NANOMATERIAL INHALATION EXPOSURE FROM NANOTECHNOLOGY-BASED CONSUMER PRODUCTS

by

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ABSTRACT OF THE DISSERTATION

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Certain kinds of nanomaterials have been shown to cause serious health effects. When various nanomaterials are introduced into consumer products, their use could lead to nanomaterial inhalation exposure with possible health effects. We explored the potential of this exposure for several consumer sprays and cosmetic powders including products marketed as nanotechnology-based and alternative non-nanotechnology-based products. Actual application of real world products was realistically simulated and the inhaled aerosol was measured directly. We described: 1) the nanoparticles and nano-agglomerates in products, to which exposure could occur during application by consumers; 2) the potential for inhalation exposure to nanomaterial-containing particulate matter, generated during product application; and 3) the quantitative nanomaterial inhalation exposure as both inhaled dose and dose deposited in different regions of the human respiratory tract (for the cosmetic powders only). Particles in the products were investigated using transmission electron microscopy (TEM), photon correlation spectroscopy (PCS) and laser diffraction spectroscopy (LDS). We then realistically simulated the use of the products by spraying them in the vicinity of a female mannequin

head or applying directly onto its face in the case of cosmetic powders. A Scanning Mobility Particle Sizer (SMPS) and an Aerosol Particle Sizer (APS) were used to measure the "inhaled" aerosol particle size distributions by drawing aerosol through the mannequin's nostrils. The measurement data for powders were also used in an inhalation exposure model. Nanoparticles were found in both the nanotechnology-based and regular products. We could not, however, determine their engineered status. It was concluded that the highest inhalation exposure to nanomaterials in the investigated consumer products would occur due to inhalation and deposition of nanoparticle agglomerates larger than 100 nm – not individual nanoparticles or nanosized agglomerates. For the cosmetic powders, inhaled particle deposition in the head airways constituted the dominant portion (85-93%) of the total deposited dose overwhelming the deposition in the tracheobronchial and the alveolar regions. Hence, the future toxicology studies of nanotechnology-based consumer products should take into account exposures not only to single nanoparticles, but also to much larger nanoparticle agglomerates and investigate the potential biological effects in all regions of the respiratory tract.

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Table of Contents

List of Tables

List of Figures

Chapter 1

Background, Motivation, and Dissertation Overview

1.1. Background and Motivation

1.1.1. Nanotechnology and Its Distinct Position in Research

The US National Nanotechnology Initiative (NNI) defines nanotechnology as "the understanding and control of matter at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications" (National Science and Technology Council 2007). It is necessary to note that other alternative definitions exist (Balogh 2010; Dionysios 2004; Romig Jr et al. 2007; Schummer 2007) and any specific dimensional boundaries of nanotechnology should not always be considered strictly limiting since the effects attributed to the dimensional parameters between the atomic (approximately 0.2 nm) and "bulk" levels are also observed outside of the 1 – 100 nm range (Cedervall et al. 2007; Hu et al. 2006; Kim et al. 2004; Konan et al. 2002; Perrault and Chan 2009; Shaw 2011; Vayssieres 2003).

Despite the ongoing debate regarding the definition and the extension of dispersion size-related effects of materials beyond certain official nanotechnology definitions, the size range that is receiving the most attention from researchers working in the field of nanotechnology is between approximately 1 and 100 nm. It is also approximately in this size range that surface and interfacial phenomena may cause higher biological activity of substances when compared to their bulk form. Incidentally, below 10 nm, the optical, magnetic, and electronic properties of materials are altered by quantum effects (Haglund Jr. 1998). These various effects stemming from the

dimensional parameters of material dispersions or the potential expectation of products' special properties by consumers stimulate the development and/or marketing of nanotechnology-based products (Wardak et al. 2008).

The dimensional characteristics of nanomaterials are better defined according to ISO/TS 27687 as cited by Iavicoli et al. (2010). There, the term "nano-object" is defined as material with one, two or three external dimensions in the size range of approximately 1 – 100 nm. A "nanoplate" is a nano-object with one external dimension of 1 – 100 nm. A "nanofiber" is a nano-object with two external dimensions of 1 – 100 nm with a "nanotube" being defined as a hollow nanofiber and a "nanorod" defined as a solid nanofiber. Finally, a "nanoparticle" is a nano-object with all of the three external dimensions of 1 – 100 nm. "Nanomaterial" then describes any kind of nano-objects in the pure form or incorporated into a larger matrix or substrate.

1.1.2. Nanomaterial Production and Use

Over the past years, we have seen an intense and increasing interest in developing technologies based on the unique behavior of nanoscale (1-100 nanometers) materials and structures. The research-driven and industrial production of nanoparticles and nano-engineered structures as well as their introduction into common consumer products are undergoing a rapid growth (Baxter et al. 2009; Sarma 2008). This increasing production and use of nanoscale products raises the risk of nanoparticle exposure and substantial releases into the environment, including indoor air and the atmosphere (Hansen et al. 2008; Majestic et al. 2010; Maynard and Aitken 2007; McCall 2011). Such release is of great concern due to potential negative environmental and health effects caused by nanoparticles (Colvin 2003; Dionysios 2004; Drobne 2007; Gwinn and Vallyathan 2006;

Hoet 2004; Holgate 2010; McCall 2011; Nel et al. 2006; Nowack and Bucheli 2007; Riediker 2009; Schmid et al. 2009; Teow et al. 2011).

Deliberately added nanomaterial ingredients are already in an extensive variety of products on the market including personal care and cosmetic sprays and powders, dietary supplements and medications, cleaning and disinfectant liquids and sprays, sports equipment, and clothing (Bradford et al. 2009; Maynard 2007; Woodrow Wilson International Center for Scholars 2009). It is hard to estimate the exact number of nanotechnology-based products in any local markets and worldwide as their registration and adequate labeling and marketing often range from non-existent to limited (Chatterjee 2008; Fischer 2008; Michelson 2008). The best attempt to estimate the number of nanotechnology-based consumer products on the market and catalogue them was done through the creation of an inventory of nanotechnology-based consumer products within The Project on Emerging Nanotechnologies that as of 31, May 2012 listed "1317 products, produced by 587 companies, located in 30 countries" (Woodrow Wilson International Center for Scholars 2011). At the same time, a search we conducted for just one type of nanotechnology-based consumer products – nanosilver consumer sprays – using the same methodology as used by the Nanotechnology Consumer Products Inventory (Fauss 2008), revealed a much higher number of such products available for purchase on the end consumer market than listed in the above-mentioned Inventory. Moreover, the fact that many of the products still listed in this database are no longer present on the market points to the current inadequacy of this widely used and cited resource for the purpose of consumer nanoproduct identification at present.

At the same time, there also has been little research targeted at exploring the possibility of nanoparticle presence in the "regular" (non-nanotechnology-based) products. The way some regular products are made can lead to some ingredients being dispersed in them at nanoscale creating a possibility for nanoparticle inhalation exposure during application of such products. Also, the development and commercialization of nanotechnologies is progressing in the absence of specific regulations or legal guidelines for labeling (Paull and Lyons 2008), and most importantly, with limited knowledge about the potential for exposure to nanoparticles from such products, which is also critical for the development of regulations and safety guidelines.

1.1.3. Concerns about Potential Implications of Nanomaterial Exposure

The concern about exposure to particles in the size range between approximately 1 and 100 nm is based on the above-mentioned fact that the physical and chemical properties of nano-sized matter differ substantially from the properties of the same materials in bulk, including their toxicity, biological and health effects (Maynard et al. 2006).

Moreover, not all physico-chemical parameters that biological effects likely depend on are usually taken into account in toxicity studies. These physico-chemical properties of nanoparticles that may play a key role in causing the toxic effects of nanomaterials include particle size and size distribution, agglomeration state, shape, crystalline structure, chemical composition, surface area, surface chemistry, surface charge, and porosity (Oberdörster et al. 2005a). Instead, studies usually consider only one or two of these parameters, e.g., only particle size and crystalline structure or most frequent particle size and most abundant chemical substance comprising the particles.

Structure-based hazards associated with engineered nanomaterials are challenging conventional approaches to risk assessment and management (Maynard 2007; Wittmaack 2007). More specifically, the few completed studies of the properties of nanomaterials in the context of their biological and health effects due to the size-related characteristics show a whole range of potential problems including incursion, retention, and mobility of nanoparticles within an organism (Oberdörster et al. 2005b). It is important to note that these studies had been limited to the pure materials and not consumer products, containing them.

These studies have shown, for example, that nanoparticles can be translocated from various parts of the respiratory system and gastrointestinal tract through the blood stream and nervous tissue into other organs of the rodent body and nanomaterial translocation was found to be dependent on the size of nanoparticles (Borm et al. 2006; Kreyling et al. 2011; Kreyling et al. 2007; Oberdörster et al. 2004; Singh and Nalwa 2007). Resulting deposition of nanoparticles following inhalation exposure occurs in the cardiovascular system, liver, brain, testes, spleen, stomach, and kidneys (Bakand et al. 2012; El-Ansary and Al-Daihan 2009; Reijnders 2012). There is evidence that certain nanoparticles penetrate the placental barrier as well (Keelan 2011; Wick et al. 2009). Numerous mechanisms are suspected to cause the negative health effects on the systemic and cellular levels. Metabolic stressors and platelet-leukocyte aggregates, which originate in inflammatory lung disease, may arise from inhalation exposure to nanoparticles (Plummer et al. 2011; Reijnders 2012; Xiong et al. 2012). These in turn have been linked with chronic inflammation in organs, cardiovascular disease, and arteriosclerosis and negatively affect development of the fetus (Gomez-Mejiba et al. 2008; Jackson et al.

2012; Reijnders 2012; Tabuchi and Kuebler 2008; Tedgui and Mallat 2006). The effects of nanoparticle aggregation and agglomeration in many cases have been shown to only slightly alter the negative biological effects of nanomaterials and were also thought to assist in particle uptake and translocation (Borm et al. 2006; Reijnders 2012).

More specifically, ^{13}C particles (36 nm count median diameter), generated by electric discharge from [^{13}C] graphite rods, can be translocated to the olfactory bulb of the rat central nervous system following whole-body exposure (Oberdörster et al. 2004), as can manganese oxide nanoparticles (30 nm, ~ 500 μg/m^3) with resulting inflammatory changes following whole-body exposure or intranasal instillation (Elder et al. 2006). Titanium dioxide aerosol particles of 22 nm count median diameter, inhaled by rats, were later (1 hr and 24 hr) found on the luminal side of airways and alveoli, in all major lung tissue compartments and cells, and within capillaries (Geiser et al. 2005). When pulmonary macrophages and red blood cells were exposed to fluorescent polystyrene microspheres of three different sizes including one nanoscale (1, 0.2, and 0.078 μm), particle uptake occurred by diffusion or adhesive interactions significantly greater for the nanosize fraction of particles compared with the 0.2 μm particles. Specifically, on average, 77 ± 15% (mean ± SD) of the macrophages contained 0.078 μm particles, 21 ± 11% contained 0.2 μm particles, and 56 ± 30% contained 1 μm particles (Geiser et al. 2005). Particle size and agglomeration of the same material nanoparticles (titanium dioxide), administered through various routes, have been shown to affect inflammatory response in various tissues of mice (Grassian et al. 2007a; Grassian et al. 2007b; Wang et al. 2007). Another study investigated pulmonary effects (pulmonary inflammation, cytotoxicity and adverse lung tissue effects) following rodent exposure to chemically

identical similarly sized (~25 and ~100 nm primary particle size) nano-TiO_2 particles that had different crystalline structure – rutile, anatase, or their combination. The researchers concluded that the different pulmonary responses could be related to crystal structure, inherent pH of the particles, or surface chemical reactivity (Warheit et al. 2007). Nanoparticulate zero-valent iron (nZVI; $Fe^0_{(s)}$) toxicity to bronchial epithelial cells has been shown and its mechanisms investigated (Keenan et al. 2009). Penetration of maghemite (γ-Fe_2O_3), iron (Baroli et al. 2007), and zinc oxide (Cross et al. 2007) nanoparticles into (although not through) the human skin has also been observed.

A number of acute and chronic effects of nanoparticle exposure in humans have been suggested based on the animal and animal cell studies. These include inflammation, exacerbation of asthma and metal fume fever, fibrosis and chronic inflammatory lung diseases, and carcinogenesis. Still, a clear conclusion about the toxicity mechanisms behind these health effects remains to be reached (Bakand et al. 2012).

1.1.4. Nanomaterials in Consumer Products

While biological effects and potential for exposure to pure nanomaterials have been explored to some degree, exposure to nanomaterials from the actual consumer products during their application and through the associated waste presents unknown health and environmental risks (Bradford et al. 2009; Keenan et al. 2009).

We have a very limited understanding of the potential for exposure to nanomaterials from such products and resulting health effects, which is critical for the development of safety regulations and guidelines (Drobne 2007; Frater et al. 2006; Schmid et al. 2009; Segal 2004; Van Calster 2006; Warheit et al. 2007a).

When nanomaterial(s) are incorporated into a product, their size, surface area, surface chemistry, solubility, and possibly shape, all possibly affecting the potential toxicology (Kanarek 2007; Maynard et al. 2006; Shrader-Frechette 2007; Warheit et al. 2007) are not the only determinants. The location and size characteristics of nanomaterial(s) in a product along with other components as well as concentration of nanomaterial(s) all affect the potential for different ways of exposure and adverse health effects (Hansen et al. 2008). Agglomeration of nanoparticles in a product is another very important parameter where larger particles of the same material, composed of adjoined nanoparticles, can exhibit different biological effects compared with monolithic particles of similar size (Bermudez et al. 2004).

The so called free nanoparticles that are not fixed within a given solid material are of a special concern within the context of potential exposure through inhalation and dermal routes (Hansen et al. 2008; Shimada et al. 2009). Public exposure to such free nanoparticles is most likely to occur through the use of commercially available nanoparticle-containing consumer products in the form of liquid or powder dispersions (Hansen et al. 2008; Oberdörster et al. 2005a; Shimada et al. 2009; Wardak et al. 2008). Human exposure to nanoparticles through various routes, in particular inhalation, is especially elevated in this case when nanoparticle-containing liquids or powders are aerosolized in close proximity to the breathing zone.

How consumer products in the form of nanoparticle-containing liquids or powders are aerosolized is a very important factor affecting the extent of resulting exposure (Shimada et al. 2009; Wolff and Niven 1994). For example, using a pump sprayer or a gas propellant sprayer results in very different aerosol particle size distributions with

different profiles of inhalability and deposition across the different regions of the respiratory tract (Hansen et al. 2008; Wolff and Niven 1994). Using a nanosilver product, Hansen et al. (2008) showed no measurable release of nanosized particles when a pump sprayer was used. In the case of a gas propellant sprayer, they observed a substantial release of silver nanoparticles. The researchers concluded that the release of nanoparticles correlated with the generated aerosol droplet size distribution. Hansen et al. (2008), however, did not measure specifically the possible release of nanosilver in the agglomerated form when the pump sprayer was used.

There is a famous example of a German bathroom cleaning product "Magic-Nano" (Kleinmann GmbH; Sonnenbühl, Germany) that illustrates several above-mentioned issues that nanotechnology-based consumer products present: 1) inadequate labeling and marketing of the products, 2) effect of the total formula of the product – not just the nano-ingredient(s) and 3) the mode of product application or dispensing. "Magic-Nano" product caused at least 90 people to report severe respiratory problems, 6 of whom were hospitalized with pulmonary edema following use of this product. The manufacturer apparently tested the product, dispensed in pump bottles instead of as a propellant aerosol, in which form it later went on sale to the public (Kanarek 2007). According to another source (Wolinsky 2006), the original formulation of the product was supposed to have pH of 2.4, but turned out to be pH 8 due to the addition of sodium hydroxide into the product that went on sale. This led to precipitation of the nano-component, so it was later reported (Wolinsky 2006) that "Magic-Nano" did not contain nanoparticles. The case is an indication of how important testing the actual final products "from the shelf" can be as far as exposure is concerned – the approach used in this dissertation project. In

the absence of strict regulations, a manufacturer's claim that a given product does or does not contain nanomaterial(s) cannot be trusted without independent testing with the purpose of finding certain kinds of nanosized or nanostructured materials in it. At the same time, different methods are only able to detect certain types of nanoparticles and a negative result of such testing is not a guarantee of the absence of nanocomponents in a given product.

The current problem where the manufacturers rarely present information about the content of nanomaterials in their products (Hansen et al. 2008) can render impossible exposure assessment that considers the concentration of nanomaterial as a parameter. Composition of the final product, and the form in which it is delivered to the consumers, can lead to chemical modification of the nanoingrediet(s), coagulation, agglomeration, and other processes that can have a substantial effect on exposure and health hazard of a given "nanoproduct". In other words, it is impossible to unmistakably predict nanomaterial exposure and health effects of a consumer "nanoproduct" solely based on the characteristics of the nanosized and (or) nanostructured components within (Maynard 2007). Therefore, characterization of the final consumer "nanoproducts" as they are delivered to the end users is a must when determining hazard potential and performing exposure and health risk assessment.

1.2. Goals and Hypotheses

The **overall goal** of this dissertation project was to fill in the knowledge gaps in understanding of the risks associated with introduction of nanomaterials into consumer products, specifically consumer sprays and cosmetic powders. This overall goal was fulfilled by work with the three main objectives. The first objective was to develop experimental techniques and methodologies of nanomaterial analysis, necessary to acquire the kind of data needed for nanomaterial exposure assessment from the two above-mentioned categories of consumer products. The second objective was to successfully use the developed experimental approaches to characterize the products and perform a typical exposure scenario simulation of product application and inhalation. The third goal was to use the collected data to calculate human exposure in this typical realistic exposure scenario.

The following **two hypotheses** were tested in this dissertation project:

1. Nanoparticles and their agglomerates in the form of aerosol are released from certain nanotechnology-based products during their use by consumers. This leads to human inhalation exposure during product use.
2. Nano-sized particles and their agglomerates may be present in aerosols resulting from consumer products that are not claimed as nanotechnology-based by the manufacturers.

1.3. Dissertation Overview

Chapter 1 introduces nanotechnologies and describes nanomaterial use in consumer products. It also describes the potential human and environmental health problems associated with the development of nanotechnologies. We review here the research published to date that outlines the current state of science in understanding of the potential human health risks of nanotechnologies. Particularly, the problem of human exposure to nanomaterials from nanotechnology-based consumer products is introduced and explained.

In **Chapter 2**, the results of our investigation of the potential nanomaterial exposure from consumer spray products including cleaning and disinfectant sprays, cosmetic mists and hair sprays are presented. This research on nanotechnology-based sprays focused on the following objectives: 1) characterize nanoparticles (if any) in several nanotechnology-based and alternative "non-nano" consumer spray products currently on the market; 2) characterize potential exposure to airborne nanomaterials due to the use of spray products in a realistic exposure scenario; and 3) compare regular spray products that perform similar functions with their nanotechnology-based counterparts.

Chapter 3 describes our research on potential nanomaterial exposure due to the use of cosmetic powder products including a powder sunscreen, blotting powders, a blusher, a finishing powder, and moisturizing powders. The research on the powder products had the same three objectives as with the spray products: 1) characterize nanoparticles (if any) in several nanotechnology-based and alternative "non-nano" cosmetic powders acquired from the public consumer market; 2) characterize potential exposure to airborne nanomaterials due to the use of these cosmetic powders in a realistic

exposure scenario; and 3) compare the regular cosmetic powders that perform similar functions with their nanotechnology-based counterparts.

Chapter 4 presents the results of our quantitative inhalation exposure assessment for cosmetic powders, calculated for a typical consumer – a female 18 – 60 years old. The data obtained from the cosmetic powders in Chapter 3 were used to quantify the exposures. The following objectives were set: 1) further characterize the primary nanoparticles (if any) in the nanotechnology-based and alternative "non-nano" cosmetic powders previously investigated in Chapter 3; 2) calculate inhalation exposure per one cosmetic powder application as mass dose of inhaled aerosol and also as mass dose of aerosol deposited in the three regions of the human respiratory system: the head airways, the tracheobronchial and the alveolar regions; and 3) compare the deposition in different regions of the respiratory system.

The data presented in Chapter 4 can be used to calculate exposure for a different exposure scenario or user profile with reasonable accuracy while still basing the calculations on the same aerosol measurement results, presented in Chapter 3.

In **Chapter 5**, we summarize the results of our investigations and outline the implications for future research. We also discuss potential impact of our work.

The work presented in this thesis is a response to the call for independent safety research of nanomaterials and nanotechnology-based consumer products that are free of conflict-of-interest and published in the open scientific literature (Maynard 2007; Maynard and Aitken 2007; Michelson 2008; Shrader-Frechette 2007).

The Curriculum Vitae of the PhD Candidate is attached at the end of the dissertation.

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Chapter 2

Potential for Exposure to Engineered Nanoparticles from Nanotechnology-based Consumer Spray Products*

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2.1. Abstract

The potential for human exposure to engineered nanoparticles due to the use of nanotechnology-based consumer sprays (categorized as such by the Nanotechnology Consumer Products Inventory) is examined along with analogous products, which are not specified as nanotechnology-based (regular products).

Photon correlation spectroscopy was used to obtain particle size distributions in the initial liquid products. Transmission electron microscopy was used to determine particle size, shape, and agglomeration of the particles. Realistic application of the spray products near the human breathing zone characterized airborne particles that are released during use of the sprays. Aerosolization of sprays with standard nebulizers was used to determine their potential for inhalation exposure.

Electron microscopy detected the presence of nanoparticles in some nanotechnology-based sprays as well as in several regular products, whereas the photon correlation spectroscopy indicated the presence of particles <100 nm in all investigated products. During the use of most nanotechnology-based and regular sprays, particles ranging from 13 nm to 20 μm were released, indicating that they could he inhaled and consequently deposited in all regions of the respiratory system. The results indicate that exposures to nanoparticles as well as micrometer-sized particles can be encountered owing to the use of nanotechnology-based sprays as well as regular spray products.

2.2. Introduction

The US National Nanotechnology Initiative defines nanotechnology as “the understanding and control of matter at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications” (National Science and Technology Council 2007). Given the unique properties of materials at such scale, the development of nanotechnologies and their implementation in consumer products are undergoing rapid growth. Regardless of the public's perception of nanotechnology as largely a future issue, nanosized ingredients have already been incorporated in an extensive variety of products in the market (Bradford et al. 2009; Maynard 2007; Woodrow Wilson International Center for Scholars 2011a). The Project on Emerging nanotechnologies (Woodrow Wilson International Center for Scholars 2011b) currently lists over 1000 nanotechnology-based consumer products. The development and commercial application of nanotechnologies are progressing in the absence of specific regulations or legal guidelines for labeling (Paull and Lyons 2008). More importantly, we have a very limited understanding of the potential for exposure to nanoparticles from such products and resulting health effects, which is critical for the development of safety regulations and guidelines (Drobne 2007; Frater et al. 2006; Schmid et al. 2009; Segal 2004; Van Calster 2006; Warheit et al. 2007a).

The concern about exposure to particles in the size range between 1 and 100 nm is based on the fact that the physical and chemical properties of nanosized matter differ substantially from the properties of the same materials in bulk, including their toxicity, biological, and health effects (Maynard et al. 2006). Studies analyzing biological and health effects of nanoparticles have shown a whole array of alarming issues, including

incursion, retention, and mobility of nanoparticles within living organisms and tissues (Oberdörster et al. 2005b). For example, 13C-graphite-derived carbon nanoparticles (median diameter=36 nm) were found to translocate from the respiratory system to the olfactory bulb of the rat central nervous system (Oberdörster et al. 2004). The same effect was found for the manganese oxide nanoparticles (median diameter = 30nm) with resulting inflammatory changes (Elder et al. 2006). Titanium dioxide aerosol particles of 22nm count median diameter inhaled by rats were later (1 and 24h) found on the luminal side of airways and alveoli, in all major lung tissue compartments and cells, and within capillaries (Geiser et al. 2005). Size and state of agglomeration of titanium dioxide nanoparticles administered through various routes have been shown to affect inflammatory response in various mice tissues (Vicki H Grassian et al. 2007; Vicki H. Grassian et al. 2007; Wang et al. 2007). Differential pulmonary effects following rodent exposure to various nanosized TiO_2 particle types (rutile, anatase and their combination) were also documented (Warheit et al. 2007b).

Notably, these toxicological studies investigated only pure nanomaterials. Hagendorfer et al. (2010)investigated the release of nanoparticles from one nanotechnology-based silver spray product. Their approach addressed only one kind of spray product and did not consider a realistic application scenario. It is important to consider the exposure and health effects associated with the use of various available types of nanotechnology-based consumer products. In contrast to pure fresh nanomaterials, consumer product use can lead to the release of both nanoparticles incorporated in a product and the particles from the product's holding matrix, that is, solvent and other ingredients. Therefore, exposure to nanoparticles from consumer products during their

handling, application, and disposal still presents unknown health and environmental risks (Bradford et al. 2009; Keenan et al. 2009; Lioy et al. 2010). Characteristics of nanomaterials incorporated into the consumer products, including their size, surface area and chemistry, solubility, possibly shape, as well as location and concentration of nanoparticles in the product, can affect the potential for exposure by different pathways and the resulting adverse health effects (Shrader-Frechette 2007). The free nanoparticles that are not fixed within a given material are of special concern in the context of potential exposure through inhalation and dermal routes (Hansen et al. 2008; Shimada et al. 2009). In addition, nanoparticle agglomerates can exhibit different biological effects compared with uniform particles of similar size (Bermudez et al. 2004). Interaction with other ingredients of a nanomaterial-containing product and the form in which it is used by the consumers (liquid, powder, spray, and so on) can lead to chemical modification, agglomeration, and other processes affecting the nanosized and/or nanostructured ingredient(s). This can have a substantial effect on the extent of exposure and health hazard of a given product compared with the pure nanomaterial ingredient(s). Thus, it is very difficult to predict the exposure and health effects of a particular nanotechnology-based consumer product with any certainty solely based on the characteristics of nanosized and/or nanostructured components within such a product (Lioy et al. 2010; Maynard 2007).Therefore, characterization of nanotechnology-based consumer products in their final form as well as of particle releases during the products' use is absolutely necessary when performing exposure and health risk assessment. However, there are virtually no studies examining compositions of nanotechnology-based consumer products as well as the exposure of consumers to nanoparticles owing to the use of such products

in realistic application scenarios. Given the discussed differences between pure nanoparticles and those incorporated and released from products' matrices during application, simulation of realistic exposure scenarios is necessary for reducing the uncertainties in exposure characterization and where necessary improve the products in commerce to reduce exposure (Lioy 2010).

