OCCUPATIONAL DERMAL EXPOSURE TO NANOPARTICLES AND NANO-ENABLED PRODUCTS: Part I - Factors affecting skin absorption

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Abstract

The paper reviews and critically assesses the evidence on the relevance of various skin uptake pathways for engineered nanoparticles, nano-objects, their agglomerates and aggregates (NOAA). It focuses especially in occupational settings, in the context of nanotoxicology, risk assessment, occupational medicine, medical/epidemiological surveillance efforts, and the development of relevant exposure assessment strategies.

Skin uptake of nanoparticles is presented in the context of local and systemic health effects, especially contact dermatitis, skin barrier integrity, physico-chemical properties of NOAA,

and predisposing risk factors, such as stratum corneum disruption due to occupational co-exposure to chemicals, and the presence of occupational skin diseases. Attention should be given to: 1) Metal NOAA, since the potential release of ions may induce local skin effects (e.g. irritation and contact dermatitis) and absorption of toxic or sensitizing metals; 2) NOAA with metal catalytic residue, since potential release of ions may also induce local skin effects and absorption of toxic metals; 3) rigid NOAA smaller than 45 nm that can penetrate and permeate the skin; 4) non rigid or flexible NOAA, where due to their flexibility liposomes and micelles can penetrate and permeate the intact skin; 5) impaired skin condition of exposed workers.

Furthermore, we outline possible situations where health surveillance could be appropriate where there is NOAA occupational skin exposures, e.g. when working with nanoparticles made of sensitizer metals, NOAA containing sensitizer impurities, and/or in occupations with a high prevalence of disrupted skin barrier integrity. The paper furthermore recommends a stepwise approach to evaluate risk related to NOAA to be applied in occupational exposure and risk assessment, and discusses implications related to health surveillance, labelling, and risk communication.

Introduction

The potential for nanoparticles, nano-objects, their agglomerates and aggregates, (NOAA, defined as having at least one dimension <100nm) to enter the body through intact skin has been a controversial issue, with some authors asserting that nanoparticles can pass through the stratum corneum, while others disputing this conclusion (Oberdoster et al., 2005, SCCP 2007; Crosera et al., 2009, Labouta and Schneider 2013, Larese Filon et al., 2015),.

The skin is a complex organ system comprising the epidermis and dermis, with hair follicles and sweat glands providing pathways across these layers, and peripheral blood flowing into the dermis. The epidermis mainly comprises keratinocytes that migrate from the basal layer towards the skin surface forming the outer protective layer (stratum corneum). The intact stratum corneum provides an effective barrier against bacteria, viruses and most exogenous chemicals. However, the barrier is not completely impervious and it is possible for relatively small molecules, and in theory very small particles, to diffuse across the stratum corneum via cellular and/or inter-cellular pathways. If the barrier is damaged (disrupted) then permeation may be enhanced.

The focus of this work is to review and critically assess the evidence on the relevance and relative significance of various skin uptake pathways for NOAA, especially in occupational settings, in the context of nanotoxicology, risk assessment, occupational medicine, medical and epidemiological surveillance efforts, and in development of relevant exposure assessment strategies. Skin uptake of nanoparticles is presented in the context of local and systemic health effects, especially contact dermatitis, skin barrier integrity, physico-chemical properties of NOAA, and predisposing risk factors, such as stratum corneum disruption due to work, co- chemical exposures, and presence of occupational skin diseases. In the accompanying paper by Brouwer et al., (2016) these findings are integrated in an approach for evaluating occupational dermal exposure to nanoparticles. Dermal exposure is approached both conceptually and from the perspective of evidence for exposure, by linking the use of NOAA and nano-enabled products in industrial sectors to job titles. In addition, we flagged specific job titles where there is often a high incidence rate of skin barrier disruption and skin disease. We conclude with recommendations for occupational health practitioners and risk assessors.

