., Bradford, B., Castelli, D., Dufour, E., Nash, J.F., Pape, W., Schulte, S., Tooley, I., van den Bosch, J., Schellauf, F., 2010. Human safety review of "nano" titanium dioxide and zinc oxide. Photochem. Photobiol. Sci. 9, 495.

Scientific Committee on Emerging and Newly Identified Health, Risks (SCENIHR), 2009. Risk assessment of products of nanotechnologies. European Commission Health and Consumer Protection Directorate-General, Directorate C— public health and risk assessment, C7—risk assessment. European Commission, Brussels, Belgium.

Seager, T.P., Linkov, I., 2008. Coupling Multicriteria Decision Analysis and Life Cycle Assessment for Nanomaterials. J. Ind. Ecol. 12, 282–285.

Shandilya, N., Le Bihan, O., Bressot, C., Morgeneyer, M., 2014. Evaluation of the Particle Aerosolization from n-TiO2 Photocatalytic Nanocoatings under Abrasion. J. Nanomater. 2014, 1–11.

Smirnova, V. V, Krasnoiarova, O. V, Pridvorova, S.M., Zherdev, A. V, Gmoshinskiĭ, I. V, Kazydub, G. V, Popov, K.I., Khotimchenko, S.A., 2012. [Characterization of silver nanoparticles migration from package materials destined for contact with foods]. Vopr. Pitan. 81, 34–9.

Som, C., Berges, M., Chaudhry, Q., Dusinska, M., Fernandes, T.F., Olsen, S.I., Nowack, B., 2010. The importance of life cycle concepts for the development of safe nanoproducts. Toxicology 269, 160–169.

Song, H., Li, B., Lin, Q.-B., Wu, H.-J., Chen, Y., 2011. Migration of silver from nanosilver–polyethylene composite packaging into food simulants. Food Addit. Contam. Part A 49, 1–5.

Stefaniak, A.B., Duling, M.G., Lawrence, R.B., Thomas, T.A., LeBouf, R.F., Wade, E.E., Abbas Virji, M., 2014. Dermal exposure potential from textiles that contain silver nanoparticles. Int. J. Occup. Environ. Health 20, 220–234.

The Nanodatabase, 2016. The Nanodatabase [WWW Document]. URL http://nanodb.dk/ (accessed 7.4.16).

Thomas, T., Thomas, K., Sadrieh, N., Savage, N., Adair, P., Bronaugh, R., 2006. Research Strategies for Safety Evaluation of Nanomaterials, Part VII: Evaluating Consumer Exposure to Nanoscale Materials. Toxicol. Sci. 91, 14–19.

Tiede, K., Boxall, A.B.A., Tear, S.P., Lewis, J., David, H., Hassello, M., 2008. Detection and characterization of engineered nanoparticles in food and the environment. Food Addit. Contam. 25, 795–821.

Tuoriniemi, J., Cornelis, G., Hasselov, M., 2012. Size Discrimination and Detection Capabilities of Single-Particle ICPMS for Environmental Analysis of Silver Nanoparticles. Anal. Chem. 84(9), 3965-3972.

Vance, M.E., Kuiken, T., Vejerano, E.P., McGinnis, S.P., Hochella, M.F., Hull, D.R., 2015. Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. Beilstein J. Nanotechnol. 6, 1769–1780.

Varner, K., 2010. State of the Science Literature Review: Nano Titanium Dioxide Environmental Matters. EPA/600/R-10/089. Environmental Protection Agency, Washington, DC, USA.

Vílchez, A., Fernández-Rosas, E., González-Gálvez, D., Vázquez-Campos, S., 2015. Nanomaterials Release from Nano-Enabled Products, in: Viana, M. (Ed.), Indoor and Outdoor Nanoparticles: Determinants of Release and Exposure Scenarios. Springer International Publishing, Switzerland.

von der Kammer, F., Ferguson, P.L., Holden, P.A., Masion, A., Rogers, K.R., Klaine, S.J., Koelmans, A.A., Horne, N., Unrine, J.M., 2012. Analysis of engineered nanomaterials in complex matrices (environment and biota): General considerations and conceptual case studies. Environ. Toxicol. Chem. 31, 32–49.

von Goetz, N., Fabricius, L., Glaus, R., Weitbrecht, V., Günther, D., Hungerbühler, K., 2013a. Migration of silver from commercial plastic food containers and implications for consumer exposure assessment. Food Addit. Contam. Part A 30, 612–620.

von Goetz, N., Lorenz, C., Windler, L., Nowack, B., Heuberger, M., Hungerbühler, K., 2013b. Migration of Ag- and TiO2-(Nano)particles from Textiles into Artificial Sweat under Physical Stress: Experiments and Exposure Modeling. Environ. Sci. Technol. 47, 9979–9987.