The route and extent of exposure will depend on the type and application mode of the nanotechnology-based products (Oberdörster et al. 2005a). Application of nanotechnology-based sprays, which are used as cleaners, disinfectants, and cosmetic mists, is likely to result in inhalation exposure as their application yields aerosol emissions in the breathing zone of the user. A report by the Nanomaterial Toxicity Screening Working Group stressed the need to assess exposure to nanoparticles by various exposure routes, including the airborne (inhalation) route (Oberdörster et al. 2005a).

Given the lack of data on exposure to nanoparticles due to the use of consumer products, this study focuses on one product category, namely nanotechnology-based sprays with the following objectives: (1) characterize nanoparticles in several nanotechnology-based consumer spray products currently in the market; (2) characterize potential exposure to airborne nanoparticles due to the use of spray products in a realistic exposure scenario; and (3) study the regular spray products that perform similar functions and compare them with their nanotechnology-based counterparts. The five types of "nanoproduct–regular product" pairs were acquired and tested for potential exposures. They were personal care silver sprays, vitamin-containing facial mists, antioxidant-containing body mists, hair care sprays, and multi-purpose disinfectants. In addition, a

cleaning nanoproduct was also tested. Overall, we believe this project responds to the call for independent, conflict of interest-free and open scientific literature published nanomaterial safety research (Maynard 2007; Maynard and Aitken 2007; Michelson 2008; Shrader-Frechette 2007; Thomas et al. 2009) and attempts to set the ground for more thorough quantitative exposure studies.

2.3. Materials and Methods

2.3.1. Tested Sprays

Inclusion of the nanotechnology-based consumer sprays in this study was based on the presence of those sprays in the Woodrow Wilson Nanotechnology Consumer Products Inventory (Woodrow Wilson International Center for Scholars 2011b). It should be noted that inclusion of products in this Inventory is based on the manufacturer's report regarding the presence of nanocomponents in them. There is no certainty that any given product in the inventory includes nanotechnological components (Hansen et al. 2008; Som et al. 2010).

A common consumer spray, in which the use of nanotechnology has not been specified by the manufacturer (regular product), was selected to match each "nanoproduct" based on the application purpose. The list of both nano and regular products examined in this study along with their compositions and suggested applications as per the manufacturers are presented in Table 2.1. Owing to high corrosiveness of the Wheel Nanocleaner and high likelihood of its alternative product being corrosive as well, a corresponding alternative regular product was not tested.

2.3.2. Analysis of Sprays

As relatively little is known about the size and shape of particles incorporated in the consumer sprays, all the test products were analyzed in liquid state using two different methods described below. In addition, we compared the sizes of particles in liquid state with the sizes of particles from the same products in the airborne state, that is, during simulated application of the products.

2.3.3. Sample Analysis using Transmission Electron Microscopy

Size, shape, and agglomeration of electron-contrast particles (those visible in transmission electron microscopy (TEM)) in the spray products were determined using a TEM 2010F (JEOL, Tokyo, Japan). Small drops of each product were spread on HC300-Cu Holey Carbon Film on 300 Mesh Copper (Electron Microscopy Sciences, Hatfield, PA, USA) and left to dry in the ambient air for at least 1 h before testing. Particle size was measured manually (Matyi et al. 1987) from the resulting micrographs relative to automatically added scale marks. Particles found in both the nano and regular silver sprays were examined under high resolution, so that atomic grid could be seen.

Weak phase objects that have low electron contrast are not visible in TEM images; therefore, only certain types of nanoparticles, for example, certain metal, metal oxide, other inorganic, and some organic nanoparticles, could be seen using the TEM. Another particle feature that could be obtained from the TEM analysis is electron beam sensitivity. It is described as a structural alteration of the tested material owing to radiolysis (Egerton et al. 2004; Hobbs 1987). Radiolysis can visually be observed during TEM investigations. Electron beam sensitivity results from electron irradiation above a certain magnification setting (Carlo et al. 2002; Leapman and Sun 1995 ; Turgis and Coqueret 1999), which results in a higher electron beam power density per unit area of the sample. As mostly organic nanoparticles tend to be beam sensitive (Egerton et al. 2004) it can be concluded with some degree of certainty about organic or inorganic nature of nanoparticles in the products based on beam sensitivity.

2.3.4. Sample Analysis using Photon Correlation Spectroscopy

Multimodal hydrodynamic particle size distributions in the original concentration of liquid spray products were deter-mined using photon correlation spectroscopy (Allen 2003; Bruce J. Berne 2000). A ZetaPALS 90 Plus with included Particle Sizing Software (both by Brookhaven Instruments Corporation, Holtsville, NY, USA) was used for this analysis. Hydrodynamic particle diameter includes the electric double layer around the particle and is the diameter of a hypothetical sphere that would diffuse at the same rate as the particle under examination. This diameter may also be called the equivalent sphere diameter (Brookhaven Instruments Corporation 1995). The software uses Stokes–Einstein equation to transform diffusion coefficients, determined by dynamic light scattering, into hydrodynamic diameters presented as measurement results (Bodycomb 2009).

Particles of any nature can be registered by this technique as long as their refractive index differs from that of the liquid medium. The Particle Sizing Software calculates multimodal particle size distributions as relative scattering intensity and relative number concentration for each registered hydrodynamic diameter. The highest intensity or the highest number concentration is expressed as 100%. All other intensity or number concentration values are expressed in percentage relative to the corresponding highest values. Thus, the data from these tests provide information used to infer the presence and relative concentration of particles of various hydrodynamic diameters. When discussing the results, the number concentration-and intensity-based hydrodynamic mode diameters have indices “N” and “I”, respectively.

For silver-containing products (Silver Nanospray and Regular Silver Spray), refractive index of 0.18 was used (that of metallic silver). For other products, refractive index of 1.60 was used based on the instrument manual's (Brookhaven Instruments Corporation 1995) recommendation for non-absorbing, white, opaque particles in the visible spectral region. Imaginary refractive indices were set to 0.00 (this value assumes absence of light absorption by particles at the used wavelength) for all samples based on the manual's recommendation as well. The actual refractive indices of different particles within each product are unknown as the composition of most products is rather complex. However, even with these assumed indices, the data are expected to be of very high quality because particle refractive index is not used to calculate any intensity-or number concentration-weighted distributions. Also for particle sizes below ~60 nm, the spherical Mie factors (used by the software for calculating mass and number fractions from the measured intensity fractions) are independent of the particle refractive index (Brookhaven Instruments Corporation 1995).

2.3.5. Analysis of the Released Particles in the Airborne State

2.3.5.1. Particle Release during Simulated Use

The first experimental setup shown in Figure 2.1a was used to measure the airborne particles released during a realistic product application, when a spray product is used near a person's breathing zone. Here, a spray was applied near a commercially available mannequin's head (Image Supply House, Endicott, NY, USA) and the inhalation of airborne particles during the spray application was simulated by sampling through two stainless-steel tubes installed in the mannequin's nostrils. This particular sampling approach mimics the proximity of the spray cone to the breathing zone as it is

in the real-life product use. The approach minimizes settling losses of larger droplets that may contain nanoparticles. The two aerosol streams drawn through the mannequin's nostrils were combined into one at the mannequin's nape using a stainless-steel Y-connector. The resulting aerosol stream was then split using a flow-splitter (TSI, Shoreview, MN, USA) and drawn by conductive tubing into a Scanning Mobility Particle Sizer (SMPS) consisting of model 3081 Differential Mobility Analyzer and model 3786 Condensation Particle Counter (TSI) and an Aerodynamic Particle Sizer (APS) model 3321 (TSI). The SMPS has an aspiration rate ($Q_{a(SMPS)}$) of 0.3 l/min. For the APS, $Q_{a(APS)}$ = 1.0 l/min. Thus, the combined aspiration rate through the nostrils of the mannequin was 1.3 l/min ($Q_a = Q_{a(SMPS)} + Q_{a(APS)}$). The SMPS system was used with a 0.0457 cm impactor ($D50$ = 0.656 µm). For the SMPS, particle density was set as 1.0 g/cm^3 as it was impossible to determine the actual density of measured aerosol droplets. In addition, use of 1.0 g/cm^3 density allowed easy comparison of the SMPS particle size distribution, expressed as electric mobility diameter, with the APS data expressed as aerodynamic diameter. The plotted range of particle size distributions was between 14.1 and 685.4 nm for SMPS and 723 nm and 19.81 µm for APS. The actual measurement range of the APS was broader: between 542 nm and 19.81 µm.

The setup was placed inside a Level II Biosafety cabinet (NuAire, Plymouth, MN, USA) with the mannequin head facing the back of the cabinet, approximately 20 cm from it. The inside dimensions of the cabinet are 178.4 cm width, 71.8 cm height, and 57.2 cm depth. The front of the biosafety cabinet was covered with a near-airtight plastic curtain with installed glove sleeves to handle and operate the sprayers. Before each test, the blower of the biosafety cabinet was operated for 15 min with the curtain open to remove

most of the particles inside the cabinet. The background particle concentration was also monitored. Then, the curtain was closed, a spray product was positioned about 10 cm to the right of the mannequin's head, and operated by hand using a provided sprayer (if available). The spray cone was directed towards the back wall of the cabinet. The lever of a sprayer was fully pressed with a frequency of ~$1s^{-1}$ and the spraying lasted for 3 min. Although the duration of a typical application may vary for different products or different users, 3 min is the minimum time needed for the SMPS to measure the entire size spectrum. The concentration and size distribution of aerosolized particles were continuously monitored by the APS and SMPS during the application of each spray. After the end of spraying, the particles inside the biosafety cabinet were removed by turning on the cabinet's filtration system again.

Although most of the products were used with the provided sprayers, in the case of Silver Nanospray, the sprayer from another randomly selected nanoproduct was used as a sprayer was not supplied by the manufacturer. For the same reason, the sprayer from Disinfectant Nanospray was used with Regular Disinfectant Spray.

2.3.5.2. Particle Aerosolization using Standard Nebulizers

The concentration and size distribution of aerosolized liquid particles depend on the spraying mechanism and spraying intensity. The same consumer product may be supplied with different spraying mechanisms, thus affecting the size of the resulting particles and consequently exposure parameters. Therefore, we examined the range of particle concentrations and sizes a user could potentially be exposed to by aerosolizing all tested liquid products with two different constant output aerosol generators. The setup for this experimental approach is shown in Figure 2.1b. The following two constant output

nebulizers were used: C-Flow PFA Nebulizer 800-1-020-01-00 (Savillex, Minnetonka, MN, USA) and three-hole Collison Nebulizer. The following accessories facilitating nebulization of small quantities of liquids were used with the Collison Nebulizer: CN41 precious fluids extension sleeve and CN70 polycarbonate precious fluids bottle (BGI, Waltham, MA, USA). The aerosol produced by each nebulizer was diluted with high-efficiency particulate air-filtered air and mixed using a passive box-type mixing element (Han et al., 2007), and then dried using a diffusion dryer model 3062 (TSI) and released into a horizontal test chamber of approximately 10 cm in diameter. The air was isokinetically sampled and measured with the APS and SMPS. The C-Flow PFA Nebulizer was operated in the self-aspirating mode at aerosol flow rate (Q_a) of 1.0 l/min at 38 psi air pressure and the dilution air flow rate (Q_d) was set to 14 l/min. The three-hole Collison Nebulizer was operated at Q_a = 4.8 l/min at 10 psi and the dilution air flow rate (Q_d) was set to 10.2 l/min. Thus, in both cases, the total aerosol output flow rate ($Q_t = Q_a + Q_d$) was 15 l/min. Flow rates were measured with a Mass Flow Meter model 3063 (TSI), which adjusts for standard temperature and pressure.

Background data were obtained before each testing session by operating the C-Flow and Collison Nebulizers without any liquid feed, and then with ultrapure water 18.2 MΩ·cm obtained with Milli-Q Academic System (Millipore, Billerica, MA, USA) feed. The system was placed into a Class II Biosafety Cabinet (NuAire) and the blower of the cabinet was constantly operated throughout the experiments.

Experiments with each product and each test protocol (hand spraying or aerosolization with constant output nebulizers) were repeated at least three times.

2.4. Results

2.4.1. Sample Analysis using TEM

A summary of the TEM image analysis results for the spray products is shown in the second column of Table 2.2. Electron beam sensitivity category was introduced as an additional characteristic of the particles in the products because it was observed when attempting to view samples at higher magnifications (around 40,000 × with dark current of 97 μA). Electron-contrast particles were found in three nanoproducts: Silver Nanospray, Disinfectant Nanospray, and Wheel Nanocleaner and five non-nanoproducts: Regular Silver Spray, Regular Disinfectant Spray, Regular Hair Spray, Regular Skin Hydrating Mist, and Regular Facial Spray.

Representative micrographs from a pool of different-magnification micrographs are shown in Figure 2.2.

2.4.1.1. Nanotechnology-Based Products

High-contrast single particles and well-defined separated nanoparticle agglomerates were found in the Silver Nanospray sample (Figure 2.2a). Low-contrast single particles with no agglomerates were found in the Disinfectant Nanospray sample (Figure 2.2b). While examining the sample of Wheel Nanocleaner at different magnifications, we found a wide size range of slightly electron beam-sensitive nanoparticles and nanoparticle agglomerates ranging from <20 nm to more than 1 μm (Figure 2.2c). No electron-contrast particles were detected in other nanoproduct samples: Facial Nanospray, Hair Nanospray, and Skin Hydrating Nanomist.

2.4.1.2. Regular Products

Single particles as well as large agglomerates were found in the Regular Silver Spray sample (Figure 2.2d and e). Compared with its nanoproduct counterpart, Silver Nanospray, the Regular Silver Spray sample looked much less refined. The sample of Regular Disinfectant Spray contained nanosized particles of approximately100 nm in diameter (Figure 2.2f). High-magnification micrographs (Figure 2.2g) show that these particles are composed of smaller particles of approximately 3–5nm in diameter. The sample of Regular Hair Spray contained single low-contrast particles with the minimum identified diameter of 16.5 nm and up to approximately 200 nm (Figure 2.2h and i). Elliptical high-contrast beam-sensitive particles were found in Regular Skin Hydrating Mist (Figure 2.2j). No particles of less than 100 nm in diameter were present in the sample, but some particles had diameter of less than 200 nm and majority of the particles had lengths between 250 to 600 nm. Most particles were found in a highly agglomerated state. The size and agglomeration, shape and the degree of electron transparency of particles found in Regular Facial Spray (Figure 2.2k and l) were similar to the particles in Regular Skin Hydrating Mist.

2.4.2. Analysis of Products Using Photon Correlation Spectroscopy

Figure 2.3 shows multimodal hydrodynamic diameter distributions for eight products (four nanotechnology-based and four regular products). For three other products (Facial Nanospray, Regular Disinfectant Spray, and Wheel Nanocleaner), the instrument could not produce valid data, most likely due to the high levels of large particles that distort the autocorrelation function (Bodycomb 2009). The hydrodynamic diameter distributions are presented in two ways: as relative scattering intensity (a, c) and as

particle number concentration (b, d). As the relative scattering intensity is proportional to particle radius to the sixth power, this method allows detecting the presence of large particles. On the other hand, the particle number size distribution indicates the relative presence of particles as a function of their diameter. The mode diameters of the intensity (I) and number (N) distributions are listed in Table 2.2. From the data acquired, it can be seen that particles of less than 100 nm in diameter were found in all tested products. Numberwise, the distributions of both nanoproducts and regular products were dominated by particles <20 nm in hydrodynamic diameter. The nanoproduct Disinfectant Nanospray contained particles <5 nm in hydro-dynamic diameter. In cases where particles larger than 100 nm were found in the nanotechnology-based products, Skin Hydrating Nanomist had the largest diameters, with peaks observed in the size ranges 0.1–1 μm (100% relative scattering intensity) and 1–5 μm (61% relative scattering intensity) (Figure 3a). Silver Nanospray was observed to have some particles larger than 4 μm. Among the regular products, all were observed to have particles larger than 100 nm, except Regular Facial Spray. Regular Silver Spray had a wide relative scattering intensity peak (max relative scattering intensity) centered on 100 nm with particles as large as 300 nm also present. Regular Hair Spray had a relative scattering intensity peak (maximum relative scattering intensity) around 1 μm. Particles >5 μm were registered in Regular Silver Spray, Regular Hair Spray, and Regular Skin Hydrating Mist.

2.4.3. Size Distribution of Airborne Particles Released from Spray Products

Figure 2.4 shows size distributions of aerosol particles released during the simulated application of spray products when hand-held spraying was used. Each graph contains particle size distributions averaged over three repeats for one nanoproduct and a

corresponding regular product with the same application purpose. The SMPS and the APS size ranges are plotted together in the same graphs and presented as a function of the electrical mobility diameter. Although the APS measures the aerodynamic particle diameter, for spherical particles with density of 1.0 g/cm^3, it is theoretically equivalent to the electrical mobility diameter. Wheel Nanocleaner and its alternative product were not tested owing to their corrosiveness and potential to damage the equipment.

For all but one nanoproduct–regular product pairs, the particle concentrations in the 14–500 nm range were similar within the pairs and ranged from 10^2 to 10^3 cm^{-3} (expressed as $\Delta N/\Delta \log D_p$,where D_p is particle diameter). The exception was Disinfectant Nanospray–Regular Disinfectant Spray pair where the regular product had the higher particle concentration by about one order of magnitude. As the same sprayer was used for both products (supplied with Disinfectant Nanospray), this concentration difference can be attributed solely to the product properties, including higher concentration of small particles in the liquid. The particle concentration measured by the APS (above 723 nm) was also substantially higher for the regular product. The higher concentration of airborne super-micron particles may be attributed to higher concentration of those particles in the regular product.

A substantial difference in the concentration of airborne super-micron particles was also observed for Skin Hydrating Nanomist–Regular Skin Hydrating Mist product pair. Similar to the product pair mentioned above, the use of regular product resulted in a higher concentration of particles >1 µm – by several orders of magnitude.

Particle size distributions obtained by aerosolizing spray products using the constant output nebulizers are presented in Figure 2.5. Each graph contains particle size

distributions for one nanotechnology-based product and its corresponding regular product obtained using both constant output atomizers. Consistent with Figure 2.4, the data are presented as $\Delta N/\Delta \log D_p$ cm^{-3}, where D_p is particle diameter. The mode diameters for all products and both nebulizers are presented in Table 2.2. The size distributions are averages of three repeats, except for Wheel Nanocleaner and its regular counterpart where only one test was performed due to their high corrosiveness to the APS and SMPS systems. Due to the excessive foaming, the use of the Collison Nebulizer was impossible for Hair Nanospray, Disinfectant Nanospray, and Regular Disinfectant Spray.

The airborne concentrations of the Silver Nanospray and Regular Silver Spray pair aerosolized by the C-Flow Nebulizer were similar for the entire size range. The difference became more pronounced when these two products were aerosolized using the Collison Nebulizer: the concentration of Regular Silver Spray was higher by 1–2 orders of magnitude for particles of 100 nm and larger. Also, particles as large as 20 μm were detected from both products. For the Hair Nanospray and Regular Hair Spray, the shapes of their size distributions looked very similar with a local minimum around 20–25 nm, a mode diameter between 300–400 nm and a gradual decrease in particle number concentration with increasing particle size. Particles of 20 μm in diameter were detected for both products and both aerosolization techniques. The aerosol from Hair Nanospray had a somewhat higher concentration than its regular product alternative in the diameter range from 20 to 1000 nm.

For the product pair Disinfectant Nanospray and Regular Disinfectant Spray, C-Flow Nebulizer-generated aerosol concentration was higher for Disinfectant Nanospray for particles of 60 nm and less, but generally lower for particles larger than 60 nm. A

similar situation was observed for the product pair of Skin Hydrating Nanomist and Regular Skin Hydrating Mist with the latter product's aerosol having higher concentration than its alternative in the regions below ~43 nm (Collison Nebulizer) and ~66 nm (C-Flow Nebulizer). However, the nanotechnology-based product had much higher concentration of particles in the range from 300 to 700 nm. Interestingly, the nanotechnology-based Skin Hydrating Nanomist had a much higher concentration of particles larger than 10 μm compared to its regular counterpart.

The size distributions of Facial Nanospray and Regular Facial Spray were similar almost over the entire size range. The Collison Nebulizer produced substantially higher concentration in the 15–400 nm range compared to the C-Flow Nebulizer for both these products. The particle size distribution of Wheel Nanocleaner aerosol, generated using only the C-Flow Nebulizer had a local minimum at approximately 35 nm, similarly to the distributions of the ethyl alcohol-containing Hair Nanospray and Regular Hair Spray. Although we did not have information on the solvent used in Wheel Nanocleaner, such a distribution suggests the presence of an organic solvent.

In general, there was a wide particle size distribution with the particle diameters ranging from 14 nm to 20 μm obtained for all products. The only exception was Silver Nanospray, aerosolized with the C-Flow Nebulizer, where the maximum observed diameter was 2 μm. Particle number concentrations in the 14-500 nm size ranged from 103 to 106 $\Delta N/\Delta \log D_p$ cm^{-3} depending on the product and aerosolization method. Collison Nebulizer typically produced higher particle concentrations compared with the C-Flow Nebulizer for all tested products.

The data for ultrapure water nebulization showed that Collison Nebulizer produced approximately 10-fold higher particle concentrations than C-Flow Nebulizer in almost all particle size channels. For both nebulizers, the airborne particle concentrations were lower compared to those from the aerosolized spray products in all size channels. One exception was silver sprays, where particle concentrations above 1 μm were similar to those for ultrapure water.

2.5. Discussion

A very interesting and rather surprising result of the study is the detection of nano-sized (1–100 nm) particles not only in nanotechnology-based, but also in regular (non-nanotechnology) products using different particle analysis techniques.

The data obtained using the TEM and ZetaPALS are important since they describe the particles that are incorporated in spray products. However, during the actual use of those products, droplet formation and dynamics come into play; thus, the size of the particles that a product user could be exposed to will be different than that found in the initial product. Analysis of the aerosol formed during simulated product application showed the presence of nano-sized particles produced from both nano and regular consumer spray products (Table 2.2, Figures 2.4 and 2.5). In addition, coarse (2.5–10 μm) and super-coarse (410 μm) (as defined in Lioy et al. 2002) were released from all products. Since the products were sprayed by hand using the supplied sprayers and the released particles were sampled in a way that simulates particle inhalation by the user, these data show the size distributions and concentrations of particles, to which human exposure would occur during the actual product use. This wide size distribution of aerosolized particles (14 nm–20 μm) indicates that particles would be inhaled and deposit in all regions of the respiratory system: extrathoracic, thoracic and alveolar (Hinds 1999). The detected large particles are likely to carry material from product matrix and agglomerates of nano-sized particles. Due to the importance of agglomerates in examining biological effects (Qu et al. 2009; Wick et al. 2007; Wirnitzer et al. 2009), subsequent research will need to examine the internal structure and composition of the released super-micron particles including the stability of the nanomaterials in

agglomerates at the site of deposition in the lung. Also, testing the effect of spray cone orientation within the breathing zone will be a subject of future studies.

Experiments with two different nebulizers show that the concentration and size distribution of the particles released during product use depend on the spraying technique and thus the exposure of users would be affected by the product packaging and the supplied sprayer.

Use of the constant output nebulizers in conjunction with the diffusion dryer produced lower concentrations of large particles compared to hand-held spraying. The nebulizers produce smaller initial droplets and the diffusion dryer removes most of the solvent from the droplets. Therefore, this method allows simulating cases when a product's sprayer produces smaller droplets and/or released droplets have a longer residence time, so most of the carrier liquid evaporates. In these cases, the user would be exposed to higher concentrations of smaller particles, which are able to penetrate deeper into the lung.

When looking at the data presented in Figures 2.4 and 2.5, one notices a less than smooth transition in the 600–700 nm range where the data from the SMPS and APS overlap. We chose to present the SMPS and APS data in the exact form as they were generated by the instruments without the use of the TSI Data Merge Software Module (Han et al., 2005). For the particle size distribution to achieve a smooth transition from the SMPS measurement range (14–700 nm) to the APS measurement range (0.5–20 μm) using the Merge Software, one needs to know a range of particle parameters (Khlystov et al. 2004), which would be difficult to determine for the diverse range of particle types in the tested spray products. Since the SMPS measurements are based on the electrical

properties and APS measurements are based on the aerodynamic properties of particles, the different detection principles can result in different detection efficiency in the transition size range (0.5–0.7 μm) depending on various aerosol characteristics (Hand and Kreidenweis 2002; Pant et al. 2009). Based on our data, it seems that the extent of this effect depends on the tested spray product and can probably be explained by different properties of carrier liquid and particles, including their density and shape, which are largely unknown. Although these researchers reported undercounting by the APS in the transition range compared to the SMPS, with one product – Regular Skin Hydrating Mist – we observed the opposite in the transition size range.

A major difference was found between the size distributions of particles produced from water-based versus alcohol-containing products using constant output aerosolization techniques (Figure 2.5). In the case of alcohol-based products, Hair Nanospray and Regular Hair Spray, much lower particle concentrations in 15–100 nm region with a local minimum around 20–25 nm were observed, whereas such a dip was not present in particle size distributions of water-based products. A similar result – a local minimum in the region between 25 and 45 nm – was observed for Wheel Nanocleaner. The composition of this product, including information about main solvent, was not obtainable, but based on similarity of the size distribution to the Hair Nanospray, the data suggest a volatile organic solvent-based solution.

The mode diameters measured by ZetaPALS were smaller compared with the mode diameters of airborne size distributions for all aerosolization methods. For those products where the TEM data indicated prevalence of nanosized particles, the analysis of aerosolized particles did not show the same results. This can be explained by aerosolized

particles primarily consisting of larger droplets from the product matrix that contain multiple single particles as well as their agglomerates. The super coarse particles (above 10 μm diameter) could also be a result of particle agglomeration during their release from the sprays. This observation suggests that a comprehensive analysis of nanotechnology-based products should include analysis of particles within a product as well as analysis of particles that are emitted during product use to understand potential exposure (Lioy 2010).