In this paper, the term nanoparticle includes both engineered and incidental nanoparticles, as well as their agglomerates and aggregates (ISO, 2011). Nanoparticles embedded in nano-enabled products, such as pastes, paints, glues, etc., are potential sources of dermal exposure to nanoparticles (Aitken et al., 2004, 2006). The term NOAA (nano-objects, and their aggregates and agglomerates) is used throughout the paper to refer inclusively to such nanoparticles. The terms penetration and permeation are used throughout the paper to mean that NOAA can reach the skin layers and pass through the skin respectively.

Methods

Literature review: An extensive literature search was conducted in major databases, including Pubmed, Thompson Reuters Web of Science (ISI), and Google Scholar using search terms ‘‘skin absorption nanoparticles’’ or ‘‘skin penetration nanoparticles’’ or “skin exposure nanoparticles”,, “sensitizer and nanoparticles”, “engineered nanoparticles and skin” and similar terms. The period taken into consideration was from 1999 to 31st-12-2015. A total of 810 papers were selected and 132 analysed. The skin absorption data were presented in detail an earlier paper by the authors (Larese et al., 2015) and are summarized here for completeness.

The search for available studies on contact dermatitis in workers was performed on the same database using the term “occupational contact dermatitis” and epidemiology, “irritant contact dermatitis” and epidemiology. A total of 176 papers were selected and 127 were analyzed. Additional searches on these same databases and internal databases available at co-authors’ institutions were performed for occupational skin disorders and occupational disease burden by industry sectors. Additional relevant information not available in the peer-reviewed literature (such as reports, white papers, personal communications) from authors’ bibliographies were also analysed.

The abstracts of all studies were reviewed and only papers that were deemed relevant to the current objectives were analysed in detail. 132 and 127 papers were included in the final analysis.

Summary data on physico-chemical (PC) properties of NOAA and impurities. Certain metals (e.g. nickel Ni) are known to cause allergic contact dermatitis and such metals can be found as engineered nanomaterials, or as impurities in NOAA (Bello et al., 2009). For this reason, we conducted a detailed analysis for metals in NOAA. In generating summary data on PC properties of NOAA and their impurities, authors conducted summary statistical analysis using a large dataset of their own ENM (Hsieh et al,. 2013). Some data on PC properties of subclasses of NOAA have been presented in earlier work in the context of exploring links between PC properties and biological oxidative damage, in vitro nanotoxicology, and exposure assessment (Bello et al., 2009; Hsieh et al., 2013). The summary analysis across all available NOAA is new, and utilizes in part a substantial subset of unpublished PC data. The methods for chemical analysis of metals (total and water soluble), organic and elemental carbon, and polycyclic aromatic hydrocarbon (PAHs) have been presented elsewhere (Bello et al., 2009) and includes sector field inductively coupled plasma mass spectrometry (SF ICP-

MS), thermogravimetric analysis for carbon speciation into organic and elemental (OC/EC), and gas chromatography mass spectrometry GC-MS for PAHs.

RESULTS

Penetration of NOAA through the skin

NOAAs on the skin may penetrate stratum corneum reaching viable epidermis using different pathways, namely: a) via sweat glands and hair follicles (Lademann et al., 2009), which are probably the most efficient way for penetration and permeation of large molecules and nanoparticles; b) via the intercellular route, which is likely only possible for very small NOAAs (<1 to 4 nm, the size of intercellular keratinocyte space) or under conditions where the skin barrier is disrupted. The intracellular pathway (Scheuplein et al., 1965, 1967) used by chemical substances and ions is not relevant for NOAAs. Skin properties per body parts are relevant for one of the pathways mentioned above. Follicular density varies greatly between different body parts, highly in forehead and lower in calf and thigh. The surface density of hair follicles, which varies by anatomical site and ethnicity, can cover up to 13.7% of skin surface on the forehead but only 0.95% on the forearm (Otberg et al., 2004). Thickness of the skin also varies by body parts. The stratum corneum is thicker in palms and soles (up to 175 and 500 µm, respectively), and much thinner in other anatomical sites (e.g. 22.6-6.4 µm on the abdomen with differences related to the method used; Holbrook and Odland, 1974; Egawa et al., 2007, Robertson and Rees, 2010, Huzaira et al., 2001).