Wagener, S., Dommershausen, N., Jungnickel, H., Laux, P., Mitrano, D., Nowack, B., Schneider, G., Luch, A., 2016. Textile Functionalization and Its Effects on the Release of Silver Nanoparticles into Artificial Sweat. Environ. Sci. Technol. 50, 5927–5934.

WHO, 2013. Nanotechnology and human health: Scientific evidence and risk governance. Report of the WHO expert meeting 10–11 December 2012, Bonn, Germany.

Wijnhoven, S., 2007. Nanomaterials in consumer products. Availability on the European market and adequacy of the regulatory framework. RIVM/SIR Advis. Rep. 11014 (IP/A/ENVI/IC/2006- 193, 49.

Wijnhoven, S.W.P., Peijnenburg, W.J.G.M., Herberts, C.A., Hagens, W.I., Oomen, A.G., Heugens, E.H.W., Roszek, B., Bisschops, J., Gosens, I., Van De Meent, D., Dekkers, S., De Jong, W.H., van Zijverden, M., Sips, A.J.A.M., Geertsma, R.E., 2009. Nano-silver – a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology 3, 109–138.

Windler, L., Lorenz, C., Goetz, N. Von, Hungerbühler, K., Amberg, M., Heuberger, M., Nowack, B., 2012. Release of titanium dioxide from textiles during washing. Environ. Sci. Technol. 46, 8181−8188.

Wohlleben, W., Brill, S., Meier, M.W., Mertler, M., Cox, G., Hirth, S., Von Vacano, B., Strauss, V., Treumann, S., Wiench, K., Ma-Hock, L., Landsiedel, R., 2011. On the lifecycle of nanocomposites: Comparing released fragments and their in-vivo hazards from three release mechanisms and four nanocomposites. Small 7, 2384–2395.

Yan, Y., Yang, H., Li, J., Lu, X., Wang, C., 2012. Release behavior of nano-silver textiles in simulated perspiration fluids. Text. Res. J. 82, 1422–1429.

Zhou, S., Gu, G., 2004. Study on the morphology and tribological properties of acrylic based polyurethane / fumed silica composite coatings. Physics (College. Park. Md). 9, 1593–1600.

10 Papers

I Hansen, S.F., Heggelund, L.R., Besora, P.R., **Mackevica, A.**, Boldrin, A. and Baun, A., 2016. Nanoproducts–what is actually available to European consumers? *Environmental Science: Nano*, 3(1), 169-180.

II **Mackevica, A.** and Hansen, S.F., 2016. Release of nanomaterials from solid nanocomposites and consumer exposure assessment–a forward-looking review. *Nanotoxicology*, 10(6), 641-653.

III **Mackevica, A.**, Besora, P.R., Brinch, A., and Hansen, S.F., 2016a. Current uses of nanomaterials in biocidal products and treated articles in the EU. *Environmental Science: Nano. In press.*

IV **Mackevica, A.,** Olsson, M.E. and Hansen, S.F., 2016b. Silver nanoparticle release from commercially available plastic food containers into food simulants. *Journal of Nanoparticle Research*, 18(1), 1-11.

V **Mackevica, A.,** Olsson, M.E. and Hansen, S.F., 2016c. The release of silver nanoparticles from commercial toothbrushes. *Journal of Hazardous Materials. In press.*

VI **Mackevica, A.,** Olsson, M.E. and Hansen, S.F., 2016d. Quantitative Characterization of Nano-TiO_2 Release from Fabrics by Single Particle ICP-MS. *Manuscript.*

VII **Mackevica, A.,** Olsson, M.E., Mines, P.D., Heggelund, L.R. and Hansen, S.F., 2016e. Estimation of dermal transfer of nanoparticles from consumer articles by wipe sampling. *Manuscript.*