On the basis of the obtained data, it is difficult to conclude whether the nanoparticles released during the product use are actually engineered nanoparticles that were incorporated into the product or they are derivatives from natural product ingredients, such as from herbal oil emulsification (Abismaïl et al. 1999; Kim et al. 1996), or they are particles from product carrier liquid. Chemical and/or structural particle analysis could provide only some of that information and only for the particle materials that can be analyzed using specific methods. Therefore, making a definite conclusion about the composition and structure of some released nanoparticles is difficult without information from the manufacturers on the nature and concentration of nanomaterials in their products.

2.6. Conclusions

This research examined the potential for nanoparticle exposure due to the use of nanotechnology-based and regular same-purpose consumer spray products.

Electron microscopy showed the presence of free nano-particles and agglomerates in several examined consumer products, including those that are not designated as nanoproducts. Similarly, simulated use of sprays resulted in the release of nanosized particles in both nano and regular spray products, even though the manufactures do not specify the "nanosized" of ingredients or even may not know that nanoparticles are present or formed during manufacturing of their products. As an example, the manufacturer lists only chemically synthesized ingredients in the composition of Regular Disinfectant Spray – this product is also not reported as containing nanomaterials.

On the whole, use of spray products resulted in the release of particles with a wide size distribution. The toxicological implications of the human respiratory system deposition and possible translocation of these particles are not known.

Experiments with hand spraying and constant output atomizers have shown that the spraying technique affects the concentration and size distribution of the released particles. Thus, the exposure to particles from nanotechnology-based and regular products would be affected by the sprayer type.

Overall, the data suggest that the use of investigated nanotechnology-based as well as regular consumer sprays would result in inhalation exposures to single nanosized particles and multi-sized agglomerates, including complex nanoparticle-containing composites.

Future experiments will examine the structure and composition of the released particles more closely. We will also examine the patterns of particle deposition in the lung owing to short-and long-term product uses. However, the most important conclusion is that controlled human exposure studies and product emission studies are essential for reducing exposures of the general public to nano-based materials, especially those that have shown some mechanistic effects in toxicological studies.

2.7. Acknowledgements

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Table 2.1. Tested consumer spray products.

Product[1]	**Composition**[2]	**Purpose**[2]
Silver Nanospray	Silver nanoparticles, Purified water	Used topically or internally as a traditional defense against bacteria
Regular Silver Spray	99.99% Pure Silver suspended in demineralised water	Used topically to treat burns, rashes, as a nasal spray for hay fever, and deodorant; also for plants and pets
Facial Nanospray	Distilled water, Vitamin C, Nano size particles of: Copper, Calcium, Magnesium, Zinc	Applied topically for younger looking skin
Regular Facial Spray	Water, Butylene Glycol, Glycerin, Panthenol, Tocopheryl Acetate, Phenoxyethanol, Alcohol Denat., Methylparaben, Lecithin, Rosa Centifolia (Rose) Water, Butylparaben, Ethylparaben, Isobutylparaben, Propylparaben	On-call moisture and vitamin E protection for the face skin
Hair Nanospray	Alcohol Denat., Aqua, PVP/VA Copolymer, Isopropyl Alcohol, Mytrimonium Bromide, Parfum	Used to hold Nanofibre hair additions to scalp
Regular Hair Spray	SDA alcohol 40-B, water, VA/Crotonates/Vinyl neodecanoate copolymer, octylacrylamide/acrylates/butylaminoethyl, methacrylatecopolymer, aminomethanol propanol, lauryl pyrrolidone, PEG-75 lanolin, cyclopentasiloxane, fragrance	Hair spray for men
Disinfectant Nanospray	Parachlorometaxylenol – 0.20%, Other ingredients – 99.80%	Multi-purpose, ready-to-use disinfectant, sanitizer, and deodorizing cleaner for use on hard surfaces
Regular Disinfectant Spray	o-Phenylphenol – 0.22%, Diisobutylphenoxyethoxy ethyl dimethyl benzyl ammonium chloride monohydrate – 0.70%, inert ingredients – 99.08%	Multi-purpose, ready-to-use disinfectant for use on hard surfaces
Skin Hydrating Nanomist	Purified water, Dimethicone, Copolyol, Algae Extract, Mugwort (Artemisia Vulgaris) Extract, Aloe Barbadensis Gel, Fucogel, Plankton Extract, Lavander (Lavendula Angustifolia) Oil, Calcium PCA, Zinc PCA, Phenoxyethanol, Methylparaben, Propylparaben	Face and body mist that helps reverse UV damage while adding powerful anti-oxidants and anti-inflammatory properties to sun parched skin
Regular Skin Hydrating Mist	Water, Glycerin, Hyaluronic Acid, Diazolidinyl Urea, Polysorbate 80, Ergothioneine, Aloe Barbadensis Leaf Juice, Sodium Carboxymethyl Beta-Glucan, Camellia Sinensis Leaf Extract, Tetrasodium EDTA, Allantoin, Citrus Aurantium Bergamia (Bergamot) Fruit Oil, Citric Acid, Kinetin, Iodopropynyl Butylcarbamate	Provides toning action and delivers antioxidant and hydrating benefits to the skin
Wheel Nanocleaner	Composition unavailable	Advanced nanotechnology formula quickly penetrates and removes tough brake dust and road grime from all wheel surfaces

[1]Nanoproduct: as per the Woodrow Wilson Nanotechnology Consumer Products Inventory; [2]As per manufacturer

Table 2.2. Mode diameters of particle size distributions and characterization of the tested consumer spray products, obtained using different analysis methods.

Product[1]	**TEM Range of Particle Diameters, Agglomeration, Shape, Structure, Electron Beam Sensitivity**	**Hydrodynamic Mode Diameter(s) < 1µm: number – (N), intensity – (I)**	**C-Flow Nebulizer used: Mode Diameter**	**Collison Nebulizer used: Mode Diameter**
Silver Nanospray	3 - 65 nm, single particles and agglomerates, spheroidal, solid, beam insensitive	N: 5.6 nm I: 5.6, 17.8, 100 nm	37 nm	30 nm
Regular Silver Spray	<3 - 435 nm, agglomerate and single particles, various shapes, solid, beam insensitive	N: 3.2 nm I: 17.8, 100.0 nm	41 nm	41 nm
Facial Nanospray	No particles detected	Unable to measure	98 nm	61 nm
Regular Facial Spray	82 - >6000 nm, single particles and agglomerates, spheroidal and elliptical, beam sensitive	N: 3.0, 8.6 nm I: 3.0, 9.5, 30.1 nm	102 nm	No peak (concentration below water background)
Hair Nanospray	No particles detected	N: 5.3 nm I: 5.6, 24.1 nm	311 nm	No data (foaming)
Regular Hair Spray	16.5 – 683 nm, single particles and agglomerates (two types), spheroidal, solid, beam insensitive	N: 2.4 nm I: 4.2, 31.6, 749.9 nm	429 nm	334 nm
Disinfectant Nanospray	71 - 214 nm, single particles, spheroidal, solid, beam insensitive	N: 1.0 nm I: 1.2, 3.3, 34.9, 101.2 nm	85 nm	No data (foaming)
Regular Disinfectant Spray	3.7 - >725 nm, single particles and agglomerates, spheroidal, nanostructured, beam insensitive	Unable to measure	157 nm	No data (foaming)
Skin Hydrating Nanomist	No particles detected	N: 8.5 nm I: 17.3, 474.4 nm	157 nm	113 nm
Regular Skin Hydrating Mist	146 - >2500 nm, single particles and agglomerates, spheroidal and elliptical, beam sensitive	N: 4.4 nm I: 9.5 nm	102 nm	No peak (concentration below water background)
Wheel Nanocleaner	<20 – >1000 nm, single particles and agglomerates, various shapes, beam sensitive	Unable to measure	181 nm	No data (foaming)

[1]Nanoproduct: as per the Woodrow Wilson Nanotechnology Consumer Products Inventory

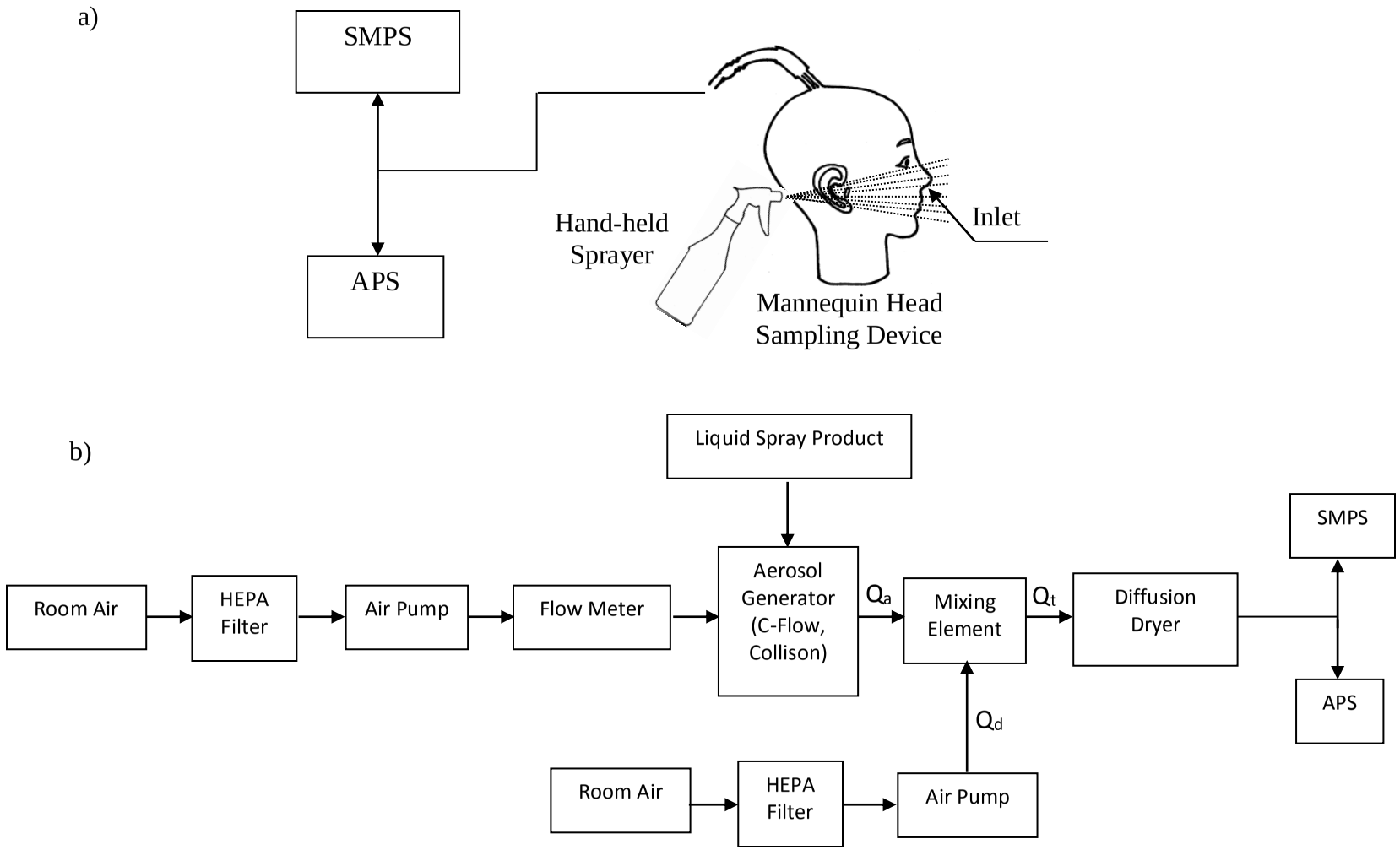
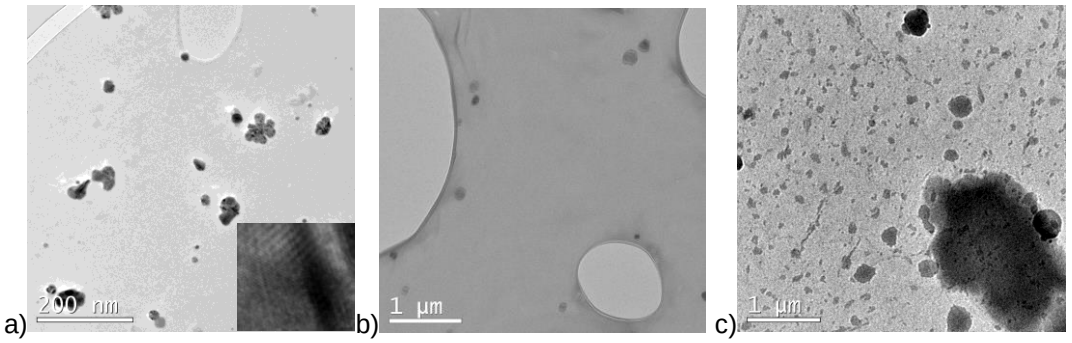
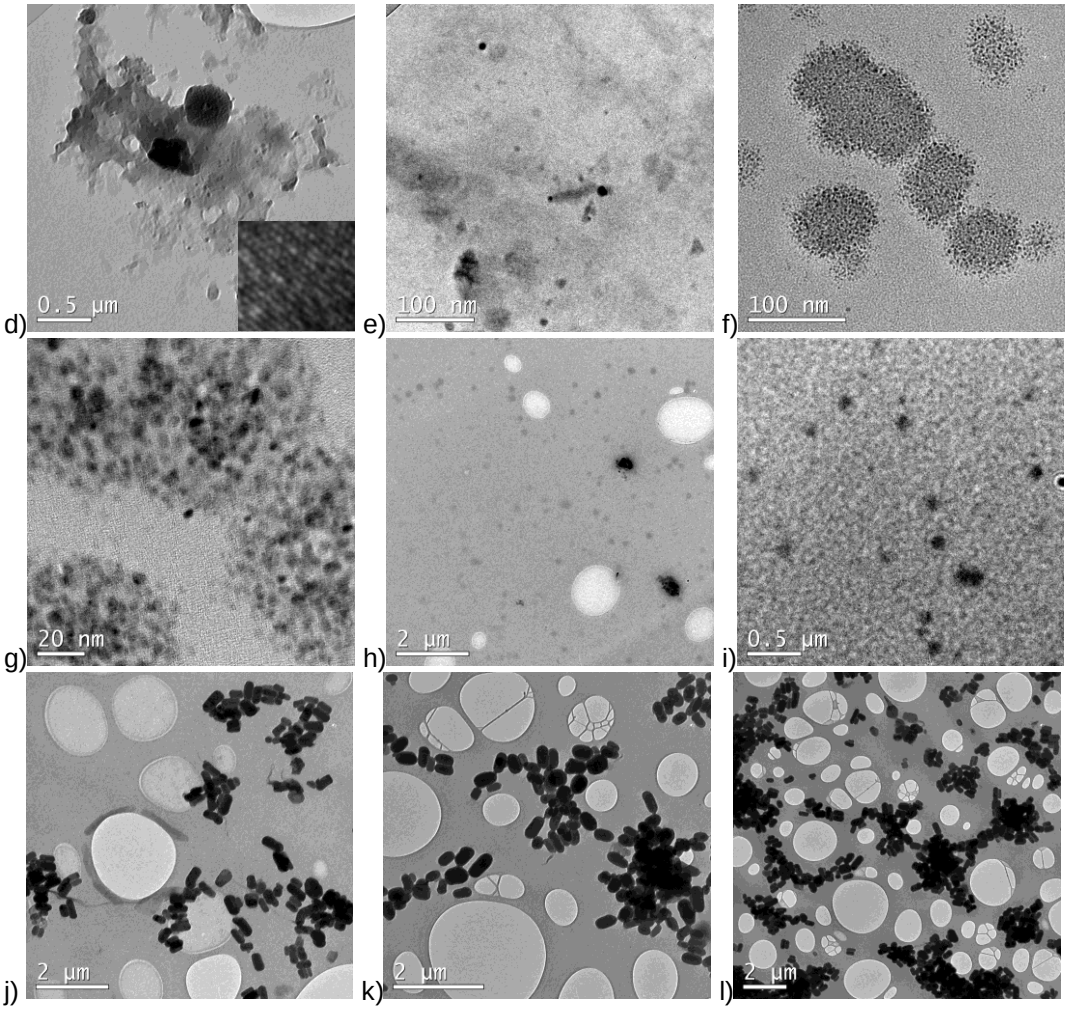


Figure. 2.1. Aerosol generation and analysis experimental setup for simulated application of the spray products (a) and constant output aerosolization (b).



(a) – (c): nanotechnology-based products as per the
Woodrow Wilson Nanotechnology Consumer Products Inventory



(d) – (l): non-nanotechnology-based products

Figure 2.2. Transmission electron micrographs of Silver Nanospray (a), Disinfectant Nanospray (b), Wheel Nanocleaner (c);
Regular Silver Spray (d, e), Regular Disinfectant Spray (f, g), Regular Hair Spray (h, i), Regular Skin Hydrating Mist (j), and Regular Facial Spray (k, l).

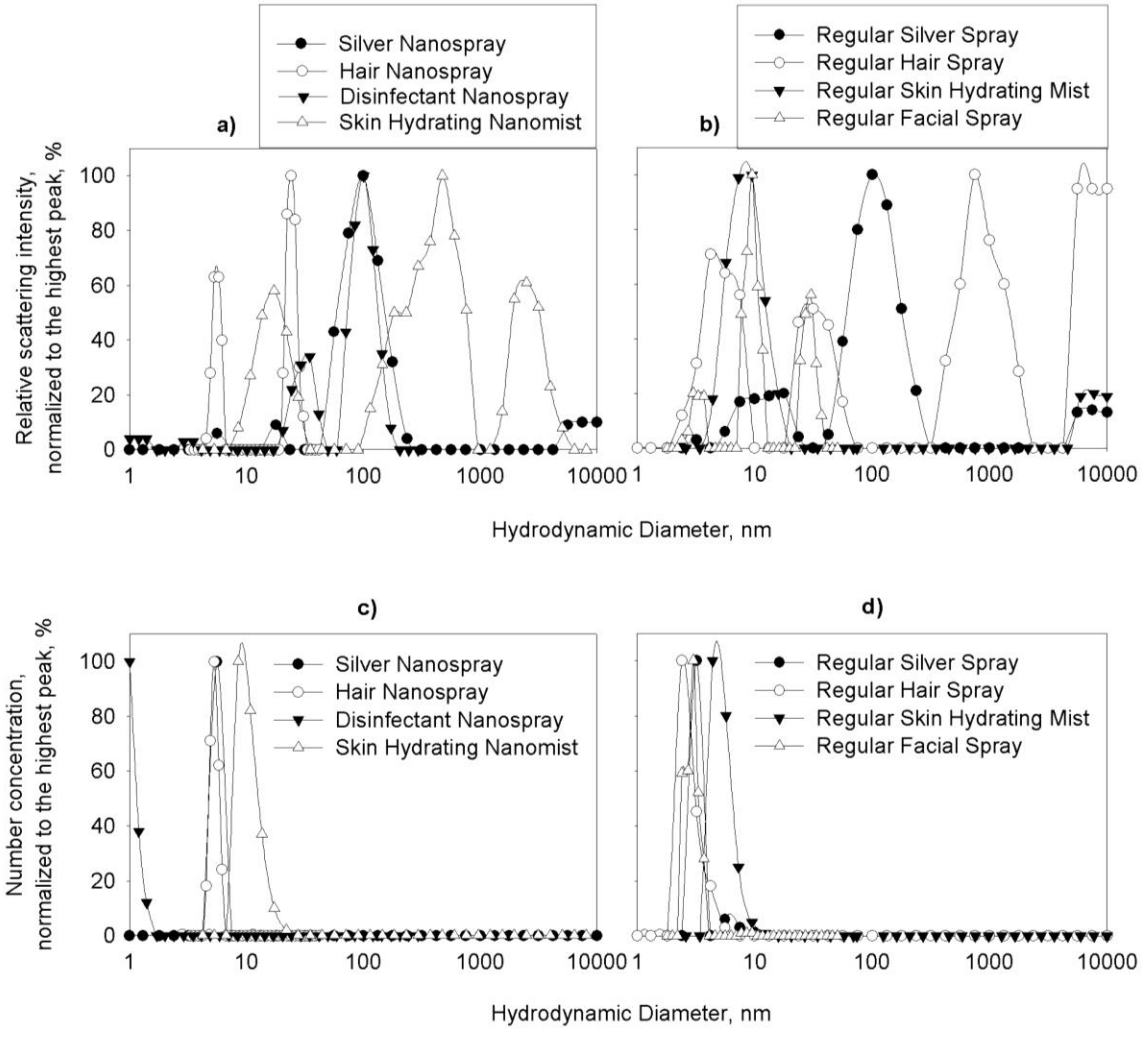


Figure 2.3. Hydrodynamic diameter of nanotechnology-based (a, c) and corresponding regular (b, d) consumer spray products: relative scattering intensity (a, b) and relative number concentration (c, d).

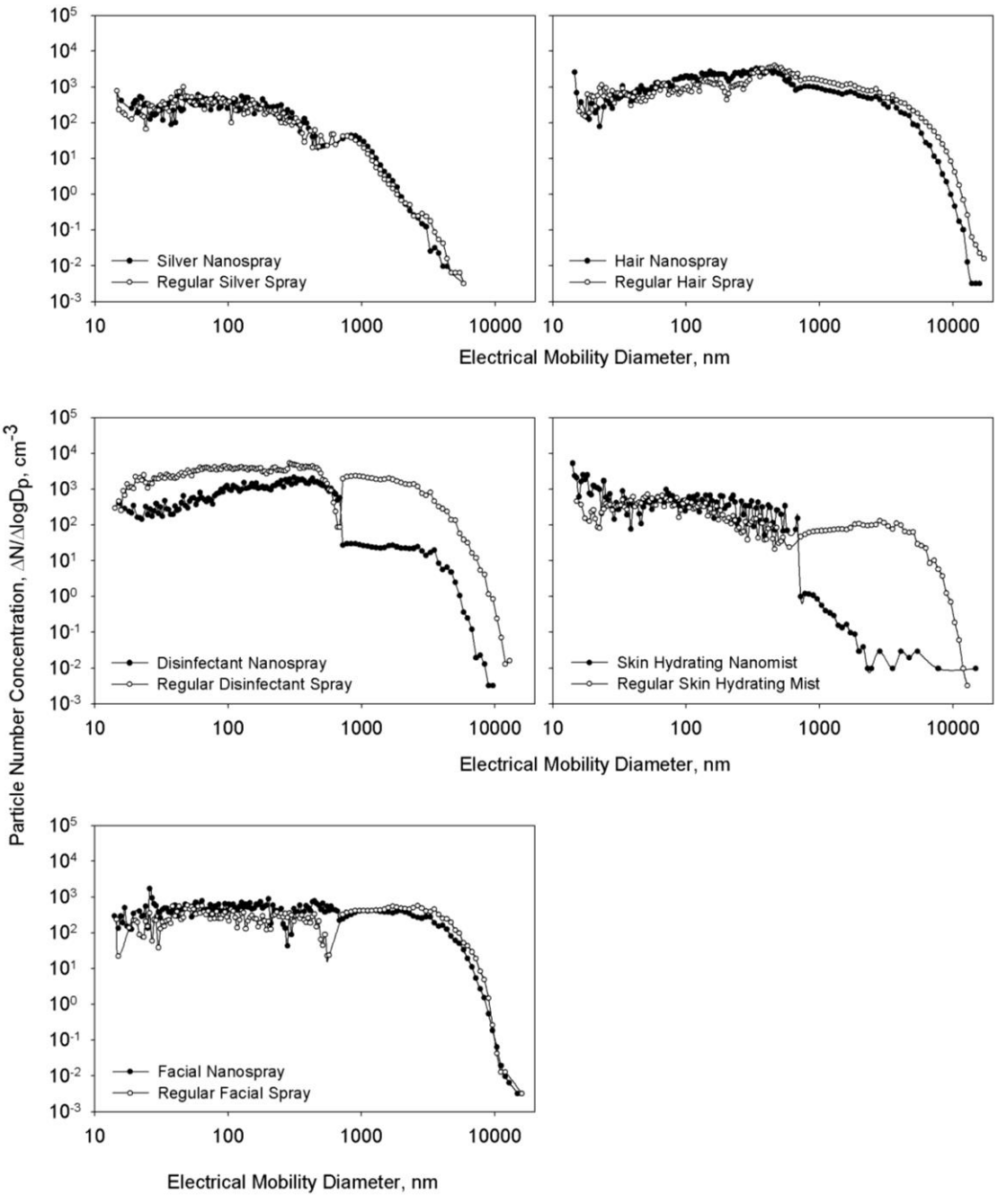


Figure 2.4. Size distributions of spray consumer products aerosolized by hand-held spraying. Nanotechnology-based products are shown in dark symbols, whereas regular products are presented in open symbols. The data present averages of three repeats.