Watkinson et al. (2013) considered that NOAAs behave like large molecules and modelled their rate of penetration using diffusion theory. They concluded that only particles of 1 nm or less are small enough to pass through intact skin. One would further assume that in healthy, intact skin, nanoparticles larger than ~4 nm (maximum intercellular space) cannot normally penetrate. However, there are experimental data that show that NOAAs larger than this size can pass through disrupted skin where intercellular gaps are larger than in normal skin (Labouta et al., 2013; Monteiro-Riveira & Larese Filon, 2012; Monteiro-Riveira & Riviere 2009, Larese Filon et al., 2009-2013, Poland et al. 2013).

The skin penetration and permeation of NOAAs is affected by many factors, including NOAA primary size, NOAA physico-chemical properties (such as rigidity/flexibility of the nanostructure, dissolution rate in water/sweat, and morphology), and skin health. Such factors have been analysed and presented in the following sections.

1. *NOAA size*

NOAAs characteristics may change considerably when they interact with physiological media. .Airborne NOAAs, which are emitted as individual nanoparticles, can subsequently agglomerate and settle on the skin and or surfaces. Therefore, the skin will come into contact mostly with agglomerates of NOAAs, especially because skin contact with contaminated surfaces and objects is a major exposure pathway. Direct contact of individual airborne NOAAs with the skin can be approached in a manner similar to gases, a process controlled by laws of diffusion (see Brouwer et al., 2016). The forces that control this deposition process depend on the primary particle size and aerodynamic behaviour of NOAAs. Once on the skin, biokinetics and transformation of NOAAs will depend on adhesion forces to the skin, interaction with sebaceous fluids and sweat, chemical stability and dissolution behaviour following such interactions. For that reason, it is critically important to characterise NOAAs behaviour in physiological media relevant to skin (i.e. sweat) to verify size modifications and rate of size change of NOAA. Changes in size towards smaller nanoparticles can enable NOAAs to pass thought the skin more easily than the original NOAAs. Sonavane (2008) for example reported a greater permeation through top layers of rat skin for 15 nm AuPN compared to 102 nm particles. Rancan (2012) demonstrated that only silica NOAAs smaller than 42 nm can penetrate the skin through hair follicles and be internalized by Langherans cells (mostly) and keratinocytes in a damaged skin model. Larger NOAAs did not pass into hair follicles. Quantum dots (QD) of 37 nm were observed to permeate the mouse skin only if the skin barrier was disrupted by dermo-abrasion (Gopee et al., 2009). Smaller QD (4 nm) have been shown to penetrate intact skin (Chu et al., 2007). Some flexible NOAA (liposomes and micelles) due to their flexibility can penetrate and permeate the intact skin also at sizes >4 nm. Larese et al. published a detailed review (2015) on this topic and defined those critical sizes.

Therefore, it can be concluded from available data and anatomical and physiological considerations of normal intact human skin that for rigid NOAA size is perhaps one of the most, if not the most, important factor influencing skin permeation/penetration. Figure 1 illustrates these concepts and table 1 summarized some relevant data from literature.

- For NOAA greater than 45 nm (primary or agglomerate size), no skin penetration and permeation is expected in healthy skin with normal barrier properties. However, penetration and permeation of NOAA > 45 nm, up to a few microns) can happen in severely damaged skin.
- For NOAA 21-45 nm, penetration and permeation can happen only in damaged skin.
- For NOAA 4-20 nm, there is possible permeation and penetration, which happens mostly through the hair follicles.
- For NOAA <4 nm: skin penetration has been demonstrated and this is consistent with expectations based on skin physiology and diffusion theory (for <1 nm) (see Larese et al., 2015 for detail).