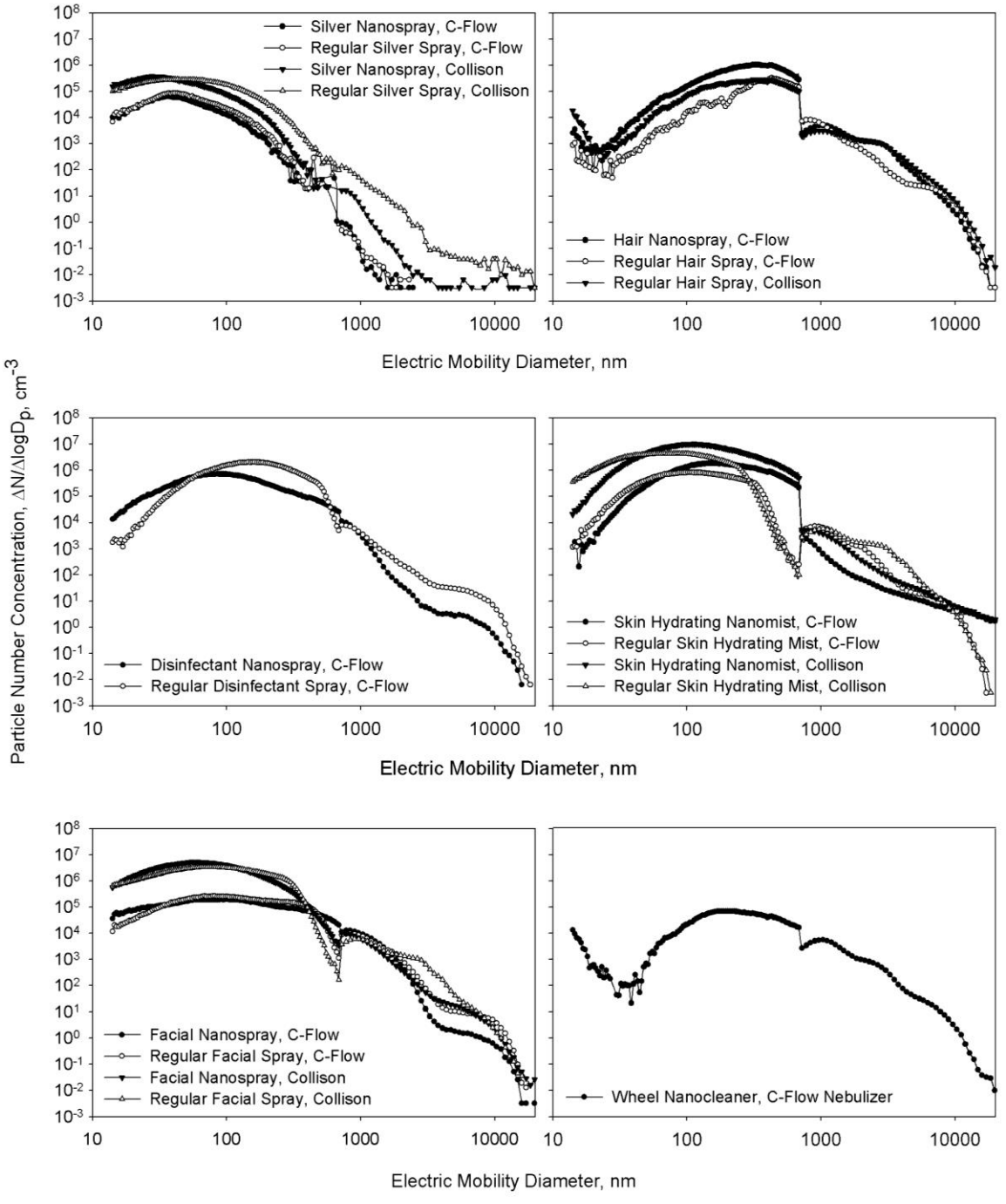


Figure 2.5. Size distributions of spray consumer products aerosolized by Collison and C-Flow Nebulizers. Nanotechnology-based products are shown in dark symbols, whereas regular products are presented in open symbols. The data present averages of three repeats.

Chapter 3

Potential for Inhalation Exposure to Engineered Nanoparticles from Nanotechnology-Based Cosmetic Powders[†]

[†]This chapter is modified from the manuscript by Yevgen Nazarenko, Huajun Zhen, Taewon Han, Paul Lioy, and Gediminas Mainelis. 2012. Potential for Inhalation Exposure to Engineered Nanoparticles from Nanotechnology-Based Cosmetic Powders. *Environmental Health Perspectives*. **120 (6)**: 885-892; doi:10.1289/ehp.1104350.

3.1. Abstract

The market of nanotechnology-based consumer products is rapidly expanding, and the lack of scientific evidence describing the accompanying exposure and health risks stalls the discussion regarding its guidance and regulation.

We investigated the potential for human contact and inhalation exposure to nanomaterials when using nanotechnology-based cosmetic powders and compare them with analogous products, not marketed as nanotechnology based.

We characterized the products using transmission electron microscopy (TEM) and laser diffraction spectroscopy and found nanoparticles in five of six tested products. TEM photomicrographs showed highly agglomerated states of nanoparticles in the products. We realistically simulated the use of cosmetic powders by applying them to the face of a human mannequin head while simultaneously sampling the released airborne particles through the ports installed in the mannequin's nostrils.

We found that a user would be exposed to nanomaterial predominantly through nanoparticle-containing agglomerates larger than the 1–100-nm aerosol fraction.

Predominant deposition of nanomaterial(s) will occur in the tracheobronchial and head airways – not in the alveolar region as would be expected based on the size of primary nanoparticles. This could potentially lead to different health effects than expected based on the current understanding of nanoparticle behavior and toxicology studies for the alveolar region.

3.2. Introduction

The development of nanotechnologies leads to the incorporation of nanomaterials into common consumer products because of the novelty and distinctive properties of materials at nanoscale. The number of nanotechnology-based consumer products listed in the Nanotechnology Consumer Products Inventory (Woodrow Wilson International Center for Scholars 2011a) is currently > 1,300. These products are manufactured by nearly 600 companies in 30 countries. Because this online database lists only a subset of products advertised on the Internet as nanotechnology based (Woodrow Wilson International Center for Scholars 2011b), the actual number is probably higher. The expansion of the nanotechnology-based consumer products market (Bradford et al. 2009; Kessler 2011; Maynard 2007; Paull and Lyons 2008; Woodrow Wilson International Center for Scholars 2011b) is cause for concern regarding potential human exposure to nanomaterials and possible health risks. The potential for exposure is still poorly understood, and potential health effects are unknown (Drobne 2007; Frater et al. 2006; Segal 2004; Van Calster 2006; Warheit et al. 2007). This impedes the development of appropriate consumer safety regulations and guidelines (Maynard et al. 2006; Oberdörster et al. 2005a; Paull and Lyons 2008; Riediker 2009).

A nanoproduct's type and intended use determine the most plausible routes and extent of exposure (Oberdörster et al. 2005b; Wardak et al. 2008). Use of nanotechnology-based cosmetic powders and sprays could lead to especially high levels of dermal and inhalation exposure, the latter being a consequence of product application leading to aerosol generation in the personal breathing zone (Hansen et al. 2008; Shimada et al. 2009). Contradictory conclusions regarding dermal absorption and toxicity of

nanoparticles have been reported (Baroli 2009; Baroli et al. 2007; Crosera et al. 2009; Larese et al. 2009; Senzui et al. 2010), and additional research has been recommended to better characterize and determine health concerns associated with dermal nanomaterial exposure (Crosera et al. 2009). At the same time, inhalation exposure to nanomaterials is a serious health concern (Savolainen et al. 2010). During consumer use, nanomaterials can be released and enter the respiratory system as free nanoparticles, nanoparticle agglomerates, and nanoparticles within or attached to larger particles. Additionally, other substances present in the applied nanoproduct could be physically transported on the nanoparticles themselves (Nowack and Bucheli 2007).

Many studies investigating the toxicity of pure nanomaterials have already been performed and summarized (Holgate 2010; Johnston et al. 2010; Marambio-Jones and Hoek 2010; Ostrowski et al. 2009; Savolainen et al. 2010; Schilling et al. 2010). However, the potential for consumer exposure to nanoparticles from actual nanotechnology-based products where nanomaterials exist in a product matrix with other ingredients has so far been addressed to only a limited degree.

Potential exposures and associated health effects are expected to depend on the dispersed particle size, agglomeration state, surface area and chemistry, solubility, concentration, and possibly the shape characteristics of nanomaterial(s) in a product (Bermudez et al. 2004; Shrader-Frechette 2007). Initial nanomaterial ingredients in consumer products might be chemically and physically modified through interactions with other ingredients in the product or through nanoparticle surface treatment during production, which may also affect their toxicity (Kessler 2011; Warheit et al. 2005). Therefore, properties of original nanomaterial ingredients cannot serve as the sole basis

for predicting exposure and health effects of a particular nanotechnology-based consumer product (Lioy et al. 2010; Maynard 2007). The size distribution of aerosol particles released and potentially inhaled during product use may also depend on the composition of the product, which in turn would affect the deposition of nanomaterial(s) in the respiratory system. Thus, one should characterize not only the in-product nanomaterials but also their characteristics during actual use by simulation and investigation of realistic exposure scenarios (Lioy 2010; Nazarenko et al. 2011).

In our earlier research, we investigated nanotechnology-based consumer spray products as well as their regular, non–nanotechnology-based, counterparts in a realistic exposure scenario, including a simulated application of the sprays (Nazarenko et al. 2011). The study demonstrated the potential for inhalation exposure to nanosize particles from all investigated products. Release of airborne silver nanoparticles during propellant-facilitated spraying of one nanotechnology-based silver spray was also shown in another study (Hagendorfer et al. 2010). The magnitude and prevalence of such exposures and associated risks are still unknown (Bradford et al. 2009; Keenan et al. 2009; Lioy et al. 2010).

In this study we focused on cosmetic powders, including nanotechnology-based- and non–nanotechnology-based powders, another category of consumer products with a high probability of inhalation exposure. The study had the following objectives: a) to characterize nanoparticles in several nanotechnology-based cosmetic powders currently in the market, b) to determine the potential for exposures to airborne nanoparticles and their agglomerates during the use of cosmetic powders in a realistic exposure scenario, and c) to compare investigated nanotechnology-based cosmetic powders with their

regular (non-nanotechnology) counterparts. This study responds to the call for independent nanotechnology-based commercial consumer product research, free of potential conflict of interest (Maynard 2007; Maynard and Aitken 2007; Michelson 2008; Shrader-Frechette 2007; Thomas et al. 2009).

To the best of our knowledge, this study is the first to determine the potential for human inhalation exposure to nanomaterials released from nanotechnology-based and regular cosmetic powders in a realistic exposure simulation.

3.3. Materials and Methods

3.3.1. Tested Cosmetic Powders

We selected three nanotechnology-based cosmetic powders ("nanopowders") – a moisturizer, a blusher, and a loose powder sunscreen – from the Woodrow Wilson Nanotechnology Consumer Products Inventory (Woodrow Wilson International Center for Scholars 2011a) and acquired them from the manufacturers. Currently, reporting by the manufacturers is the only way to identify the "nano" status of the products, and it may not be a guarantee that any given product in the inventory contains nanotechnological components (Hansen et al. 2008; Som et al. 2010). Consequently, the authors' references to products in this project as "nanoproducts" or "nanopowders" are based on product's presence in the above-mentioned inventory as of 1 September 2008. Additionally, we selected three cosmetic powders that manufacturers do not claim include nanomaterial(s) ("regular powders") – two blot powders and a finishing powder – and tested them for comparison with the cosmetic nanopowders. The selected regular powders perform functions similar to those of their nanotechnology-based counterparts and are also applied to the face.

The studied nano- and regular products are listed in Table 3.1, along with their intended application purpose and composition as reported by the manufacturers. We tested all products in their original formulation as shipped, without any deliberate pretreatment, deagglomeration, or any other type of modification. We replaced the product brand names with letter codes.

3.3.2. Characterization of cosmetic powders in their original state

Characterization of nanoparticles in the original products is necessary because the size distribution of particles released during a simulated product application might differ from that in the original product and may not adequately reflect the nanomaterial content to which a user would be exposed. We analyzed the powders in their original state using transmission electron microscopy (TEM) and laser diffraction spectrometry (LDS).

3.3.2.1. TEM

We used a transmission electron microscope (model 2010F; JEOL Ltd., Tokyo, Japan) to determine the size, shape, and agglomeration of electron-contrast particles visible in TEM photomicrographs for the tested powders. Only certain types of nanoparticles, e.g. certain metal, metal oxide, other inorganic and some organic nanoparticles absorb and scatter electrons enough to be visible in TEM micrographs (Egerton et al. 2004). Weak phase objects (mostly organic material) have low electron contrast and are consequently not visible in TEM images. We spread small quantities of each product on HC300-Cu grids (Electron Microscopy Sciences, Hatfield, PA) and performed manual particle size measurement (Matyi et al. 1987) from the resulting photomicrographs using the automatically inserted scale marks.

3.3.2.2. LDS

We used a laser diffraction particle size analyzer (Mastersizer 2000; Malvern Instruments Ltd., Worcestershire, UK) with a dry powder feeder (Scirocco 2000; Malvern Instruments Ltd.) to disperse the cosmetic powders in the air inside the device and determine their particle size distributions. The dry powder feeder employs a vibrating tray, which continuously feeds a powder into a Venturi tube, where it is accelerated close

to the speed of sound. This separates loose agglomerates by shear forces (Jones 2002). Mastersizer 2000 uses the red helium neon laser (633 nm) to measure particle size from 2,000 μm down to 100 nm (Malvern Instruments Ltd 2011). The laser light undergoes scattering, diffraction, and absorption by the airborne material, which results in varying intensities of the signal measured by large angle, focal plane, and backscatter detectors (Malvern Instruments Ltd 2011). The size, shape, and nature of the particles determine light scattering nanoparticles, e.g. certain metal, metal oxide, other inorganic and some organic nanoparticles absorb (Hackley et al. 2004). Mie theory is applied to determine particle size distribution.

The primary measurement unit is particle volume concentration. The instrument's software converts volume based scattering data into a particle size frequency distribution. For non-spherical particles, their size is reported as volume-equivalent diameter of a sphere.

The size distributions were generated by the Malvern Application (version 5.60) using the general purpose enhanced model for fine powders. All but one (i.e., regular powder E) of the products are mixtures of substances with different refractive indexes (RIs). The RI used in LDS is mathematically expressed as a complex number consisting of real and imaginary parts. The real part is the ratio of phase velocity of light in vacuum versus phase velocity of light in the bulk material, whereas the imaginary part, which describes absorption, depends on the nature and shape of particles (Gillespie and Lindberg 1992). Although the RIs of calcium carbonate, talc, and silica are around 1.5–1.7, the RI of titanium dioxide, a component of many cosmetic powders including one of the products tested in this study (nanopowder K), is 2.741. Because LDS performs

analysis of a given powder based on a single RI, the accuracy of measurements may be undermined depending on the selected RI when particles with different RIs are present. To minimize the measurement error, and on the basis of the composition of the cosmetic powders (see Table 3.1), we chose to perform our analyses using the RI of silica (1.544) for all powders with the exception of nanopowder K, for which we used the RI of zinc oxide (2.0041), which is a second active ingredient in this product along with titanium dioxide. This was considered a reasonable approach because the manufacturer did not provide the full composition of nanopowder K but listed only the active ingredients constituting 45% of the product: the nature of the remaining 55% of the product's ingredients remained unknown. The imaginary RI could not be determined for the products experimentally, so we used the imaginary RI value of 0.1 as advised in the Malvern Application for ground transparent materials (Malvern Instruments Ltd 2011).

3.3.3. Simulated application of cosmetic powders

We realistically simulated the application of cosmetic powders and the resulting inhalation exposures using the experimental setup shown in Figure 3.1. We placed a human female mannequin head (Image Supply House, Endicott, NY) inside a custom-built glove box with a removable cover. The inner dimensions of the glove box were 56 × 33 × 39 cm (approximately 72 L), and we covered its inner walls with aluminum foil to reduce electrostatic effects. We placed the glove box inside a level 2 biosafety cabinet (NUAIRE Inc., Plymouth, MN) with inner dimensions of 178.4 cm wide × 71.8 cm high × 57.2 cm diameter. We removed the top cover of the glove box for 5 min immediately before each experiment, to bring the concentration of background particles to below the detection limit of the instruments used, and then replaced it. We operated the HEPA

filtration system of the biosafety cabinet continuously throughout the powder application experiments. The glove box had two air inlets open to the inside of the biosafety cabinet to replace the air removed from the box by the measurement devices with particle-free air.

We applied all powders to the face of the mannequin head in a way that simulated actual product usage, using brushes or pads included with each product. Because the manufacturers did not include applicators with nanopowder M and regular powder E, we applied these two products using identical Kabuki brushes (Sephora USA Inc., San Francisco, CA). Additionally, we used a new Kabuki brush without any powder for comparison. After each application, we thoroughly cleaned the mannequin's face with 70% vol denatured ethanol. We performed background (i.e., no manipulations in the glove box) control measurements between the product tests.

3.3.4. Measurement of released particles

We installed two stainless steel tubes with an inner diameter of 5 mm into the nostrils of the mannequin head to sample the particles that would be inhaled during the application of the powders. The two aerosol streams drawn through the mannequin's nostrils were combined into one at the mannequin's nape using a stainless steel Y-connector, fitted through the back wall of the glove box, and then split using a stainless steel flow splitter (model 3708; TSI Inc., Shoreview, MN) and drawn into a scanning mobility particle sizer (SMPS) (module combination 3080/3786; TSI Inc.) and an aerosol particle sizer (APS; model 3321; TSI Inc.) via conductive tubing. These devices measured the actual airborne particle size distribution presented to the human respiratory system for inhalation, which is crucial for quantitative nanomaterial exposure assessment.

The aspiration rate of the SMPS, Q_{SMPS}, was 0.3 L/min, and that of the APS, Q_{APS}, was 4.7 L/min. An additional pump provided an auxiliary aspiration rate, Qaux, of 6.0 L/min, thus resulting in the total sampling flow rate Qa = 11.0 L/min, which corresponds to the breathing rate recommended for assessing short-term exposures for a 18 to 60-year-old female performing light activity (U.S. Environmental Protection Agency 1997). The U.S. EPA 1997 Exposure Factors Handbook (Table 5-14 in that document) recommends the used inhalation flow rate specifically for short- term exposures for our chosen user/activity profile. We believe that our choice of the recommended inhalation flow rate for short- term exposures matches the type of inhalation exposure expected during cosmetic powder application (short-term exposure) and is the most realistic relative to the activity level expected during cosmetic powder application. This inhalation flow rate slightly exceeds the inhalation flow rates referenced for sedentary activity defined as sitting and standing (Table 5-6 in that document) and as car driving and riding (Table 5-7 in that document). We find it consistent with our referenced inhalation flow rate since application of a cosmetic powder would occur during both sitting or standing, but performing the physical activity required for the application of a product and the same application but during a visit to a public bathroom or a similar place of retreat where a cosmetic powder application process would follow physical movement that would be more intense than simply standing or sitting, which would result in a somewhat higher inhalation flow rate. All connectors and sampling lines were of conductive material, and as short and as vertical as possible, to minimize potential particle losses.

Because the SMPS provides a full scan of the entire size range in 3 min, we continuously and to the best of our ability uniformly applied each test powder during this

time period. The APS measured particle concentration in all size bins simultaneously every second and provided an average concentration for each 3-min interval.

We used the SMPS system with a built-in 0.0457 cm impactor (d_{50} = 0.656 μm). This allowed us to obtain particle size distributions of 14.1–723 nm, whereas the APS measured particles in the 0.6–19.8 μm (600–19,800 nm) range. The measured particle size distributions by number are presented as $\Delta N/\Delta \log D_p$ per cubic centimeter, where ΔN is the number of particles detected in a size channel and $\Delta \log D_p$ is the difference between the logarithms of the upper and lower channel diameters. The SMPS and APS measure electrical mobility and aerodynamic diameters, respectively, which are identical for spherical particles of 1 g/cm^3 density. We assumed that all of the airborne powder particles had a density of 1 g/cm^3, which we considered to be a reasonable approach because all investigated powders except regular powder E were composites of multiple materials, mixed in mostly unknown proportions. Therefore, we interpreted SMPS and APS data as measurements on the same particle-size scale.

We subtracted the background particle concentration data from each set of APS measurements. The SMPS measurements indicated particle concentrations below the detection limit in the overwhelming majority of size channels for the background and clean brush measurements. We tested each powder three times in randomized order and calculated the average particle-number–based size distributions for each powder and instrument.

3.4. Results and Discussion

3.4.1. Analysis of Powders

3.4.1.1. TEM Analysis

TEM allows for direct viewing of solid electron-contrast primary nanoparticles or their agglomerates in consumer products. Representative TEM photomicrographs of tested powders are presented in Figure 3.2, and the summary of the TEM image analysis results is presented in Table 3.2. We found electron-contrast particles in all of the tested powders. The electron beam did not appear to alter the structure of any of the particles observed. When material is irradiated in TEM above a certain magnification setting (Carlo et al. 2002; Leapman and Sun 1995 ; Turgis and Coqueret 1999), higher electron beam power density per unit area of the sample results in physical and/or chemical alteration of the tested material (Egerton et al. 2004; Hobbs 1987). During the TEM analysis, this process can be observed visually. As mostly organic nanoparticles tend to be beam sensitive (Egerton et al. 2004), it can be concluded with some degree of certainty about organic or inorganic nature of nanoparticles in the tested products based on beam sensitivity.

All the primary particles (i.e., particles constituting the smallest dispersion level) in the sample of nanopowder M (Figure 3.2 a – c) were in the nanosize range. In fact, the largest observed particle was 45 nm in diameter. No free nanoparticles or individual agglomerates were observed – the level of agglomeration was very high, because all the nanoparticles in the samples of this product were continuously interconnected on the TEM grids. The sample of nanopowder D (Figure 3.2 d – f) contained no electron-contrast particles in the nanosize range. The only particles observed were > 5 μm (5,000

nm) in diameter and were not agglomerated. Nanopowder K (Figure 3.2 g – i) contained a wide size range of highly agglomerated particles, with most primary particles being in the nanosize range. Close examination of photomicrographs for regular powder F (Figure 3.2 j – l) showed nanosize particles in contact with larger particles. In the photomicrographs of regular powder G (Figure 3.2 m – o), most of the surface of the TEM grid was covered with particles > 5 μm in diameter, with only a few separate nanoparticles. Regular powder E (Figure 3.2 p – r) contained a large number of nanoparticles that were agglomerated and attached to larger particles.

Based on the composition of the powders as provided by the manufacturers (Table 3.1), we expect that the observed electron-contrast particles, including nanoparticles, contained silica (in all products with a possible exception of nanopowder K, for which information on composition was incomplete), talc (nanopowder D and regular powder G), mica (nanopowder D and regular powder F), aluminum hydroxide (nanopowder D), titanium dioxide and zinc oxide (nanopowder K), or kaolin and iron oxides (regular powders F and G).

Overall, based on TEM, we observed the highest abundance of nanoparticles in nanopowders M and K and in regular powder E.

3.4.1.2. LDS Analysis

The summarized results of the LDS analysis are listed in Table 3.2. The descriptive statistics of the size distributions of cosmetic powders by number as measured by the Mastersizer 2000 are presented in Table 3.3. The instrument detected particles of 100 nm in nanopowders M and K and in regular powders F, G, and E. Size distributions

of particles in these five powders were similar in shape, and all had mode diameters of 0.33 μm. Nanopowder D had a mode diameter of 0.66 μm (Figure 3.3).

Because the lower size limit of the LDS instrument used was 100 nm, particles with smaller diameter would not have been observed. However, the size distributions of these powders suggest that particles with diameters < 100 nm were likely present as well (Figure 3.3). This assumption is supported by the fact that TEM also registered particles < 100 nm in these five products.

Notably, neither TEM nor LDS indicated nanoparticles in nanopowder D, which is marketed as nanotechnology based. Conversely, the same analysis techniques detected a high number of nanoparticles in regular powder E, which is not marketed as nanotechnology based. These findings suggest that information provided regarding the presence or absence of nanomaterials in consumer products may not always be confirmed by experimental techniques.

3.4.2. Analysis of airborne particles released during powder application

The size distributions and concentrations of aerosol particles released during the simulated application of cosmetic powders based on SMPS and APS are presented in Figures 3.4 and 3.5. The mode diameters of the released particle size distributions Table 3.2. The descriptive statistics of the APS aerosol size distribution are presented in Table 3.4.

The particle concentrations for 14.1 – 700 nm size range as measured by the SMPS are shown in Figure 3.4. In the nanosize range (14.1 nm – 98.2 nm), the highest concentration reached 3.4×10^{4} cm^{-3} (at 14.1 nm for Regular Powder F). Below 25 nm, Nanopowders M and D and regular Powders F and E showed spikes of high nanoparticle

concentration. The instability of the aerosol concentration over the course of cosmetic powder application to the face of the mannequin mimics the real life situation and is not unexpected. The impact of this instability on the results is discussed in the main article.

In the rest of the nanosize range (25 – 98.2 nm), Regular Powder G remained comparatively low reaching only 2.4×10^{1} cm^{-3} (at 53.3 nm) while Regular Powder E consistently showed the highest concentrations among the investigated powders with three maxima at 61.5, 76.4, and 98.2 nm (3.1×10^{2}, 3.6×10^{2}, and 2.8×10^{3} cm^{-3} respectively).

In summary, for particles < 25 nm in diameter, which are characterized by higher alveolar deposition efficiency compared with larger particles (International Commission on Radiological Protection 1994), more variance in particle concentration was observed for nanopowders M and D and regular powders F and G (Figure 3.4) than for the rest of the products. The SMPS system is very sensitive to fluctuating particle concentrations. Therefore, we concluded that for these four cosmetic powders (M, D, F, and G), airborne nanoparticle concentration in the region < 25 nm in diameter was unstable over the course of cosmetic powder application. In general, peak nanoparticle number concentrations for particles < 25 nm in diameter were comparable to the highest concentrations observed for particles that were 25–723 nm in diameter.

Concentrations of nanoparticles between 25 and 100 nm in diameter differed among products (Figure 3.4). It is notable that the highest total particle counts were measured during the application of regular powder E, which is not marketed as a nanotechnology-based product by its manufacturer. Nevertheless, the spherical shape of the silica particles observed in this cosmetic powder using TEM (Figure 3.2B, p–r)

suggests that they may have been engineered, which, if true, would make this product *de facto* nanotechnology based.

From ~100 nm to ~700 nm, concentration of Regular Powder E was the highest reaching the order of 10^5 cm^{-3} for ~300 – 700 nm particles. Concentrations of the rest of the powders ranged from 7.2×10^{-1} cm^{-3} (at 278.8 nm) to 1.3×10^3 cm^{-3} (at 661.2 nm) both for Nanopowder D. The background SMPS measurement and the clean brush control showed concentrations mostly below the detection limit of the instrument and are therefore not shown in Figure 3.4.

Results for 0.6-20 µm particles as measured by the APS are shown in Figure 3.5. In the size range from 0.6 to 1 µm, the lowest concentrations were observed during application of Regular Powders F and G, and Nanopowder M with concentrations reaching ~10^1 cm^{-3}, while the other three powders reached concentrations up to 10^3 cm^{-3}.

The concentration of Nanopowder M was the lowest for the rest of the size range and comparable to the level of the clean brush control.

In accumulation mode (1 – 2.5 µm), moderate concentrations of particles were released during application of Powders F and G reaching only 6.5×10^1 and 4.2×10^2 cm^{-3} at 2.5 µm. The highest concentration in this range was from Regular Powder E reaching close to 10^4 cm^{-3}. For the Nanopowders D and K the concentrations were approximately 10^3 cm^{-3}.

In the coarse (2.5 – 10 µm) and supercoarse (>10 µm) size modes, the highest concentrations were observed from Regular Powder E: it peaked at 7.8×10^3 cm^{-3} at 3 µm and decreased to approximately 1.3×10^1 cm^{-3} in the supercoarse mode. The particle concentration from nanopowders D and K and regular powders F and G were

substantially higher than for Nanopowder M. At 2.5 μm size, their concentrations ranged from 6.9×10^{1} cm^{-3} to 5.1×10^{2} cm^{-3}. For larger particles, concentrations of these powders declined and separated a little bit more. At 10 μm, concentrations of these four powders ranged from 7.8×10^{-1} cm^{-3} to 1.1×10^{1} cm^{-3}.

In summary, airborne concentrations of particles between 100 nm and 20 μm in diameter (Figures 3.4 and 3.5) varied substantially among the different cosmetic powders. Particles across this entire range were measured during the application of both nanotechnology-based powders and regular powders, without obvious differences in the distributions between the nanopowders and regular powders. The products with the highest and lowest airborne concentrations varied within different particle size modes: fine (0.1–1 μm), accumulation (1–2.5 μm), coarse (2.5–10 μm), and supercoarse (> 10 μm), as defined by Lioy et al. (2006) (Figure 3.5). Notably, for particle diameters > approximately 1.5 μm, concentrations and nanopowder M had the lowest concentrations.

It is important to note, however, that application of all nanopowders resulted in the release of particles as large as 20 μm (Figure 3.5), and judging from the size distribution, even larger particles may have been released. As shown by the electron microscopy (Figure 3.2), the nanoparticles were agglomerated in the cosmetic powders, which suggests that nanomaterial may have been present in all airborne particle size fractions generated in the personal breathing cloud by cosmetic powder application.

SMPS (Figure 3.4) and APS (Figure 3.5) measurements in the overlapping size range (500–700 nm or 0.5–0.7 μm) do not always agree. A discussion of potential causes for such differences is provided elsewhere (Nazarenko et al. 2011).

3.4.3. Implications for exposure assessment and health risks

Although deposition in the alveolar region of the lung is the highest for nanoparticles and agglomerates of nanoparticles < 100 nm in diameter, particles larger than approximately 0.3 μm (300 nm) in diameter can efficiently deposit in the non–gas-exchange region of the lung, with particles > 10 μm in diameter (supercoarse particles) depositing primarily in the head airways (Hinds 1999). Therefore, inhalation of aerosol particles containing nanomaterials in both the 1–100 nm and 100 nm to 20 μm diameter size ranges, and possibly larger, and their potential deposition in all regions of the respiratory system, should be considered.

Our TEM data showed a predominance of agglomerated nanoparticles in nanopowders M and K, and a high number of agglomerated nanoparticles that were in contact with the surface of larger particles in regular powder E. Based on the TEM and aerosol measurement data, we expect that most of the airborne nanomaterial from cosmetic powders, especially by mass (Figure 3.6), will be in agglomerated form in particle size fractions > 100 nm, which are usually not the focus of most toxicology studies involving nanomaterials. A similar phenomenon could be predicted for many other nanotechnology-based consumer products that release nanoparticles as agglomerates and/or as composites with larger particles.

Most toxicological studies of potential health effects of inhaled nanoparticles, including studies of murine models, have used aerosols in which individual nanoparticles or nanosize agglomerates are a dominant fraction. For example, Geiser et al. (2005) administered a pure conditioned titanium dioxide aerosol with a 22-nm count median diameter into the rat respiratory system through an endotracheal tube. Sayes et al. (2010)

used a freshly generated silica aerosol with 37- and 83-nm mode diameter aerosols for nose inhalation exposure of rats. Based on size, such particles would primarily deposit in deep regions of the respiratory system.

By contrast, in consumer products such as cosmetic powders, our findings suggest that primary particles would likely coagulate among themselves and with other ingredients present in the product before its application. As a result, aerosols produced when cosmetic powders are used may be dominated by much larger particles, including agglomerates ≥ 10 μm in diameter. Consequently, application of cosmetic powders may result in inhaled nanomaterial deposition not only in the gas-exchange region of the lung (alveoli) but also in the non–gas-exchange regions (tracheobronchial and head airways). For example, nanoparticles ≤ 100 nm may form agglomerates > 10 μm (supercoarse-size particles) that deposit much higher up in the respiratory system than do nonagglomerated nanoparticles, that is, in the head airways rather than the alveolar and tracheobronchial regions (International Commission on Radiological Protection 1994), resulting in completely different health effects. Use of pure nanomaterials, as in the experimental studies cited above, would lead to a much higher nanomaterial deposition in the deeper regions of the respiratory system than could be expected based on product exposure simulation. As a result, such studies would have a diminished capacity to predict human health effects due to exposure to actual nanotechnology-based products.

The combined surface area of nanoparticle agglomerates exceeds that of solid particles of the same size by orders of magnitude. Thus, such agglomerates would present a much higher potential for surface-based reactivity within live tissue, potentially leading

to greater health risks compared with solid particles of the same size (Brown et al. 2001; Duffin et al. 2007; Gwinn and Vallyathan 2006; Monteiller et al. 2007; Nel et al. 2006).

At the same time, depending on the breathing rate, the laryngeal jet may break up inhaled loose agglomerates as small as 1 μm in diameter (Li et al. 1996) into smaller aggregates or individual particles that could deposit throughout the entire respiratory system. Therefore, quantitative nanoparticle exposure studies should take into account the polydisperse nature of aerosol produced during the use of nanotechnology-based consumer products and examine not only the exposure to and deposition of unbound nanoparticles, but also the fate, transport, and deposition of nanoparticle agglomerates in all regions of the respiratory system, including smaller aggregates or particles that may result from the breakup of larger nanoparticle agglomerates.

Our findings on potential nanomaterial inhalation exposure due to the use of actual consumer products emphasize that properties and effects of the pure nanomaterial ingredients cannot be used to predict actual consumer exposures and resulting health effects. Therefore, experimental techniques for toxicity studies of *de facto* nanotechnology-based consumer products must be developed. Results of such studies will provide guidance for the developing market of nanotechnology-based consumer products and help clarify the need and feasibility of its regulation.

We performed our measurements indoors at comfortable relative humidity levels: 40–50%. Application of the powders at different humidity conditions, especially at very low or very high levels, could possibly affect the extent of powder agglomeration and thus its deposition in the respiratory system. The effect of relative humidity and other

environmental conditions on the extent of exposures should be addressed in future studies.

3.5. Conclusions

The release of particles > 100 nm and as large as 20 μm in diameter indicates potential exposure to nanoparticle agglomerates, especially from products in which a very large proportion of primary particles are in the nanosize range (e.g., nanopowders M and K and regular powder E, as shown by the TEM).

TEM observations and aerosol measurements suggest that exposure to nanomaterial(s) due to the use of cosmetic powders will be predominantly in the form of agglomerates or nanomaterials attached to larger particles that would deposit in the upper airways of the human respiratory system rather than in the alveolar and tracheobronchial regions of the lung, as would be expected based on the size of the primary nanoparticles. This may lead to completely different health effects than expected on the basis of nanoparticle behavior and toxicology studies for the alveolar region. Thus, predominant deposition of nanomaterials in the upper airways must be taken into account when designing inhalation toxicology studies of actual consumer products.

We conclude that the use of *de facto* nanotechnology-based cosmetic powders has a strong potential to result in inhalation exposure to single and agglomerated nanoparticles and that this potential should be quantitatively described by exposure assessment studies.

The absence of information regarding the engineered status of nanomaterials in consumer products and difficulties in determining whether engineered nanomaterials are present point to the need for legislation requiring manufacturers to report the use of engineered nanomaterials in their products. It is also important to determine whether nanosize particles present in some consumer products may be the result of a

manufacturing process rather than a consequence of the deliberate introduction of engineered nanomaterial(s) into the products.

This study provides an example of data acquisition methodology that could be used in quantitative exposure studies of nanotechnology-based consumer products, especially when simulating realistic exposure scenarios. Results from such studies could be used to estimate exposures resulting from the short-term and long-term use of cosmetic powders and to design future studies of nanoparticle deposition in the respiratory system and inhalation toxicology experiments.

3.6. Acknowledgements

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Table 3.1. Tested cosmetic powders

Product	**Composition[2]**	**Purpose[2]**
Nanopowder M[1]	Water, Butylene glycol, Sodium ascorbyl phosphate, Glycerin, Betain, Silica, Dimethicone, Citric acid, Polymethyl metacrylate, Squalane, Sodium hydroxide, Sodium metabisulfite, Capryloyl glycine, Sodium Hyaluronate, Marus Alba root extract, Rosmarinus Officinalis (Rosemary) leaf extract, olea europaea (Olive) leaf extract	Powder Moisturizer
Nanopowder D[1]	Mica, Talc, Dimethicone/Vinyl Dimethicone crosspolymer, Hydrogenated C6-14 Olefin polymers, Petrolatum, Dimethicone, Polysilicone-2, Aluminum stearate, HDI/Trimethylol Hexyllactone crosspolymer, Sorbitan sesquisostearate, Aluminum hydroxide, Methicone, Tocopherol, Silica, Triisostearin, Trimethylolpropane trioctanoate, Ethylparaben, Butylparaben, Parfum, CI 77492, CI 77947, CI 77891, CI 77491, CI 77499	Powder Blusher
Nanopowder K[1]	Active Ingredients: Titanium dioxide – 25%, Zinc Oxide – 20%	Powder Sunscreen
Regular Powder F	Dimethicone, Silica, Kaolin, Water, Hydrolyzed Soy Protein, Caprylyl glycol, Hexylene glycol, Methicone, Coconut acid, Phenoxyethanol, +/- Mica, Iron oxides (CI 77491, CI 77492, CI 77499), ILN31255	Blot Powder
Regular Powder G	Talc, C12-15 Alkyl Benzoate, Kaolin, Silica Silylate, +/- Mica, Iron oxides (CI 77491, CI 77492, CI 77499)	Blot Powder
Regular Powder E	Silica	Finishing Powder

[1]Nanoproduct as per the Woodrow Wilson Nanotechnology Consumer Products Inventory
[2]As per manufacturer

Table 3.2. Characteristics of the tested cosmetic powder products obtained using different analysis methods

Product	TEM[1] Range of Particle Diameters, Agglomeration, Shape, Structure, Electron Beam Sensitivity	Presence of particles <100 nm	LDS[2] Smallest Detected Particle Diameter, µm	LDS Mode Diameter, µm	SMPS[3], Mannequin Sampling: Mode Diameter(s), nm	APS[4], Mannequin Sampling: Mode Diameter, µm
Nanopowder M[5]	6 – 45 nm, only agglomerates, fused spheroidal and irregular, solid, beam insensitive	All particles are <100 nm and agglomerated	0.1	0.33	< 100	1.7
Nanopowder D[5]	> 5 µm, single particles , irregular, solid, beam insensitive	Not observed	0.44	0.66	< 100	1.0
Nanopowder K[5]	7 nm - > 3 µm, only agglomerates, angular spheroidal, solid, beam insensitive	Many agglomerated particles <100 nm	0.1	0.33	53.3, 101.8, 241.4, 358.7	1.5
Regular Powder F	12 nm – > 8.8 µm, single particles and agglomerates, angular composite, beam insensitive	Many particles <100 nm, but all in composites within large particles	0.1	0.33	< 100 nm, 121.9	2.6
Regular Powder G	62.5 nm – > 10 µm, single particles and agglomerates, irregular, solid, beam insensitive	Very few separate particles, unclear if larger particles are agglomerates of nanoparticles	0.1	0.33	156.8	2.6
Regular Powder E	23.3 nm – > 12.8 µm, single particles and agglomerates, spheroidal, solid, beam insensitive	Many agglomerated and attached to the surface of large particles	0.1	0.33	17.5, 61.5, 76.4, 135.8, 181.1, 429.4	3.3

[1]TEM: Transmission Electron Microscopy
[2]LDS: Laser Diffraction Spectrometry
[3]SMPS: Scanning Mobility Particle Sizer
[4]APS: Aerosol Particle Sizer
[5]Nanoproduct as per the Woodrow Wilson Nanotechnology Consumer Products Inventory

Table 3.3. Descriptive statistics of the size distributions of cosmetic powders by number as measured by the Mastersizer 2000. These size distributions are shown in Figure 3.3.

	Nanopowders			Regular Powders		
	M	D	K	F	G	E
Mode, μm	0.33	0.66	0.33	0.33	0.33	0.33
Geometric Mean (d_g), μm	0.33	1.03	0.32	0.35	0.35	0.33
Geometric Standard Deviation (σ_g)	1.76	1.83	1.72	1.79	1.86	1.73

Table 3.4. Descriptive statistics of the size distributions of cosmetic powders by number during their application to human mannequin face as measured by the Aerodynamic Particle Sizer (APS). These size distributions are shown in Figure 3.5.

		Nanopowders			Regular Powders		
	Clean Brush	M	D	K	F	G	E
Mode, µm	1.72	1.72	1.04	1.49	2.64	2.64	3.28
Geometric Mean (d_g), µm	1.75	1.64	1.44	1.45	2.86	2.79	3.12
Geometric Standard Deviation (σ_g)	1.70	1.69	1.66	1.54	1.93	1.56	1.63

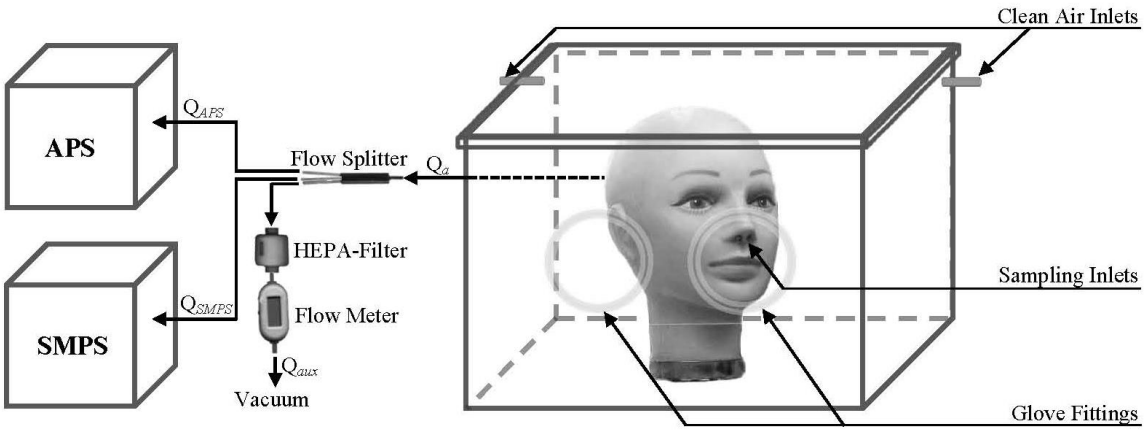

Figure. 3.1. Aerosol generation and analysis experimental setup for simulated application of the spray products (a) and constant output aerosolization (b).

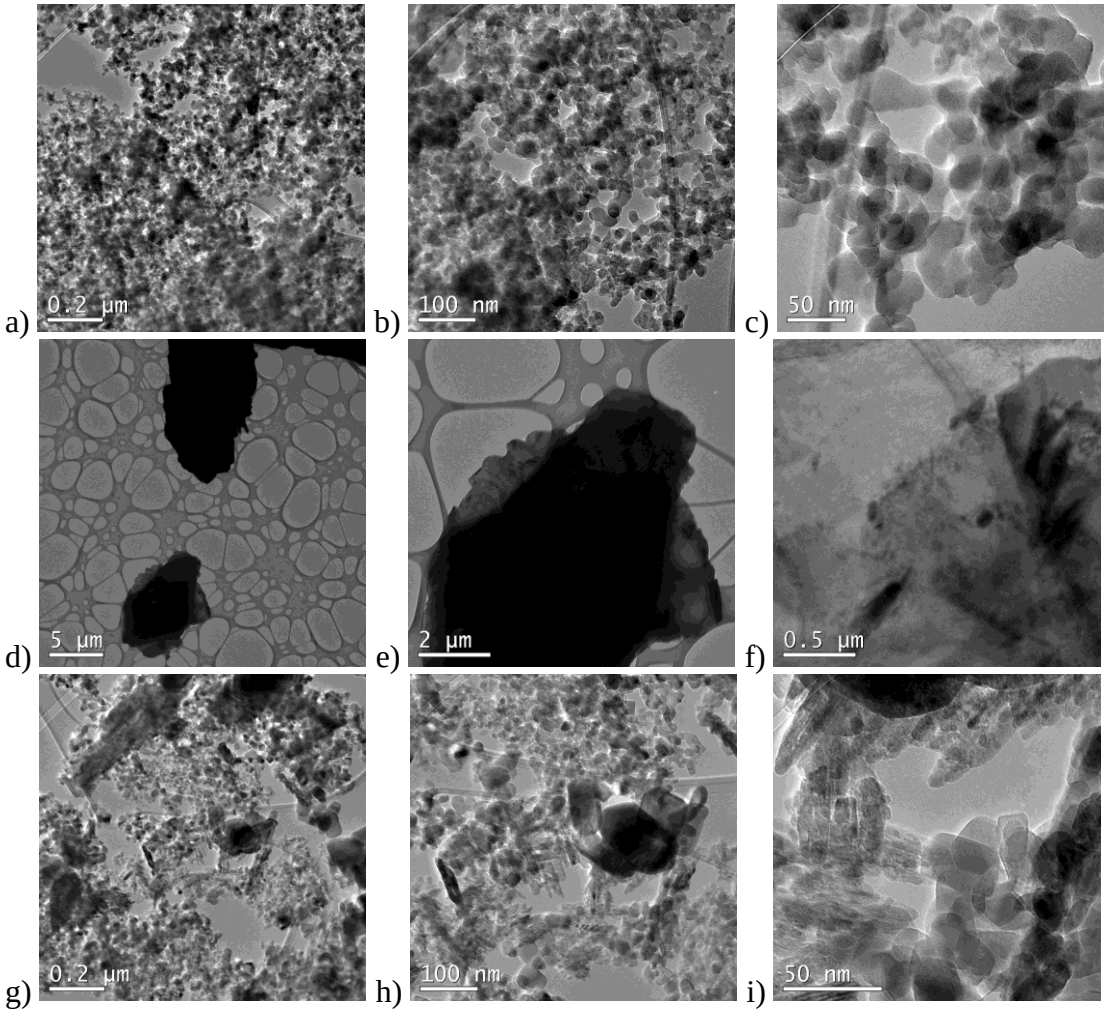

Figure 3.2A. Transmission electron micrographs of the tested cosmetic nanopowders. Nanopowder M (a – 0.2µm scale bar, b – 100nm scale bar, c – 50nm scale bar), Nanopowder D (d – 5µm scale bar, e – 2µm scale bar, f – 0.5µm scale bar), Nanopowder K (g – 0.2µm scale bar, h – 100nm scale bar, i – 50nm scale bar).

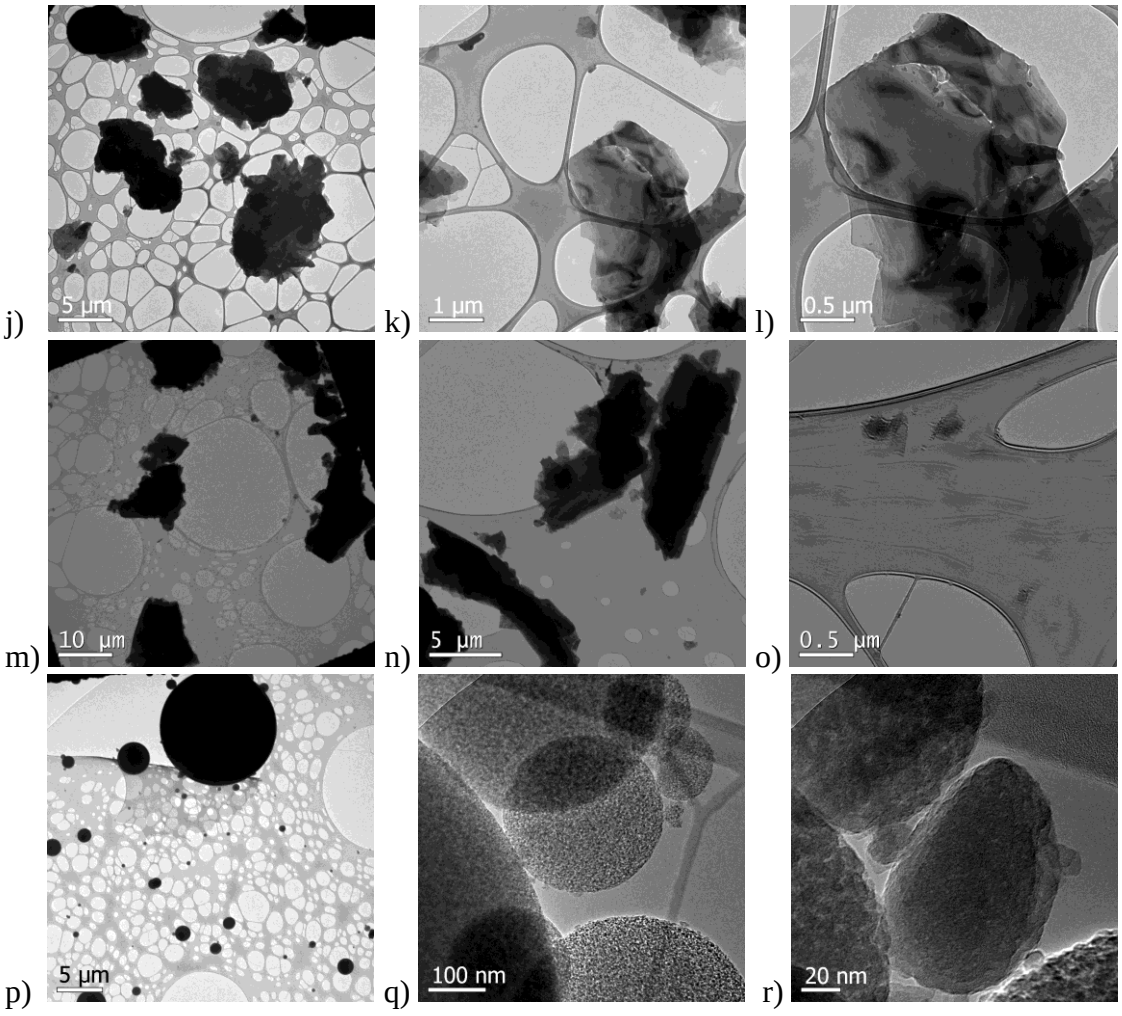

Figure 3.2B. Transmission electron micrographs of the tested cosmetic powders. Regular Powder F (j – 5µm scale bar, k – 1µm scale bar, l – 0.5µm scale bar), Regular Powder G (m – 10µm scale bar, n – 5µm scale bar, o – 0.5µm scale bar), Regular Powder E (p – 5µm scale bar, q – 100nm scale bar, r – 20nm scale bar).

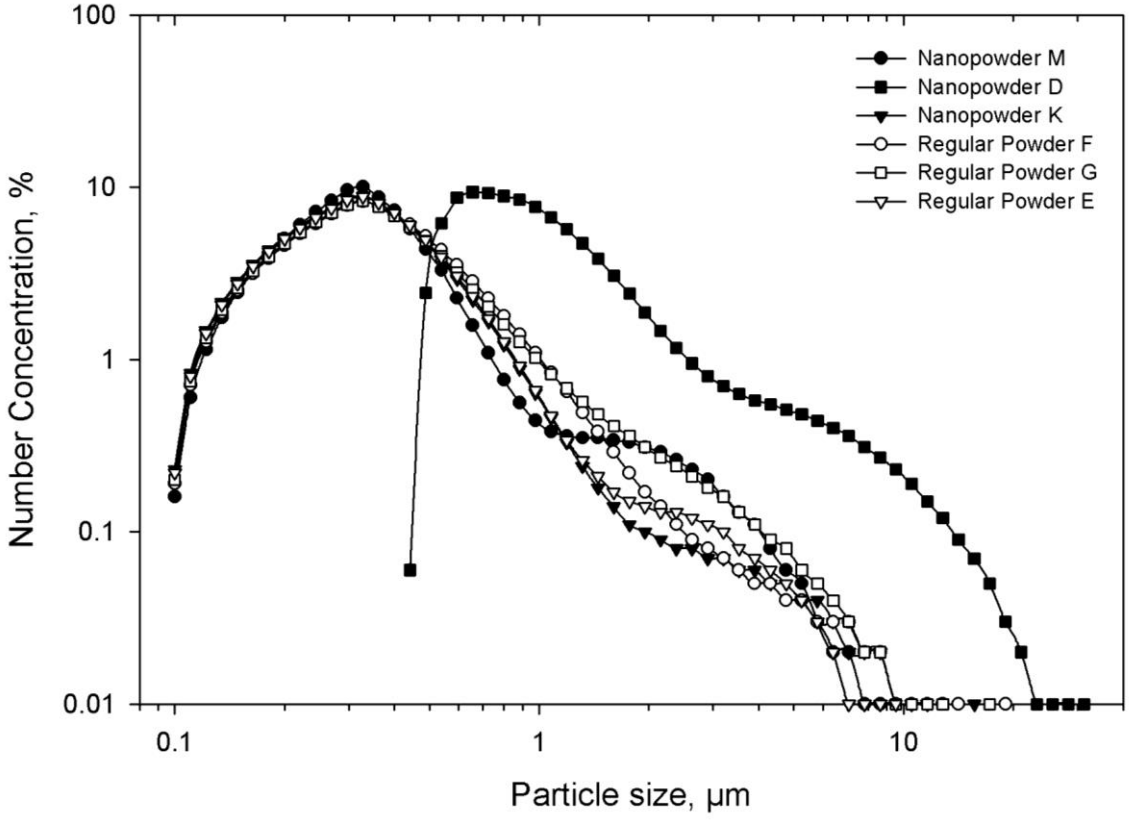

Figure 3.3. Size distributions of cosmetic powders by number as measured by the Mastersizer 2000. The data represent averages of three repeats. Nanotechnology-based products are shown in black symbols, regular ones are shown in white symbols.