2. *NOAA Surface properties*

NOAA surface properties, including surface charge, functional groups, Z potential, can influence penetration and permeation but their role in skin penetration is not clear and must be evaluated for each NOAAs. For some Quantum dots the surface charge as well as pH may influence penetration (Rymann-Rasmussen et al., 2006). Protein corona can play an important role in NOAA biokinetics and translocation inside the body, however the nature, role, and significance of protein corona on skin absorption of NOAA are poorly understood. Contact with solvents and oils can influence significantly skin absorption of NOAA by modifying skin permeability and/or nanoparticle diffusivity, and needs to be evaluated on a case-by-case basis. The data on factors related to impact of surface properties of nanoparticles on skin permeation/penetration is limited, yet highly relevant for occupational settings where co-exposures are common.

3. NOAA dissolution biokinetics, ions release and impurities

NOAA dissolution in sweat, skin-associated water and other biomolecules, is of critical importance because some metal NOAAs (such as Ni^{2+}) are known to cause skin sensitization and allergic dermatitis. Dissolution rates of NOAAs on the skin have not been investigated experimentally, however it is expected that they have higher rates (i.e. produce a higher ionic flux) than the corresponding micron sized particles, because of their much higher surface/mass ratio. NOAAs can reach hair follicles where they can reside and release ions for a long period. That may increase the risk of allergic contact dermatitis for NOAA containing sensitizing metals such as Ni, Pd, Co (Larese et al., 2013; Journeay and Goldman, 2014). Skin pH and sweat are expected to enhance NOAA dissolution, enhancing metal release.

Impurities in NOAA have received considerable attention in the context of inhalation exposures and associated respiratory and systemic diseases (Donaldson et al., 2006; Hsieh et al., 2012; Guo et al., 2007) but little attention has been paid to skin exposures and associated skin diseases. These impurities may include transition metals used as catalysts in the manufacture of carbon nanotubes (e.g. nickel, chromium, cobalt), organic impurities including polyaromatic hydrocarbons (PAHs) and other carbonyl compounds produced during the gas phase synthesis of several NOAA (especially CNTs), and inorganic impurities present in the raw materials used in the production of primary NOAA. These impurities can be carried through the skin by NOAA and then be released from NOAA leading to both localized and systemic adverse effects. Possible mechanistic interactions of impurities with nanoparticles in the development of skin disease have not been studied, but they may be particularly important in certain conditions, such as allergic contact dermatitis.

PAHs have been found in CNTs, carbonaceous ENMs (such as carbon black), and combustion by-products absorbed on surfaces of ENM (Plata et al., 2008). Supplemental Table S1 and S2 provide data on PAHs and organic carbon content (OC), respectively, in various classes of NOAA, collected as part of this work. OC is used as a surrogate for total organics and an index of organic impurity content. Note that carbon blacks in particular and refined fullerenes did contain several PAHs such as pyrene (~5 ppm), phenantrene (4.7 ppm), fluoranthene, Indeno (1,2,3-cd) pyrene (up to 18 ppm), and Benzo (ghi) perylene (up to 30 ppm). Several PAHs are known human carcinogens.

Table S3 summarized the total content of selected metals relevant to skin exposure, especially in the context of skin sensitization (see later section on skin disease) for different classes of NOAA. The distributions of such elements are typically right skewed, and geometric mean (GM), geometric standard deviation (GSD) and maximum values in a range of commercially relevant NOAAs are provided. The water-soluble fraction of these metals, an important indicator of the likelihood of metal ions release (which are believed to be involved in sensitization), is also presented. Several observations in Table S3 are important to note:

i) Ni and Cr, and to some extent Co as well, were present in appreciable amounts in many commercial CNTs; GM ranging from ~10 (µg/g) to 800 (µg/g) and maxima as high as 1.2% (Ni); Interestingly, high concentrations of several transition metals, including Ni, Cr, Co, etc. have been found in tattoo inks, which often employ nanoscale NOAA (Hogsberg et al., 2011; Forte et al., 2009).

ii) Pd and As were present mostly in trace impurities in ng/g (ppb range). One notable exception was one high volume TiO_2 commercial sample, which contained 50 µg/g As. Similarly, Zr was found only in certain metal oxide NOAA, notably ZnO, CeO_2, and TiO_2. Zr, As and certain other metals (Fe in CB for example) are likely related to impurities in raw materials (e.g. natural ores). One zirconia sample in the dataset contained 200 µg/g Cadmium (Cd), 5 µg/g platinum, and 45 µg/g Yttrium (Y, added sometimes as a stabilizer). Cd and Pd are likely impurities.