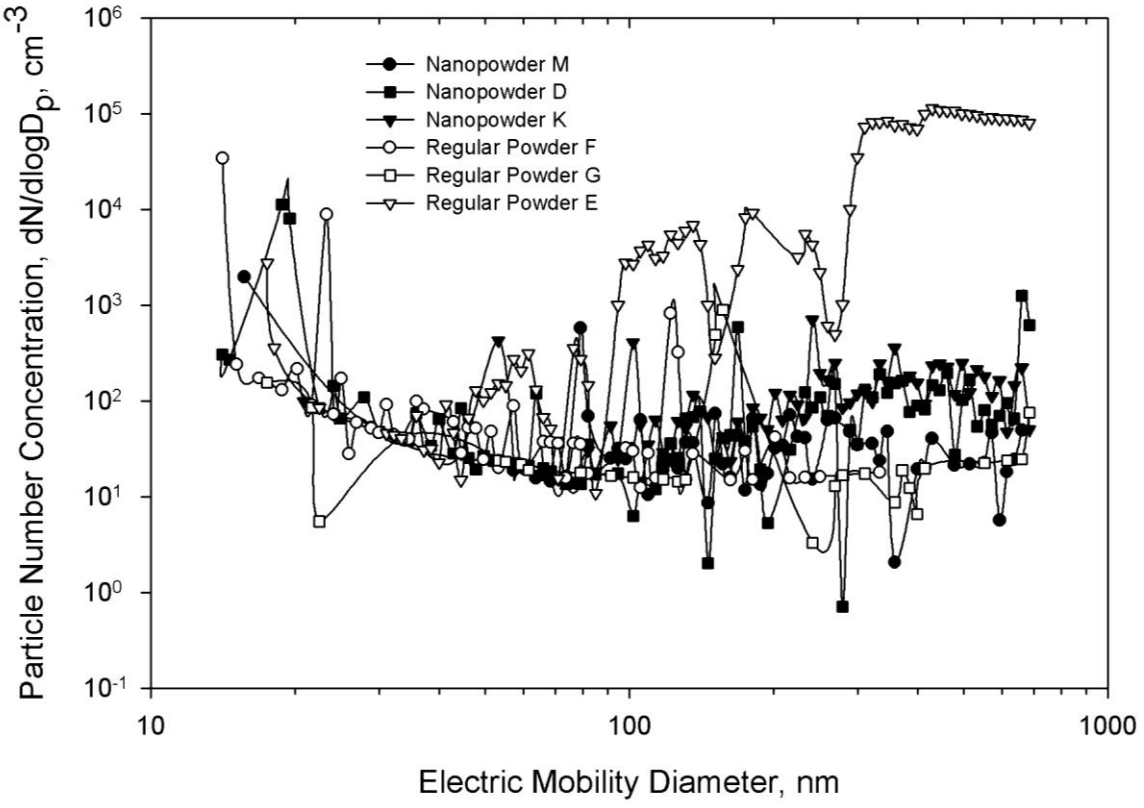

Figure 3.4. Size distributions of airborne cosmetic powders by number during their simulated application to human face as measured by the Scanning Mobility Particle Sizer (SMPS): 14.1 – 723 nm measurement size range.
The data represent averages of three repeats. Nanotechnology-based cosmetic powders are shown in black symbols, regular ones are shown in white symbols.

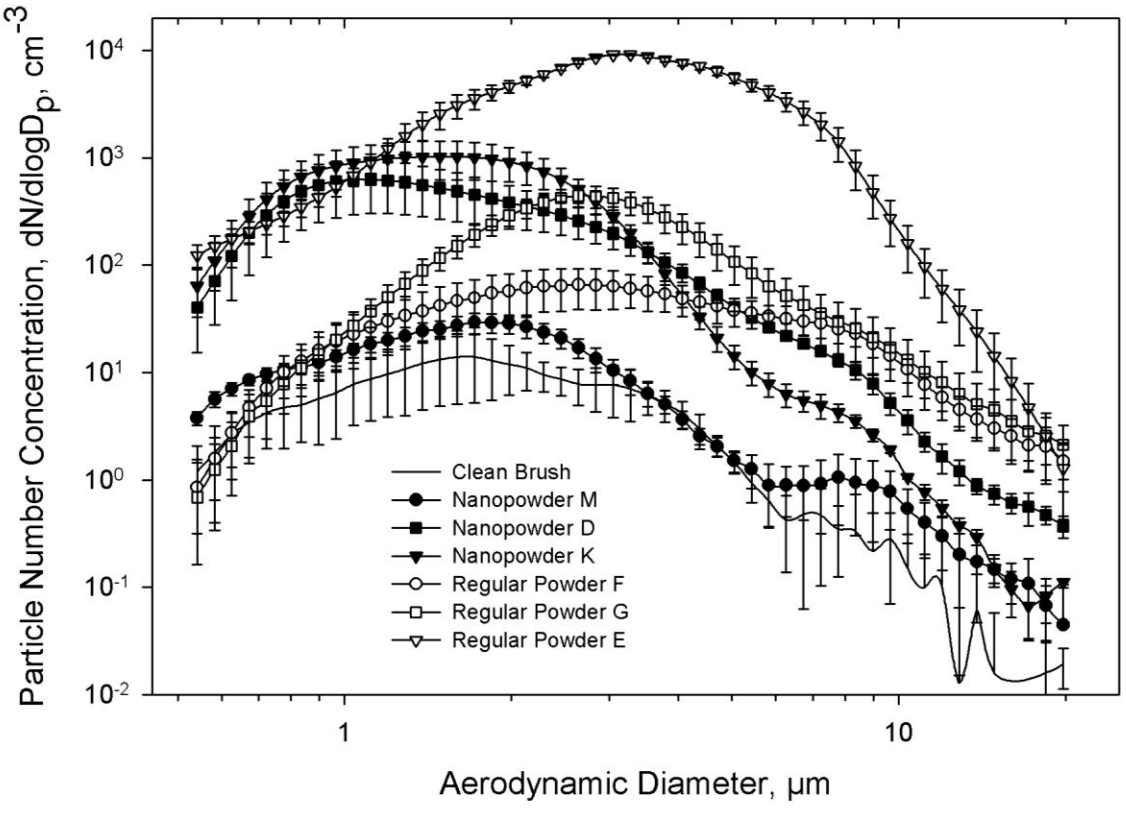

Figure 3.5. Size distributions of airborne cosmetic powders by number during their application to human mannequin face measured by the Aerodynamic Particle Sizer (APS): 0.6 – 19.8 µm measurement size range.
The data represent averages of three repeats with error bars representing ± one standard deviation. Nanotechnology-based cosmetic powders are shown in black symbols, regular ones are shown in white symbols.

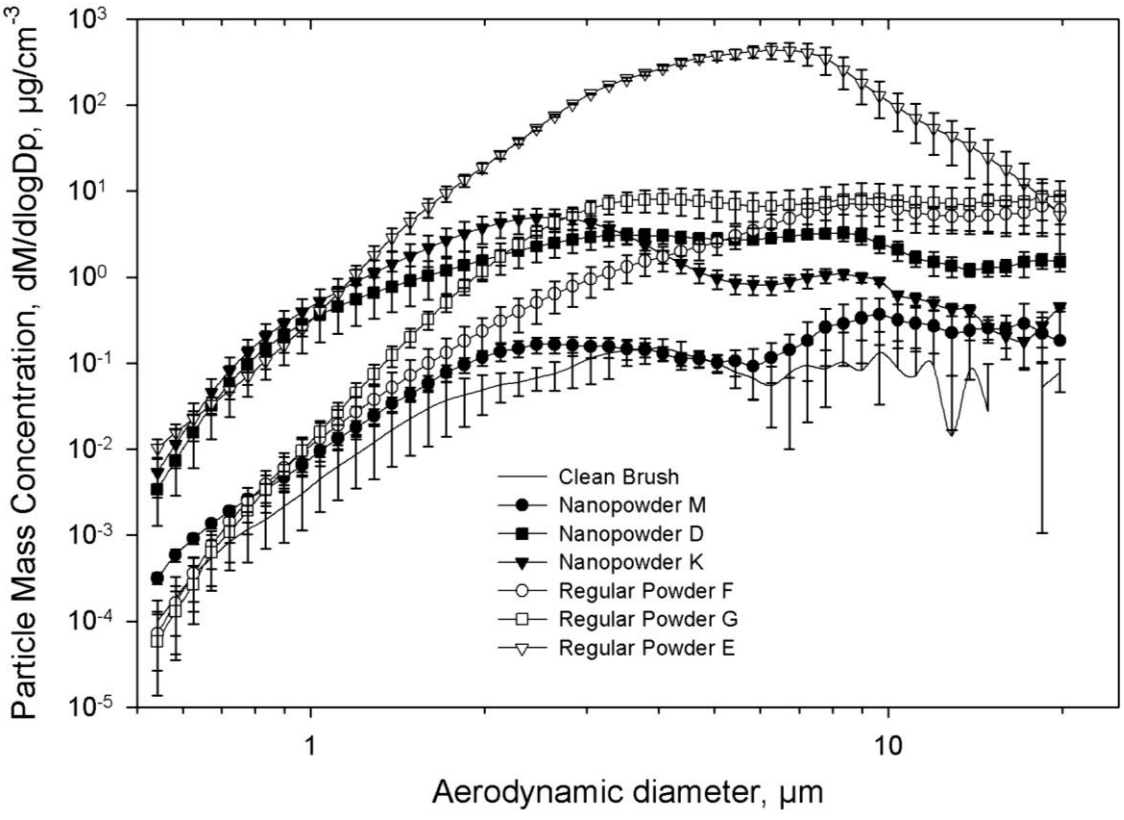

Figure 3.6. Size distributions of airborne cosmetic powders by mass during their application to human mannequin face as measured by the Aerodynamic Particle Sizer (APS): 0.6 - 19.8 µm measurement size range.
The data represent averages of three repeats with error bars representing ± one standard deviation based on these repeats.
Nanotechnology-based cosmetic powders are shown in black symbols, regular ones are shown in white symbols.

Chapter 4

Nanomaterial Inhalation Exposure from Nanotechnology-based Cosmetic Powders: a Quantitative Assessment[‡]

[‡]This chapter is modified from the manuscript by Yevgen Nazarenko, Huajun Zhen, Taewon Han, Paul Lioy, and Gediminas Mainelis. 2012. Nanomaterial Inhalation Exposure from Nanotechnology-based Cosmetic Powders: a Quantitative Assessment. *Journal of Nanoparticle Research*. **14 (11)**: Online 10, October 2012; doi: 10.1007/s11051-012-1229-2.

4.1. Abstract

In this study we quantified exposures to airborne particles ranging from 14 nm to 20 μm due to the use of nanotechnology-based cosmetic powders. Three nanotechnology-based and three regular cosmetic powders were realistically applied to a mannequin's face while measuring the concentration and size distribution of inhaled aerosol particles. Using these data we calculated that the highest inhaled particle mass was in the coarse aerosol fraction (2.5–10 μm), while particles <100 nm made minimal contribution to the inhaled particle mass. For all powders, 85–93 % of aerosol deposition occurred in the head airways, while <10 % deposited in the alveolar and <5 % in the tracheobronchial regions. Electron microscopy data suggest that nanomaterials were likely distributed as agglomerates across the entire investigated aerosol size range (14 nm–20 μm). Thus, investigation of nanoparticle health effects should consider not only the alveolar region, but also other respiratory system regions where substantial nanomaterial deposition during the actual nanotechnology-based product use would occur.

4.2. Introduction

Use of nanomaterials in consumer products has now become a widespread industry practice (Chuankrerkkul and Sangsuk 2008; Gleiche et al. 2006; Lloyd's 2007; Mihranyan et al. 2012), including extensive application of nanomaterials in cosmetics and other products (Fender 2008; Mihranyan et al. 2012; Mu and Sprando 2010; Nohynek et al. 2008). While it has now been recognized that human exposure to nanomaterials resulting from the use of certain consumer products is possible (Benn et al. 2010; Donaldson et al. 1998; Hagendorfer et al. 2010; Nazarenko et al. 2011; Nazarenko et al. 2012), the extent of this exposure and the associated risks are still unknown (Bradford et al. 2009; Keenan et al. 2009; Lioy et al. 2010) and need to be investigated in depth. Since the market of nanotechnology-based consumer products is expanding (Bradford et al. 2009; Maynard 2007; Woodrow Wilson International Center for Scholars 2011b), the prevalence of possible human exposures to nanomaterials in such products and related health risks are likely to be increasing as well. Toxicology of pure nanomaterials has been a subject of research for a number of years (Ostrowski et al. 2009). However, when it comes to nanotechnology-based consumer products where these nanomaterials are incorporated, the research community is still far from drawing substantiated conclusions about the potential associated health effects. This lack of quantitative exposure data is one of the reasons why the development of regulations and safety guidelines for nanotechnology-based consumer products is currently delayed (Maynard et al. 2006; Oberdörster et al. 2005b; Paull and Lyons 2008). This study provides a pioneering insight by quantitative assessing of inhalation exposure, which is the first step toward determining potential health effects.

Among the different kinds of nanotechnology-based consumer products, two categories – sprays and cosmetic powders – present a special concern as sources of potentially the strongest nanomaterial inhalation exposure (Hagendorfer et al. 2010; Shimada et al. 2009). When a person uses a cosmetic powder or a consumer spray, airborne particles from the generated aerosol could be inhaled and enter the respiratory system. If the products are nanotechnology-based, these inhaled airborne particles are likely to carry nanomaterials, which could be in the form of free nanoparticles and their agglomerates or nanoparticles attached or incorporated into larger particles. Our previous research on the potential of nanomaterial inhalation exposure from nanotechnology-based cosmetic powders has shown that particles ranging from 14 nm to 20 μm are aerosolized during cosmetic powder application and are likely to be inhaled thus resulting in exposure to nanomaterials (Nazarenko et al. 2012). However, the fraction and size of aerosolized particles carrying nanomaterials that would deposit in a particular region of the respiratory system remained unknown. Information about the sizes of deposited particles as well as their deposition sites in the human respiratory system is important, because chemically the same substance may have substantially different toxicity and associated biological and health effects depending on its size and structural state as well as deposition site following inhalation (Brunekreef and Holgate 2002; Lee 2011; Nel et al. 2006; Oberdörster et al. 2005a; Tsuji et al. 2009; Wardak et al. 2008). These differences can be profound even for small variations in particle size, including within the 1 – 100 nm range (Bermudez et al. 2004; Carlson et al. 2008; Grassian et al. 2007; Hussain et al. 2005; Quadros and Marr 2011).

In our earlier study, we measured number concentration of aerosol particles that would be released and inhaled during simulated application of cosmetic powders (Nazarenko et al. 2012). Here, we used those data to calculate the mass of various aerosol size fractions inhaled and deposited in different regions of the human respiratory system as a result of using nanotechnology-based cosmetic powders. For comparison, regular powders (not based on nanotechnology) were investigated as well.

To the best of our knowledge, these are the first quantitative data on nanomaterial inhalation exposure due to the use of nanotechnology-based consumer products, specifically cosmetic powders. It is hoped that these inhalation and deposition exposure data will be useful in future studies investigating health effects due to the use of nanotechnology-based consumer products.

4.3. Materials and Methods

4.3.1. Summary

The size characteristics of the tested nanotechnology-based and regular cosmetic powders were investigated using transmission electron microscopy (TEM). The powders were then realistically applied to the face of a human mannequin head. The particles released as a result of this application were sampled through the nostrils of the mannequin head and their sizes and concentrations were determined. These data were then used to determine the inhaled and deposited dose based on particle mass.

4.3.2. Investigated Products

The quantitative inhalation exposure assessment was performed for three nanotechnology-based and three regular cosmetic powders. The three nanotechnology-based cosmetic powders were selected from The Woodrow Wilson Nanotechnology Consumer Products Inventory (Woodrow Wilson International Center for Scholars 2011a). The method used to construct The Inventory is based on information provided by manufacturers as part of product marketing. The three regular cosmetic powders with a similar purpose of use as the nanopowders were selected randomly. Table 1 lists the investigated nanotechnology-based and regular cosmetic powders alongside their purpose of use and chemical compositions as reported by the manufacturers. We tested all of the cosmetic powders in their original state without any pre-treatment, deagglomeration or dilution. The brand names of the investigated cosmetic powders were replaced by letter codes. Additionally, the cosmetic powders were identified by their purpose of application.

4.3.3. TEM Characterization of Cosmetic Powders

All of the cosmetic powders were examined in their original state using a transmission electron microscope (2010F, JEOL Ltd, Tokyo, Japan). A minute quantity of each cosmetic powder was placed on a HC300-Cu TEM grid (Electron Microscopy Sciences, Hatfield, PA, USA) and a number of representative digital micrographs at different magnifications were taken for each specimen. Particle diameters, shape and the degree of agglomeration in each cosmetic powder were assessed visually using the automatically inserted scale bars on the micrographs.

4.3.4. Simulated Application

The experiment to measure the number concentration of the released and inhaled particles was designed to simulate a realistic exposure scenario when cosmetic powders are used by a consumer. The cosmetic powder application and aerosol sampling and measurement process have been described in detail elsewhere (Nazarenko et al. 2012). Briefly, as shown in Figure 1, a human mannequin head was placed inside a glove box located within a Level II Biosafety cabinet (NUAIRE, Inc., Plymouth, MN, USA). Two stainless steel tubes were installed into the nostrils of the mannequin head to allow for sampling of particles that would be inhaled during the real life application of the powders. The two aerosol streams drawn through the mannequin's nostrils were combined into one at the mannequin's nape using a stainless steel Y-connector, and then drawn into a Scanning Mobility Particle Sizer (SMPS) (module combination 3080/3786, TSI, Inc., Shoreview, MN, USA) and an Aerodynamic Particle Sizer (APS) (model 3321, TSI, Inc.) via electrically conductive tubing. The SMPS and the APS instruments

provided aerosol concentrations and size distributions in the range between 14.1 nm and 20 μm.

All the connectors and sampling lines were made as short as possible and of conductive material to minimize potential particle losses due to the electrostatic effects, diffusion and gravitational settling. Each test powder was continuously and, to our ability, uniformly applied to the face of the mannequin head during each measurement period. The applicators (brushes or pads) included with the products by the manufacturers were used. No applicators were supplied with Nanopowder M and Regular Powder E, so we used identical kabuki brushes (Sephora USA, Inc., San Francisco, CA, USA) for their application. Another clean kabuki brush was used without any cosmetic powder for comparison. Three measurement repeats were performed for each cosmetic powder. The background particle concentrations were subtracted from the SMPS and the APS measurements.

The total sampling flow rate was Q_a = 11.0 L/min corresponding to the breathing rate recommended for assessing short-term exposures for a 18 – 60 year-old female performing light activity (Yang et al. 2008). This total sampling flow rate was achieved by combining the sampling flow rates of the SMPS – $Q_{a(SMPS)}$ (0.3 L/min) and of the APS – $Q_{a(APS)}$ (4.7 L/min) with an auxiliary aspiration rate – Q_{aux} (6.0 L/min) provided by an additional pump.

Since we sampled through the nostrils of a human mannequin head, we assumed that the measured aerosol size distribution is approximately that of cosmetic powders aspirated into the human nasal airways during the real world cosmetic powder application.

4.3.5. Quantitative Exposure Assessment

Based on the SMPS and APS measurements, we calculated both the "inhaled dose" and the "deposited dose". By "inhaled dose" we mean the mass of airborne particulate matter that enters the human respiratory system. By "deposited dose" we mean the mass of particulate matter that deposits either in the entire respiratory system or in a specific region of the respiratory system: the head airways (HA), the tracheobronchial region (TB) and the alveolar region (AL).

4.3.5.1. Inhaled Dose

In order to calculate the inhalation exposure, we used the concentration of aerosol released during the simulated application of cosmetic powders as an input for the inhalation model based on the work by Hansen and colleagues (Hansen et al. 2008). While the original Hansen's calculations assumed a hypothetical inhalable fraction of the aerosol not considering particle size, we, on the other hand, used the size-resolved concentrations of airborne particles released during the realistic cosmetic powder applications accounting for the inhalability. The following equation was used (based on Hansen et al. (2008)) and assumptions about each variable are provided below:

$$ID = f_{nano} \cdot C_{inh} \cdot Q_{inh} \cdot T_{contact} / Bw \quad (1),$$

Where:

ID – inhaled dose of particulate matter per powder application (ng/kg bw/application);

C_{inh} – mass concentration of particulate matter in inhaled air (ng/L);

Q_{inh} – inhalation flow rate for a given gender/activity scenario (L/min);

$T_{contact}$ – duration of contact per application (min);

Bw – body weight (kg);

f_{nano} – mass fraction of nanomaterial(s) in the inhaled aerosol.

We assumed the duration of each application of a cosmetic powder $T_{contact}$ = 1 min. A different $T_{contact}$ can be used to recalculate for different scenarios of cosmetic powder use.

We were not able to determine the fraction of nanomaterials in each investigated product (f_{nano}). This information was not provided by the manufacturers either, despite our requests. Therefore, we decided to present the worst case scenario by assuming that the powders are completely made up of nanomaterial(s) and the released and inhaled aerosol particles would be completely made of nanomaterials, i.e., f_{nano} = 1. If and when the information on nanomaterial content in the investigated products becomes available, the doses presented here could be easily recalculated using a new f_{nano}.

Mass concentration of particulate matter in the inhaled air (C_{inh}) used in equation (1) can be described as:

$$C_{inh} = IF \cdot C_{air} \quad (2),$$

Where C_{air} is mass concentration of aerosol particulate matter in the personal breathing cloud. Inhalability fraction (IF) used in equation (2) represents the fraction of particulate matter in the personal breathing cloud that is actually inhaled into the respiratory system and is described by Hinds et al. (1999) as:

$$IF = 1 - 0.5\left(1 - \frac{1}{1 + 0.00076 d_p^{2.8}}\right) \quad (3),$$

Where d_p is particle diameter. This equation is applied for particles up to 100 µm in diameter. Since we used a human mannequin head and sampled through its nostrils at a realistic sampling flow rate, we assumed that the particle aspiration efficiency through the mannequin's nostrils approximately matches inhalability fraction IF for the investigated particle size range of 14.1 nm – 20 µm. Therefore, C_{inh} can be obtained directly from the SMPS and APS measurements, which were performed in our previous study (Nazarenko et al. 2012).

The SMPS and APS devices measure the number concentration and size distribution of the particles and the Aerosol Instrument Manager software (TSI, Inc.) can convert the data into particle mass concentration using user-provided particle density and assuming that particles are spherical.

Both SMPS and APS report aerosol size distributions by particle number and the data are presented in multiple size channels, which are defined by their midpoint. The SMPS aerosol particle concentrations in 108 size channels in the size range of 14.1 – 661.2 nm were used, while for the APS, aerosol particle concentrations in 48 size channels ranging from 673 nm to 19.81 µm were used. The Aerosol Instrument Manager software (TSI, Inc.) then converts concentration data from each size channel into particle mass concentration using the channel midpoint diameter (assuming that particles are spherical) and user-provided particle density. Since cosmetic powders are generally mixtures of multiple, both inorganic and organic substances, and are usually composites of multiple materials mixed in mostly unknown proportions, we made an assumption of the particle density to be 1.0 g/cm^3. The final exposure data can easily be recalculated for different densities of particles.

The mass concentrations from individual channels could be summed up to determine the total inhaled particle mass or the mass from several channels could be grouped into fractions based on aerosol particle size. Since particles of different sizes may present different potential health impacts and have different penetration and deposition characteristics in the respiratory system, the entire investigated size range was divided into several particulate matter (PM) size fractions of interest: $PM_{0.1\text{-}0.014}$ (particles between 14 and 100 nm, or nanoparticle aerosol fraction), $PM_{1\text{-}0.1}$ (fine particles between 0.1 and 1 μm, or submicron fraction of fine particles), $PM_{2.5\text{-}1}$ (fine particles between 1 and 2.5 μm, or micron fraction of fine particles), $PM_{10\text{-}2.5}$ (particles between 2.5 and 10 μm, or coarse particles), and finally $PM_{20\text{-}10}$ (particles between 10 and 20 μm, or supercoarse aerosol fraction). The supercoarse fraction was described by Lioy et al. (2006). For the $PM_{0.1\text{-}0.014}$ fraction, the lower limit of 14 nm represents the limit of our instruments. The inhaled particle mass was calculated for each one of these size fractions by adding the mass of particles in individual size channels within that fraction.

The body weight, Bw, and inhalation flow rate, Q_{inh}, were assumed to be those of an adult female (60 kg Bw) performing light activity level. This scenario was assumed to be the most typical for the application of cosmetic powders. For this scenario, the U.S. EPA 2011 Exposure Factors Handbook recommends using 11.0 L/min inhalation flow rate specifically for short-term exposure studies (Table 6-49 in the U.S. EPA 2011 Exposure Factors Handbook) (Yang et al. 2008).

The inhalation flow rate associated with light activity (11 L/min) slightly exceeds the inhalation flow rates referenced in the ICRP Publication 66 (International Commission on Radiological Protection 1994) and the U.S. EPA 2011 Exposure Factors

Handbook for sedentary activity defined as sitting and standing and as car driving and riding. However, since the powder application not only involves passive sitting or standing, but also involves the physical activity required to apply a cosmetic powder, we feel that selection of a slightly higher inhalation flow rate is justified. Moreover, in many cases cosmetic products are applied while visiting a public bathroom or a similar place of retreat following light activity (walking), thus resulting in a higher breathing rate than would result from simply standing or sitting.

4.3.5.2. Deposited Dose

We defined the deposited dose, DD_i, as a product of inhaled dose, *ID*, and the deposition fraction, DF_i, integrated over a particle size range, d_p:

$$DD_i = \int_{d_p} DF_i(d_p) ID(d_p) \quad (4),$$

Where *i* represents a particular region of the respiratory system: head airways, tracheobronchial region, alveolar region, or the entire respiratory system. The deposition fraction DF_i is a fraction of inhaled airborne particulate matter that is removed from the air within a particular region or the entire respiratory system. Deposition fractions for different regions of the respiratory system were calculated using equations fitted to the ICRP (International Commission on Radiological Protection 1994) model for monodisperse spheres of standard density at standard conditions (Hinds 1999).The equations were modified to exclude the inhalability fraction *IF* because, as discussed above, we assumed that it already is taken into account due to sampling through the nostrils of the human mannequin head (see Eq. 2). The modified equations for DF_i as a function of particle diameter are:

$$DF_{HA}(d_p) = \left(\frac{1}{1+\exp\left(6.84+1.183\ln d_p\right)} + \frac{1}{1+\exp\left(0.924-1.885\ln d_p\right)} \right) \quad (5),$$

$$DF_{TB}(d_p) = \frac{\left(\frac{0.00352}{d_p} \right)\left[\exp\left(-0.234\left(\ln d_p+3.40\right)^2\right)+63.9\exp\left(-0.819\left(\ln d_p-1.61\right)^2\right)\right]}{1-0.5\left(1-\frac{1}{1+0.00076d_p^{2.8}}\right)} \quad (6),$$

$$DF_{AL}(d_p) = \frac{\left(\frac{0.0155}{d_p} \right)\left[\exp\left(-0.416\left(\ln d_p+2.84\right)^2\right)+19.11\exp\left(-0.482\left(\ln d_p-1.362\right)^2\right)\right]}{1-0.5\left(1-\frac{1}{1+0.00076d_p^{2.8}}\right)} \quad (7),$$

$$DF_{T}(d_p) = \left(0.0587 + \frac{0.911}{1+\exp\left(4.77+1.485\ln d_p\right)} + \frac{0.943}{1+\exp\left(0.508-2.58\ln d_p\right)} \right) \quad (8),$$

Where:

DF_{HA} – deposition fraction for the head airways;

DF_{TB} – deposition fraction for the tracheobronchial region;

DF_{AL} – deposition fraction for the alveolar region;

DF_T –total deposition fraction, equal to the sum of DF_{HA}, DF_{TB}, and DF_{AL}.

Based on our experimental data, d_p corresponded to a midpoint diameter of an SMPS or APS size channel (μm) and the deposited dose in each region of the respiratory system or the total deposition was calculated as a sum of deposited doses for each measurement channel:

$$DD_i = \sum_{d_p} DF_i(d_p) ID(d_p) \quad (9),$$

Thus, the deposited dose was calculated as mass of particulate matter deposited in a given region of the respiratory system per 1-minute cosmetic powder application per 1 kg of body weight.

Additionally, we calculated the deposited dose for each human respiratory system region as percentage of the total deposited dose to better showcase the region of the respiratory tract with the greatest deposition of inhaled particulate matter.

The assumptions used to calculate the deposited dose were the same as discussed above when calculating inhaled dose. For both the inhaled and the deposited dose, we considered particle losses in the sampling lines to be negligible.

4.4. Results

4.4.1. TEM Characterization of Cosmetic Powders

During the TEM characterization of the cosmetic powders, we did not observe the electron beam to affect the integrity of particles in any of the cosmetic powders as was the case with particles in certain spray-type consumer products investigated previously (Nazarenko et al. 2011). This means that the chemical nature of the particles is likely inorganic (Egerton et al. 2004).

We observed Nanopowder M (Figure 2a) to contain only nanoparticles (<50 nm), spheroidal in shape, and in a highly agglomerated state. Nanopowder D (Figure 2b) did not contain any nanoparticles visible using TEM, but seemed to contain only very large irregularly shaped (>5 μm) individual non-agglomerated particles. The majority of particles in Nanopowder K (Figure 2c) were nanoscale along with larger particles (> 3 μm), angular or rod-like, all of which were highly agglomerated.

In the Regular Powder F (Figure 2d), we observed no individual or agglomerated nanoparticles, but there were nanosized electron-contrast inclusions within the larger particles if viewed at higher magnifications. Similar to Nanopowder D, Regular Powder F contained large (>1 μm) and very large (>5 μm) irregularly shaped individual non-agglomerated particles. There were a few small nanosized structures observed in the Regular Powder G (Figure 2e), however, we mostly observed 5 – 10 μm and larger irregularly shaped particles that were either agglomerated or individual. In the Regular Powder E (Figure 2f), we found both agglomerated and individual spherical particles of a very wide range of sizes up to >10 μm, and also many nanoparticles, all of which were attached to the surface of larger particles.

In summary, nanoparticles dominated in two out of three nanopowders (Nanopowder M and Nanopowder K) and constituted a considerable fraction in the Regular Powder E.

4.4.2. Quantitative Exposure Assessment

The inhaled dose, calculated as mass of inhaled particulate matter per kilogram of body weight for 1-minute application of each cosmetic powder is shown in Figure 3. Additionally, we show the inhaled dose calculated for simulated application with a clean kabuki brush, where no powder was used. Here, the particles were produced due to shedding of the brush. Inhaled dose is presented for the five different aerosol size fractions defined above: $PM_{0.1\text{-}0.014}$, $PM_{1\text{-}0.1}$, $PM_{2.5\text{-}1}$, $PM_{10\text{-}2.5}$, and $PM_{20\text{-}10}$.

In the $PM_{0.1\text{-}0.014}$ aerosol size fraction, inhaled particle dose significantly higher than the background was observed only for Nanopowder M (6×10^{-5} ng/kg bw/application) and Regular Powder E (6×10^{-3} ng/kg bw/application). Since the background aerosol concentration was subtracted from each measurement, the values above indicate the presence of particles higher than the background level.

Use of nanopowders D and K and Regular Powder E resulted in the highest inhaled dose of the $PM_{1\text{-}0.1}$ aerosol fraction, close to ~60 ng/kg bw/application for these two nanopowders and about 350 ng/kg bw/application for Regular Powder E. For the remaining products, the inhalation exposure was around 0.1 ng/kg bw/application – about an order of magnitude higher than inhaled dose from the use of a clean kabuki brush.

For nanopowders D and K and Regular Powder G, the inhaled dose in the $PM_{2.5\text{-}1}$ fraction was on the order of 10^3 ng/kg bw/application while for Nanopowder M and Regular Powder F – about an order of magnitude lower (~50 – 100 ng/kg bw/application)

– close to the level for the clean kabuki brush (~25 ng/kg bw/application). Regular Powder E showed the highest inhaled dose for the $PM_{2.5-1}$ fraction: ~1×10^4 ng/kg bw/application.

The highest inhaled dose of the $PM_{10-2.5}$ aerosol size fraction also resulted from the use of Regular Powder E (~3×10^4 ng/kg bw/application). Nanopowders D and K and regular powders F and G showed inhaled dose levels two orders of magnitude lower in the range 200 – 775 ng/kg bw/application while Nanopowder M only produced a relatively low exposure to this aerosol size fraction at 19 ng/kg bw/application, which was close to the level for the clean kabuki brush (11 ng/kg bw/application).

For the supercoarse size fraction (PM_{20-10}), the highest exposure (~2×10^3 ng/kg bw/application) was created by the use of Regular Powder E. This level of inhaled dose was about an order of magnitude higher than for the other two regular powders (F – 322 ng/kg bw/application and G – 437 ng/kg bw/application). For all of the tested nanopowders compared to the regular powders, the simulated application resulted in much lower inhaled doses of the PM_{20-10} aerosol size fraction (in the range 15 – 86 ng/kg bw/application).

The deposited dose for each cosmetic powder as well as for the clean kabuki brush is shown in Figure 4. The dose is expressed as mass of inhaled particulate matter per kilogram of body weight that would deposit in the head airways (HA), the tracheobronchial region (TB), the alveolar region (AL), as well as the total respiratory system deposition during a 1-minute application of cosmetic powders.

As can be seen in Figure 4, the highest deposited mass in all three respiratory system regions resulted from the application of Regular Powder E with the total

deposited dose of 3.2×10^4 ng/kg bw/application, which was 1 – 3 orders of magnitude higher than for the other cosmetic powders. The total deposited dose for Nanopowder M was the lowest of all the products: 37 ng/kg bw/application, only about twice as high as the use of the clean kabuki brush (15 ng/kg bw/application). The other two nanopowders (D and K) produced deposited doses around 400 ng/kg bw/application, and regular powders F and G produced doses of 684 and 1.2×10^3 ng/kg bw/application, respectively.

For all the nano and regular cosmetic powders, the mass deposited in alveolar region was by a factor of 1.5 – 2 higher compared to the tracheobronchial deposition for the same powders. Regular powder E stood out with the highest AL and TB deposited doses (2×10^3 and 1.4×10^3 ng/kg bw/application respectively). Nanopowder M showed very low levels: 2 ng/kg bw/application for AL and 1.2 ng/kg bw/application for TB. The other four cosmetic powders were in-between: the AL ranged from 31 to 61 ng/kg bw/application, the TB – from 19 to 41 ng/kg bw/application.

For all the powders, the distribution of mass deposition in the head airways was similar to that in the two other regions of the respiratory system. The dose deposited in HA due to the use of Regular Powder E was 1 – 3 orders of magnitude higher (2.9×10^4 ng/kg bw/application) compared to nanopowders D and K and regular powders F and G. For the latter four cosmetic powders, the HA deposited dose ranged from 295 to 1.1×10^3 ng/kg bw/application. For Nanopowder M, it was the lowest – ~33 ng/kg bw/application, which was consistent with the generally lower deposited dose from this product in the other two respiratory system regions.

The comparison of deposited dose in different regions of the respiratory system is shown in Figure 5. As could be seen, the dose deposited in the head airways constituted

the dominant portion of the total deposited dose; between 85 and 93% of the total deposition of inhaled particulate matter occurred in the HA region of the human respiratory system.

4.5. Discussion

The most important outcome of this study is that for all of the tested cosmetic powders, the coarse aerosol fraction ($PM_{10-2.5}$ in Figure 3) was responsible for the highest inhaled dose. It is also notable that while the TEM showed a very high abundance of nanoparticles in nanopowders M and K and the Regular Powder E, the inhaled dose of individual nanoparticles and/or nanoagglomerates, represented by the $PM_{0.1-0.014}$ aerosol fraction in Figure 3, was either very low (nanopowders M and E) or insignificant (Regular Powder K) compared to the background. If engineered nanomaterials are added to a cosmetic powder, when the powder is applied, the nanomaterials are unlikely to become dispersed as nanosized airborne particles due to insufficiency of energy needed for deagglomeration (Seekkuarachchi and Kumazawa 2008). Instead, the majority of nanomaterials should be distributed in larger size fractions due to particle agglomeration. Therefore, engineered nanomaterials can be effectively delivered into all regions of the human respiratory system in the form of agglomerates of various sizes. The quantities of these nanomaterials entering the respiratory system would be proportional to the total aerosol mass in each size fraction and the fraction of nanomaterial in it.

Since coarse particles are responsible for the highest fraction of inhaled dose, it came as no surprise that the overwhelming deposition of particulate matter was shown to occur in the head airways (Figures 4 and 5). The alveolar region was the second most exposed region of the respiratory system; however the deposited mass was only ~1/20 of that deposited in the head airways. Deposition levels in the tracheobronchial region were lower than that in the AL by a factor of 1.5-2. Although the absolute deposited dose levels differed from product to product, the above-mentioned proportion of particulate

matter deposition between the HA, TB and AL human respiratory system regions was similar for all tested cosmetic powders.

There is an active debate regarding the best particle metric to use when analyzing nanomaterial exposures: particle number, surface area or mass (Dhawan et al. 2009). Although the surface area and number of nanoparticles deposited in the respiratory system have been shown to correlate well with toxic effects for some nanoaerosols like nanoparticulate quartz, metallic cobalt and nickel, and elemental carbon ^{13}C (Duffin et al. 2002; Oberdörster et al. 2004), this was not the case with many other materials like nanoparticulate TiO_2, carbon black, polystyrene beads, and surface-modified quartz (Duffin et al. 2007; U.S.EPA 2011; Wittmaack 2007). The existing measurement techniques are still limited when it comes to the measurement of number and surface area concentration of agglomerated nanoparticles and nanoparticles in composites with larger particles, which is the case of cosmetic powders. In this study, we chose to use the mass metric because here we deal with a nanomaterial-containing aerosol where nanomaterials are distributed across all aerosol size fractions in the form of agglomerates. Hence, much greater mass of nanomaterials is delivered into the respiratory system in the form of nanomaterial-containing agglomerates and composites compared with nanomaterials in the form of nanosized particles (Nazarenko et al. 2012). The particle number metric would count each individual agglomerate containing multiple nanoparticles as a single particle while the surface area may not be accurately measured for multi-ingredient products where particulate matter is often embedded in a matrix of organic and other components.

Nanotechnology-based consumer products differ from pure nanomaterials because they usually contain many other ingredients. The presence of ingredients other than the nanomaterial component is likely to affect particle agglomeration and therefore plays a major role in determining the distribution of nanomaterials across different size fractions once the product is aerosolized. Consequently, during inhalation exposure, the multi-ingredient composition and agglomeration of particles released from a nanotechnology-based consumer product would lead to a different deposition of nanomaterial(s) and other essential materials across the human respiratory system compared to tests with pure nanomaterials. Nanomaterials may become substantially altered by their inclusion in a product matrix composed of other ingredients, and the aerosol generated during a multi-ingredient nanoproduct's use may be substantially different from the aerosol generated from a pure nanomaterial composed of the same primary nanoparticles. Hence, we suggest that the exposure and toxicology studies of pure nanomaterials should be conducted in parallel to similar studies of actual products and exposures that use nanomaterials. This parallel approach will provide the relevant data, and conclusions can be drawn about the exposure and potential health effects resulting from the use of nanotechnology-based consumer products.

Our investigation with the TEM showed that two out of the three tested nanopowders – Nanopowder M and Nanopowder K – contained exclusively (in Nanopowder M) or predominantly (in Nanopowder K) nanosized particulate matter. Regular Powder E contained a high number of nanoparticles along with larger particles. This observation indicates that when particles from these products are aerosolized during product use, there can be exposure to actual nanomaterials. At the same time, however,

the third nanopowder (D) did not contain any nanoparticles that could be detected using TEM and particles below 100 nm were virtually not detected in the air (Fig .3). These findings illustrate that manufacturers' claims regarding the inclusion of nanomaterials in their products need to be verified. Due to the current absence of any regulations mandating the reporting of nanotechnology-based ingredients in cosmetics or other consumer product types, a manufacturer's statement about the nano status of their product is not a guarantee that the product, marketed or not marketed as nanotechnology-based, contains engineered nanomaterial(s) (Hansen et al. 2008). Therefore, conducting specific analyses to detect and characterize nanomaterials in such products is essential to estimate potential of exposure to nanoparticles from such products, as well as, the exposure to agglomerates during use.

Regular Powder F presented a special case where TEM showed no separate nanoparticles, but nanosized inclusions within the larger particles were noticed. We think that in this case, the exposure and risk of nanoparticle-related effects would be minimal if nanoparticles were not released from the larger particles. However, disintegration of such larger particles and the potential release of nanoparticles from them *in vivo* cannot be completely ruled out and such a phenomenon should be a subject of future investigations.

4.6. Conclusions

We found that the levels of inhalation exposure to particulate matter associated with different aerosol size fractions varied substantially depending on the product used. Mass-based inhalation exposure to individual nanoparticles or their agglomerates smaller than 100 nm was found to be minimal compared to the inhalation exposure to larger particles. The highest mass of inhaled particles was found in the coarse aerosol fraction ($PM_{10-2.5}$) for all products. Since electron microscopy showed presence of nanosized particles in nanopowders M and K and Regular Powder E, it is likely that particles in the entire investigated aerosol size range contained nanoparticle agglomerates or nanoparticles attached to other particles.

Our data show that the vast bulk of inhaled cosmetic powders by mass, including particles containing nanomaterials, would deposit in the head airways (more than 80%), while less than 10% of deposition would occur in the alveolar region. It is, therefore, necessary to reconsider the current research overemphasis on the alveolar region for the study of nanomaterial effects. Instead, efforts must be directed to investigate those regions of the human respiratory system where majority of nanomaterial deposition during the actual product use would occur.

The methodological approach used in this study emphasizes realistic simulation of product application to determine inhalation exposures. It can serve as a model for future quantitative inhalation exposure assessments. Such assessments will be required to obtain quantitative exposure data for a wide variety of nanotechnology-based consumer products and are necessary for the ongoing development of safety guidelines and potential regulations.

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Table 4.1. Investigated Cosmetic Powders

Product	**Purpose[2]**	**Composition[2]**
Nanopowder M[1]	Moisturizer	Water, Butylene glycol, Sodium ascorbyl phosphate, Glycerin, Betain, Silica, Dimethicone, Citric acid, Polymethyl metacrylate, Squalane, Sodium hydroxide, Sodium metabisulfite, Capryloyl glycine, Sodium Hyaluronate, Marus Alba root extract, Rosmarinus Officinalis (Rosemary) leaf extract, olea europaea (Olive) leaf exctract
Nanopowder D[1]	Blusher	Mica, Talc, Dimethicone/Vinyl Dimethicone crosspolymer, Hydrogenated C6-14 Olefin polymers, Petrolatum, Dimethicone, Polysilicone-2, Aluminum stearate, HDI/Trimethylol Hexyllactone crosspolymer, Sorbitan sesquisostearate, Aluminum hydroxide, Methicone, Tocopherol, Silica, Triisostearin, Trimethylolpropane trioctanoate, Ethylparaben, Butylparaben, Parfum, CI 77492, CI 77947, CI 77891, CI 77491, CI 77499
Nanopowder K[1]	Sunscreen	Active Ingredients: Titanium dioxide – 25%, Zinc Oxide – 20%
Powder F	Blot Powder	Dimethicone, Silica, Kaolin, Water, Hydrolyzed Soy Protein, Caprylyl glycol, Hexylene glycol, Methicone, Coconut acid, Phenoxyethanol, +/- Mica, Iron oxides (CI 77491, CI 77492, CI 77499), ILN31255
Powder G	Blot Powder	Talc, C12-15 Alkyl Benzoate, Kaolin, Silica Silylate, +/- Mica, Iron oxides (CI 77491, CI 77492, CI 77499)
Powder E	Cosmetic Powder	Silica

[1]Nanoproduct as per the Woodrow Wilson Nanotechnology Consumer Products Inventory
[2]As per manufacturer

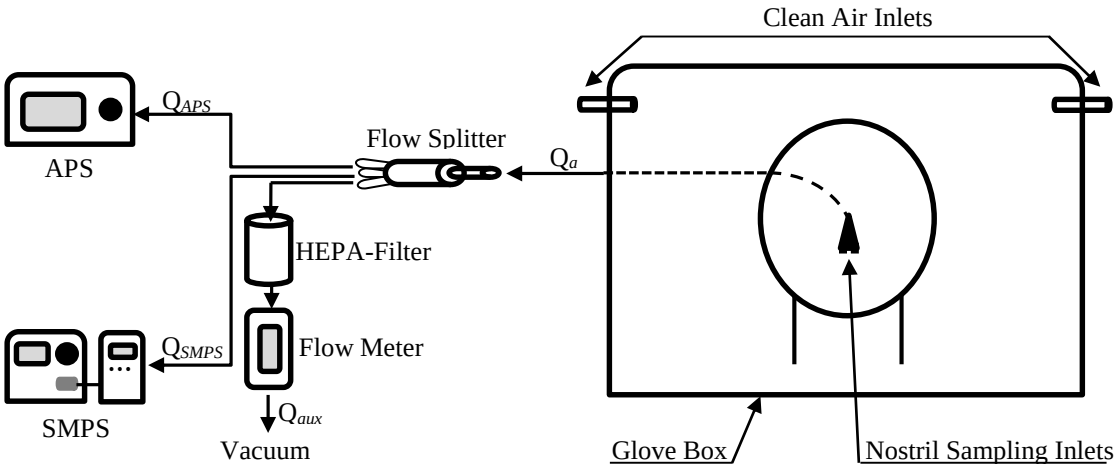

Figure. 4.1. Setup for exposure measurement of airborne particulate matter resulting from simulated cosmetic powder application.

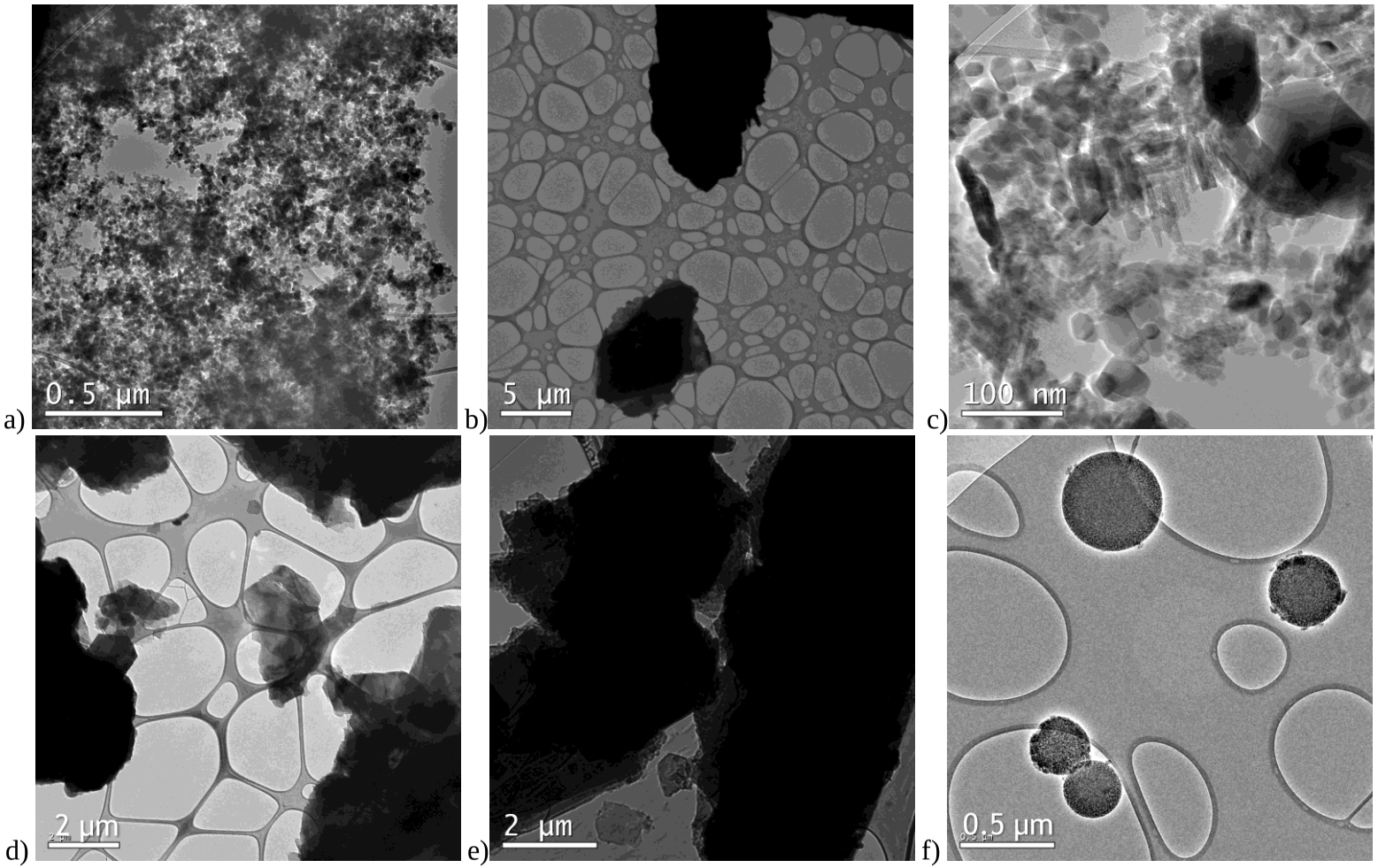

Figure 4.2. Transmission electron micrographs of the tested cosmetic nano- and regular powders: a) Nanopowder M (0.5µm scale bar), b) Nanopowder D (5µm scale bar), c) Nanopowder K (100 nm scale bar), d) Regular Powder F (2µm scale bar), e) Regular Powder G (2µm scale bar), f) Regular Powder E (0.5µm scale bar).

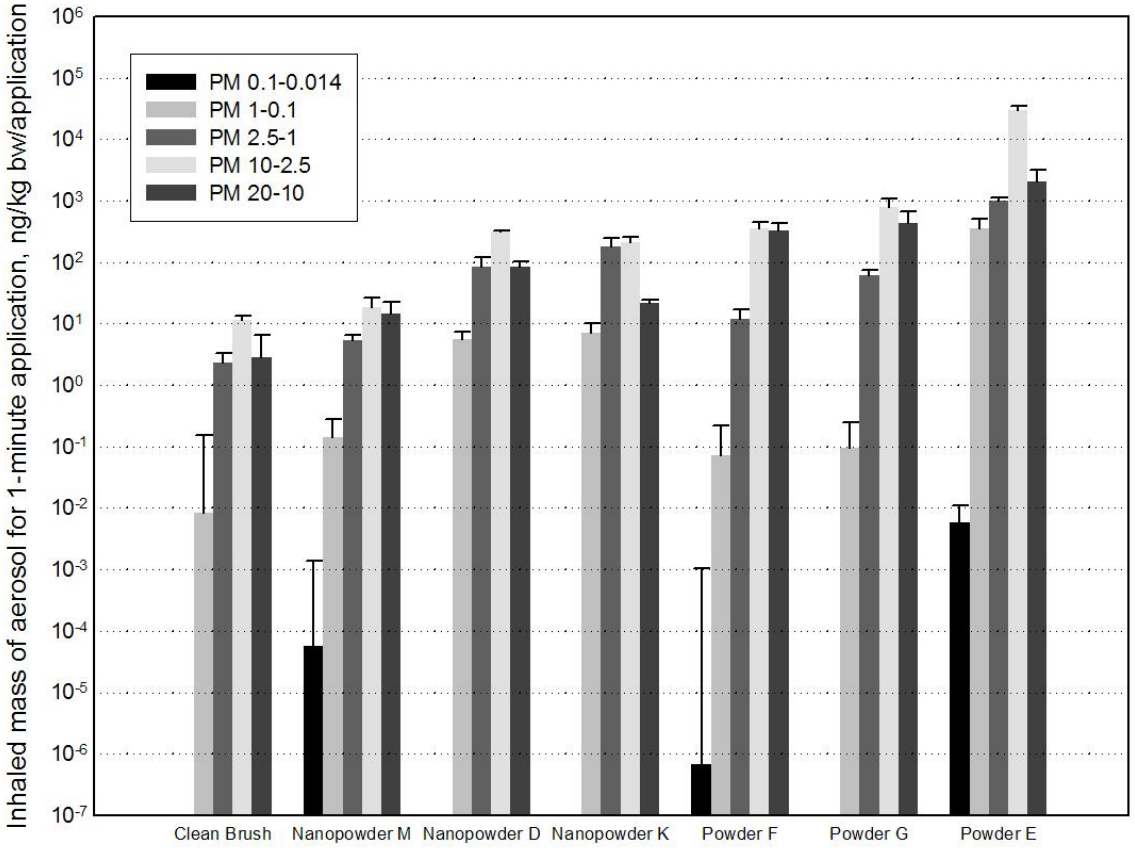

Figure 4.3. Inhaled dose of particulate matter during the use of cosmetic powders. Based on mass concentration of particulate matter in different aerosol particle size fractions as sampled with the mannequin head sampler during simulated product application. The data represent averages of three repeats. The error bars represent one standard deviation.

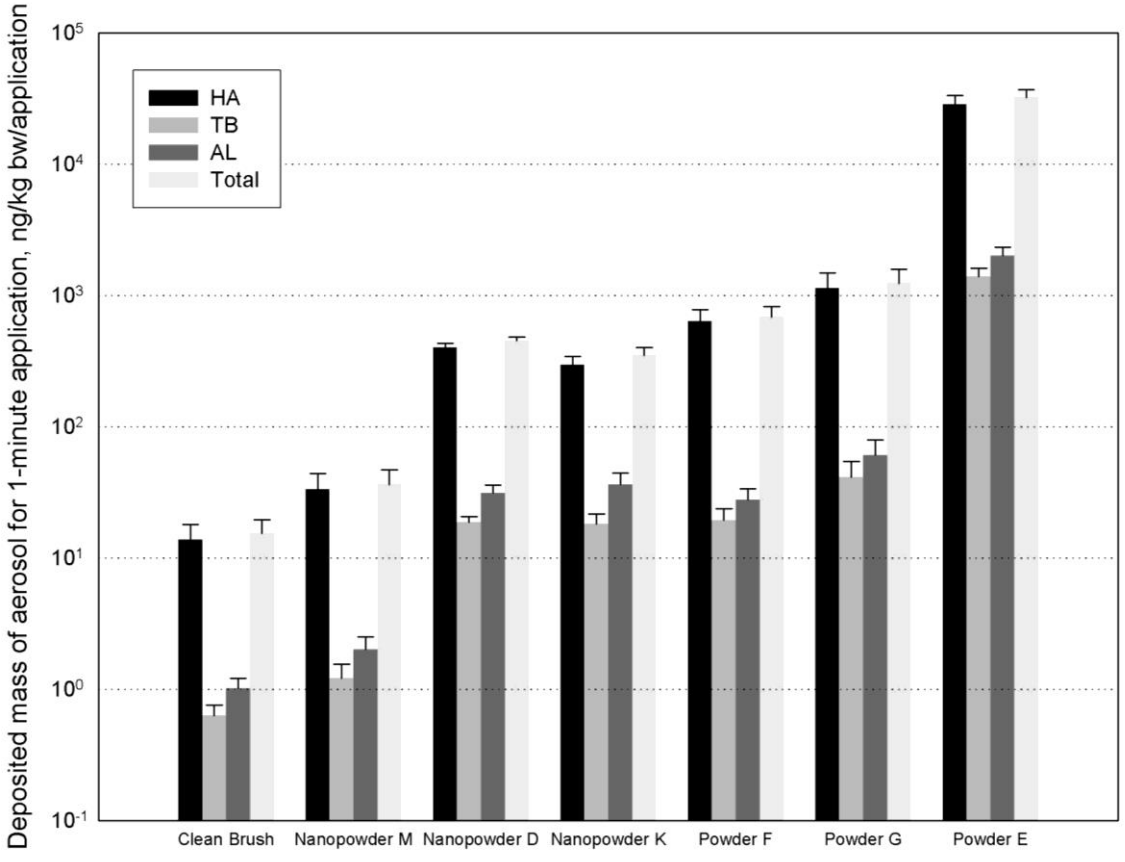

Figure 4.4. Dose of particulate matter deposited in different regions of the respiratory system during simulated application of cosmetic powders. Deposited mass was calculated for the head airways (HA), the tracheobronchial (TB), the alveolar (AL) regions, and the total respiratory system deposition (Total). The data represent averages of three repeats. The error bars represent one standard deviation.

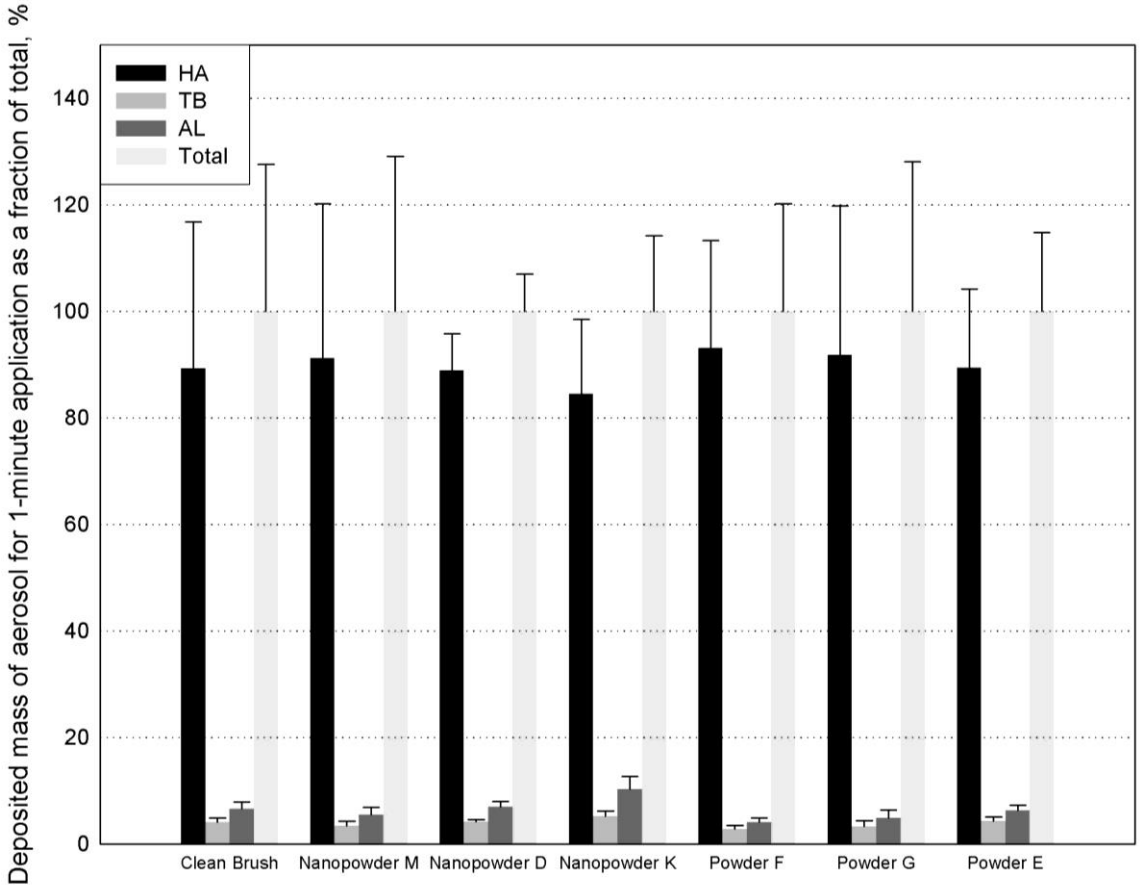

Figure 4.5. Percent distribution of particulate matter deposited in different regions of the respiratory system during simulated application of cosmetic powders. Percent deposition was calculated for the head airways (HA), the tracheobronchial (TB), and the alveolar (AL) regions. The total deposition represents the sum from the three regions. The data represent averages of three repeats. The error bars represent one standard deviation.

Chapter 5

Summary, Potential Impact and Implications for Future Research

5.1. Summary

5.1.1. Principal Conclusions and Outcomes of the Study

Nanomaterials in the form of nanoparticles and their agglomerates may be released as aerosol from certain nanotechnology-based products during use by consumers. Such aerosols span an airborne particle size range from nanoparticles (<100 nm) to supercoarse particles as large as 20 μm (size limit of the used instrumentation). Nanomaterials in an agglomerated state are expected to be present in all size fractions of this aerosol leading to inhalation exposure and nanomaterial deposition in all regions of the human respiratory system with possible health consequences.

In the case of cosmetic powders, it was shown that the highest particulate matter deposition by mass was found in the head airways followed by the alveolar and the tracheobronchial regions of the human respiratory system. A similar situation is predicted for consumer sprays as well based on the aerosol particle size distributions, measured during their simulated use. The obtained quantitative inhalation exposure data can be correlated with toxic effects of nanomaterials in future research and serve as a model for future quantitative inhalation exposure assessments of nanotechnology-based consumer products. The deposition of particulate matter that can contain nanomaterials when they are present in the original product predominantly in the head airways points to the

necessity to reconsider the current almost exclusive research emphasis on the study of nanomaterial effects in the alveolar region only.

According to our data, nanoparticles and their agglomerates may also be present in aerosols resulting from consumer products that are not claimed as nanotechnology-based by the manufacturers.

5.1.2. Research Novelty of this Dissertation

The essence and novelty of the methodology developed for inhalation exposure assessment from nanomaterial-containing consumer products, specifically consumer sprays and cosmetic powders, lies in (1) the characterization of nanomaterial(s) in a consumer product itself and (2) the realistic simulation of product use and measurement of resulting potential inhalation exposure.

Contrary to previously existing speculative information about the likely risk of nanomaterial exposure from nanomaterial-containing consumer products, this project provided concrete quantitative data about the concentrations of nanomaterial-containing airborne particulate matter that could be inhaled during product use. Moreover, for the cosmetic powders, the inhalation and deposition exposure was quantitatively assessed for a realistic consumer profile and simulated exposure scenario.

The experimental approach developed is suggested as a model approach for future extensive exposure assessment studies for nanotechnology-based consumer products including for the development of standardized procedures, which may be required should such consumer products become regulated.

To the best of our knowledge, in the only exposure study of nanotechnology-based consumer products preceding this dissertation project (Hansen et al. 2008),

researchers performed a quantitative exposure assessment for a hypothetical spray product for indoor surface treatment. In that case, both dermal and inhalation exposures were taken into account. Hansen's inhalation exposure assessment required numerous assumptions including 1) concentration of active substance in a product as well as in the breathing zone following spraying a given amount of the product and 2) characteristics of the inhalation process. In this dissertation project, actual application of real world products was realistically simulated and the inhaled aerosol measured directly. We demonstrated the possibility of and characterized inhalation exposure. Therefore, we add substantially to where Hansen et al. (2008) started with inhalation exposure analysis.

5.1.3. Summary of Results

Certain manufacturers market their consumer products as "nano", "nano-enhanced", "nano-enabled", "nanotechnology-based", or list certain nanomaterials among ingredients in their products. In this study, we considered these products as nanotechnology-based. However, we also introduced the term "*de facto* nanotechnology-based products" to identify those products that indeed contain nanomaterials no matter if they are marketed or labeled as nanotechnology-based or not. Materials dispersed at nanoscale have indeed been found in some consumer products, marketed as nanotechnology-based, in this as well as other studies (McCall 2011). At the same time, our investigations found that some consumer products marketed as nanotechnology-based did not contain any matter dispersed at the nanoscale that could be detected using employed analytical techniques, i.e., transmission electron microscopy, photon correlation spectroscopy and laser diffraction spectrometry. On the other hand, some regular products were revealed to contain matter dispersed at the nanoscale. Thus, we

exposed the inconsistency with respect to manufacturers' mentioning the "nano-status" of their products in advertising and marketing and the *de facto* "nano-status" of products that exist in the absence of regulations mandating accurate reporting of this information, particularly for the two investigated categories of consumer products: consumer sprays and cosmetic powders.

To explore the possible extent of the above-mentioned problem with correct identification of "nano-status" of consumer products, characterization of materials' dispersion states at nanoscale in consumer products was performed for both products marketed as nanotechnology-based and those without any associated information from products' manufacturers on possible nanomaterial content (regular products).

In the case of consumer spray products, electron microscopy detected the presence of nanoparticles in some nanotechnology-based sprays as well as in several regular products, while the photon correlation spectroscopy indicated presence of particles <100 nm in all investigated products. During the use of most nanotechnology-based and regular sprays, particles ranging from 14 nm to 20 μm were released indicating that they could he inhaled and consequently deposit in all regions of the human respiratory system. Thus it was shown that exposures to nanoparticles as well as micrometer-sized particles can be encountered due to the use of nanotechnology-based sprays as well as regular spray products.

In the case of cosmetic powders, we also found the potential for inhalation exposure to particles in the entire investigated size range (14 nm - 20 μm) from both nanotechnology-based and "regular" products. The mode diameters of airborne particles from several products were below 100 nm.

We also found that even the products producing relatively low particle concentrations above 1 µm, still produced high nanoaerosol concentrations (14-100 nm) comparable to the rest of the products. Therefore, we showed that the use of nanotechnology-based cosmetic powders would result in inhalation exposures to single nanoparticles and their agglomerates.

When quantifying inhalation exposure from cosmetic powders, we described the mass of inhaled aerosol particulate matter that enters the human respiratory system as "inhaled dose" and used the term "deposited dose" for the mass of inhaled particulate matter that actually deposits in a given region of the human respiratory system: the head airways, the tracheobronchial region and the alveolar region. Both the "inhaled dose" and the "deposited dose" describe inhalation exposure; however, presenting the data in terms of how much material deposits in different regions of the human respiratory system is more useful for health studies. Accordingly, one of the most important conclusions of the study came from the finding that the dominant portion of the total deposited dose is the deposited dose for the head airways. The deposition in the head airways is overwhelming compared with particulate matter that would deposit in the other two regions of the respiratory system – the tracheobronchial and the alveolar regions, so that between 85 and 93% of the total deposition of particulate matter inhaled as a result of cosmetic powder exposure occurs in the head airways region of the human respiratory system. Based on the aerosol particle size distributions measured during the consumer spray exposure simulation, we can predict a similar respiratory system deposition situation for that case as well. Based on this finding, we think it may be essential to reexamine the current almost exclusive research emphasis on the study of nanomaterial effects in the

alveolar region where predominantly the smallest particles deposit. Because the distribution of nanomaterial deposition in different regions of the respiratory system is affected by the mode of administration of nanomaterial or their preparations or nanotechnology-based consumer products, a choice of, e.g., a whole-body inhalation exposure vs. intratracheal or an intranasal instillation will likely play a great role in the toxicological study outcomes. The conclusions about potential health effects stemming from the studies using such different exposure approaches need to be interpreted with consideration for the mode of nanomaterial's or nanoproduct's administration and/or exposure.

5.1.4. Other Routes of Exposure to Nanomaterials from Consumer Products

Although we investigated only inhalation exposure in this dissertation project, other types of exposure in addition to inhalation exposure may play substantial roles in the development of health effects as a result of nanotechnology-based consumer product use. The kind of nanotechnology-based consumer product is the most important parameter determining the extent and contribution of each type of exposure including the major three routes: inhalation, dermal and ingestion. In many cases, exposure through several of these routes is likely. For example, if a nanotechnology-based cosmetic powder is applied to face, it will result in exposure through all three above-mentioned routes with ingestion possibly happening as a result of direct product application around the mouth, landing of aerosol particles in the mouth, and ingestion of inhaled particles. Dermal exposure will happen through direct contact with a product being applied to the skin. Inhalation exposure will be the result of breathing in particles generated by the

mechanical action of the product's applicator on the bulk product as well as on the product already in/on the applicator and the skin.

As with inhalation exposure, accurate nanomaterial dermal exposure assessment will require information from the manufacturer on the exact concentration of nanomaterial(s) in a product. We were unable to verify or experimentally determine nanomaterial(s) concentration in the investigated products and this will likely remain a problem in other similar investigations.

5.1.5. Implications for Future Choice of Analytical Techniques

Investigation of release and exposure to nanomaterials from consumer products requires continued development of novel experimental techniques including those that can apply existing measurement approaches to nanomaterial analysis as well as realistic exposure simulation. Advancement of safety research looking at nanotechnology-based consumer products will be impossible without continued improvement of sampling and analytical techniques. Particularly, better differentiating measurements should become available to analyze: 1) particles that are composed of different materials, 2) particles that are monolithic *versus* aggregates/agglomerates of smaller particles, 3) particles that have varying specific densities and shapes, and 4) particles dispersed in various liquid media. The measurements in various media should provide data that could be correlated with aerosol measurements.

This research project demonstrated the usefulness as well as exposed the challenges presented to state-of-the-art measurement techniques including: 1) ultrafine (<100 nm) aerosol analysis using a scanning mobility particle sizer (SMPS) and a time-of-flight aerosol particle sizer (APS) for measuring particles larger than about 0.5 μm, 2)

transmission electron microscopy (TEM), 3) photon correlation spectroscopy (PCS), and 4) laser diffraction spectrometry (LDS).

Generally, our application of the above-mentioned analytical techniques was very successful. SMPS and APS were able to effectively measure airborne particle size distributions in aerosols generated using standard nebulizers (C-Flow and Collison) as well as during simulated product use. With the help of TEM, we described very well the primary electron-contrast nanoparticles including their shape and certain physico-chemical properties based, for example, on their subjection to radiolysis. With PCS and LDS, we obtained informative particle size distributions in the original liquid and powder products.

5.1.5.1. Challenges to the Employed Analytical Techniques

There were challenges for the analytical techniques used to analyze consumer products. Particularly, SMPS, APS, PCS, and LDS could not distinguish between monolith particles and tightly fused aggregates and loose agglomerates that can easily break up under mechanical action or dissociate after deposition *in vivo*. SMPS can take up to several minutes to measure one particle size distribution. This requires an effort to stabilize aerosol generation during this time, which was especially challenging during simulated product application. The various specific densities and shapes of particles as well as their charge properties affect the reliability of SMPS and APS measurements as well as their agreement in the overlapping region (around 0.6 – 0.7 μm).

Certain materials including many organics are partially or completely invisible to TEM due to high electron transparency. Therefore, TEM is not able to analyze equally well the particles made of different materials that have a different degree of electron

contrast. Many materials undergo radiolysis, i.e., physically and/or chemically change under the action of the energetic electron beam. In some cases, the effect of radiolysis leads to fusion of otherwise loosely agglomerated particles, which can wrongly be interpreted leading to an incorrect conclusion about low likelihood of particle break-up and/or dissociation after deposition *in vivo*.

Lastly, PCS and LDS rely on autocorrelation functions using pre-set physical parameters such as particle refractive index. The current state of these analytical techniques and their instrumental implementations do not provide for an opportunity to reliably analyze mixtures of multiple materials that have different physical characteristics. These methods are also challenged by multimodal particle size distributions and very small particles. Additionally, PCS is sensitive to the nature of the solvent and does not allow for accurate analysis in the situations of complex solvent mixtures, which is often the case with liquid nanotechnology-based consumer products. Presence of very large particles distorts the autocorrelation function used in the PCS (Bodycomb 2009), thus reducing accuracy or making it impossible to measure certain colloidal solutions/suspensions. We can summarize that these challenges are mostly due to the multi-component nature of most consumer products while the above-mentioned measurement techniques are best suited for pure single-component materials. In this dissertation project, we considered all the mentioned specificities of the measurement techniques and interpreted the data with consideration for their deficiencies.

5.1.5.2. Analytical Techniques Recommended for Future Work

The other analytical methods that we can recommend for future work on characterization of nanotechnology-based consumer products are 1) Scanning Electron Microscopy (SEM), 2) Energy Dispersive Spectroscopy (EDS), 3) X-Ray Diffraction (XRD) and 4) Aerosol Mass Spectrometry (AMS) for particle elemental analysis.

While TEM effectively provides a two-dimensional image of the particles and agglomerates, the SEM provides for gaining a much better insight regarding the spatial or three-dimensional configuration of the particles and agglomerates. As we reveal nanoparticle-containing agglomerates to be of very high importance in the context of human exposure from nanotechnology-based consumer products, improved characterization of particle and agglomerate structure is particularly valuable.

EDS allows analyzing the atomic composition of the individual particles providing quantitative data as proportional abundance of various elements in each particle, which can provide insight about chemical identity of particles.

XRD analyzes the crystalline structure of particles, which allows identification of certain chemical compounds and/or mineral types (crystal structures) of the same compounds, e.g., rutile or anatase forms of titanium dioxide (TiO_2). Of course, not all materials can be analyzed by the latter two methods as, for instance, low electron contrast particles may not even be visible and the particles consisting of materials subject to radiolysis under the energetic electron beam will decompose or physico-chemically change before analysis can be finished. Naturally, amorphous particles that have no particular crystalline structure cannot be analyzed by XRD. Finally, the complex physico-chemical particle composition, i.e., particles consisting of more than one chemical

compounds and/or different crystalline structures of the same chemical compounds, is a challenge for both EDS and XRD. Notably, this kind of particle is not unlikely to be found in consumer products that are most often compositions of multiple materials. At the same time, EDS and XRD could be suitable and very useful for identification of certain simple inorganic nanomaterials that are very widespread in the nanotechnology-based consumer products currently on the market, e.g., metallic (mostly Ag), single-element (various forms of carbon including carbon nanotubes) and metal oxide (ZnO, TiO_2, SiO_2) nanoparticles.

Analysis by any type of electron microscopy, e.g., TEM and SEM, and the related methods, e.g., EDS and XRD, requires prior preparation or collection of material(s), e.g., product samples or samples of aerosols generated using the products. This preparation or collection has to be representative of the original product or aerosol formed as a result of its use, which presents a challenge on its own. Employing thermophoretic and electrostatic collectors with known collection efficiencies by particle size will decrease the effect of non-representative sampling. Although, these collectors may be challenged in terms of accounting for varying collection efficiency for different particle materials (Han et al. 2011; Sillanpää et al. 2008; Wen and Wexler 2007), so further work is needed to improve representativeness of sampling for varying particle types expected to be found in aerosols generated from application of most nanotechnology-based consumer products. In addition, in the thermophoretic collection method, exposure of aerosol particles to a high temperature of the hot plate may vaporize volatile or semi-volatile particles or chemically alter particle composition. The low temperature of the cold place may lead water to condense on the cold TEM grid (Wen and Wexler 2007).

AMS allows for a near real-time chemical analysis of particles simultaneously with their size characterization. Thus, chemical identities of individual particles can be determined. However, among the deficiencies of AMS in the context of nanomaterial-containing aerosol analysis is, as with SMPS and APS, the inability to distinguish between agglomerates of particles and equally sized monolithic particles. In other words, AMS will not analyze each nanoparticle in an agglomerate separately, but will produce an averaged result from analysis of all primary particles in it at once. Therefore, it will be a challenge to resolve the chemical composition data for primary nanoparticles if they are agglomerated, which is a likely case with consumer products. And of course, in the case of complex larger agglomerates where, for example, nanoparticles are attached to a larger monolithic particle, the challenge of interpreting the particle size-resolved chemical composition data provided by AMS will be even greater.

In addition, no universal inlets exist for the AMS instruments that would allow simultaneous sampling and analysis of particles across the entire particle size spectrum from small nanoparticles up to supercoarse particles. Different existing inlets only allow sampling in the range from 40 nm to about 1 μm, but at least one company is currently working on developing new inlets to transmit smaller and larger particle size ranges (Aerodyne Research Inc. 2012).

In summary, the plethora of existing analytical techniques that should be tried for investigations of nanotechnology-based consumer products is considerable. The goal here is to ultimately develop methodology that achieves a fully quantitative physico-chemical characterization of these products that is particle size-resolved, i.e., physico-chemical characterization of each particle size fraction at the background of the detailed

characterization of particle agglomeration. In the future, one can expect the advancement of the analytical techniques to the point that such characterization will ideally include three-dimensional chemical mapping of each particle, i.e., a three-dimensional atomic/molecular particle structure.

5.2. Potential Impact

We believe the research described in this thesis provides a substantial contribution toward general understanding of some of the potential effects of nanomaterial introduction into consumer products. In particular, the reported research has shown: (1) the potential for inhalation exposure to nanomaterials when such products are used and (2) pointed to the area of contact with the highest mass of inhaled material – the head airways.

We see our findings guiding the research community to better design health effects studies to understanding the effects that use of nanomaterials in consumer products may have on human health. This in turn contributes to a better scientific basis for legislators to debate regarding possible regulations of nanomaterials in consumer products. In addition, the findings of this project are of utility for the current and future nanotechnology-based consumer product manufacturers. One example is our finding of the substantial effect of a product's consistency (e.g., stickiness of a powder) and the spraying technique on the aerosol particle size distribution, which affects how much material can be inhaled and where it would deposit in the human respiratory system. Therefore, the manufacturers of nanotechnology-based consumer products could design product sprayers and applicators as well as product formulas in such a way as to minimize generation of unwanted aerosol during product application and disperse aerosol

with such a particle size distribution that will minimize inhalation exposure of consumers to nanomaterials in these products.

5.3. Implications for Future Research

The choice of analysis techniques for in-product nanomaterial characterization should be made based on two criteria: (1) the physical form of the consumer product, e.g., liquid or powder, and (2) the physico-chemical nature of nanomaterial(s) possibly present in the product as well as the physico-chemical nature of the non-nanotechnology-based components including both inorganic and organic ingredients, called the product's matrix. For liquid products, this also includes the solvent mixture. Sometimes information about a given product's contents can be obtained from the label or documentation supplied with the product. It can also occasionally be obtained from the manufacturer. However, in the absence of regulations that would mandate accurate manufacturers' reports on the contents of many categories of consumer products, work to resolve the problem of missing or inaccurate ingredient information should continue in two directions: (1) development of better analytical techniques for nanomaterial detection and analysis in complex mixtures such as those that are multicomponent consumer products, as well as (2) development of legislation requiring manufacturers to accurately and quantitatively report certain engineered nanomaterial ingredients in their products, so the researchers can quantify exposure, potential risk if any, and potential health and environmental effects. If the latter is not possible in full, the problem could be mitigated by creating regulation with a dynamic and constantly updated list of nanomaterial ingredients, content of which must be quantitatively reported by consumer product manufacturers.

Together with results of current and future pure nanomaterial toxicity studies, our quantitative nanomaterial exposure data have the potential to provide a more comprehensive idea about potential health effects of extensive public use of nanotechnology-based consumer products. The methodology for realistic exposure simulation combined with in-product nanomaterial characterization can serve as a model for future extensive studies of a much wider spectrum of various categories of nanotechnology-based consumer products. Such future studies will address the specific environmental and human health issues arising from production of nanomaterials, their introduction into consumer products and subsequent transfer of nanomaterials into waste streams. The current speculations within both the research and the socio-political communities regarding potential public health and environmental consequences of nanomaterial use will be replaced by decision-making based on quantitative studies offering concrete data for benefits vs. hazard considerations based on novel methodology for nanoparticle analysis and exposure assessment.

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Curriculum Vitae

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Publications

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