iii) The water-soluble content of Ni, Cr, Co varied by NOAA type, with GM in the 0.001-7 (µg/g) range. Water solubility varied by metal and NOAA type. The GM ratio of water soluble to total metal size distributions (i.e. GM water soluble/GM total metal) varied in the 0.2-28% range for Ni, 0.05-8% (Cr) and 0.3-80% for Co (Table 2). In CNTs, where these elements were in higher concentrations, this GM ratio was <1%; however, much higher water solubility has been observed for these metals when they appear as impurities in other NOAA (e.g. TiO_2, CeO_2 or ZnO).

Effects of NOAA on the skin

Irritation

Mechanical friction between solid objects and the skin can cause abrasion, damage to the thickness of the SC, and skin irritation. Early on Eedy (1996) reported irritant contact dermatitis in workers exposed to relatively coarse carbon fibers in micrometer range. However, more recent data shows no dermal irritation in guinea pigs exposed to carbon nanotubes (Khisore et al., 2009).

Experimental evidence regarding NOAA skin exposure and disease is also limited. Ema et al (2011) investigated acute skin and eye irritation and skin sensitization potential of three types of CNTs in rabbits and guinea pigs respectively and demonstrated that only one MWCNT (out of three tested) was a very weak acute irritant to the skin and eyes (Ema et al., 2011). Similarly, Park et al. (2011) demonstrated that polystyrene and titania nanoparticles did not induce phototoxicity, acute skin irritation, or skin sensitization in animals (rabbits, mice). However, subchronic skin exposures to TiO_2 could induce inflammation of the epidermis, leading to effects such as focal parakeratosis (flattened keratinocyte nuclei within the stratum corneum) and spongiosis (intercellular oedema between keratinocytes), (Adachi et al. 2013) whereas chronic exposures to TiO_2 may accelerate skin aging (Wu et al. 2009). Highly purified fullerenes were shown to be 'minimally irritating' to the skin and eyes, and did not present a problem with regard to skin irritation, skin sensitization, skin photosensitization or contact phototoxicity (Aoshima et al. 2009). Overall the available limited evidence suggests minimal effects of NOAA in human intact skin.

Metal (ions) of Ni, Co, Hg, and Cr (as soluble salts, e.g. sulfate or chloride), as well as antimony (Sb, as trioxide), and arsenic (as trioxide) are known skin irritants (Cohen and Moore 2007).

Sensitization

Several transition metals are known to cause sensitization and allergic contact dermatitis. There is further evidence of possible risk from exposure to metal NOAA or metal impurities

in NOAA. Several metals, including nickel (Ni), chromium (Cr), cobalt (Co), beryllium (Be), and palladium (Pd), are well-known skin allergens (Cohen and Moore et al., 2007; Rice & Mauro, 2008). Nickel, Cr, Co, Au, and Pd are available commercially as metallic engineered nanoparticles of various sizes. Most of these elements, except for Be, Hg and As, are commercially available as metal oxides nanoparticles, or as components of more complex nanoparticle chemistries (http://www.nanowerk.com/phpscripts/n_dbsearch.php). Q-dots, another type of engineered nanoparticle, often contain cadmium selenide (CdSe) or cadmium sulfide (CdS), sometime mixed with other metals (e.g. Zn). They can release Cd causing intoxication, as already demonstrated in animals (Chu et al., 2007; Liu et al., 2011).
Nickel in jewellery is a classic example of Ni ions leaching over time and reaching the epidermis, leading to development of allergic contact dermatitis in various individuals. One case report already describes nickel NOAAs as causing asthma and skin diseases (Journeay et al., 2014). NOAAs can release ions in higher amounts than bulk material due to their high surface/mass ratio. For that reason, NOAAs containing sensitizing metal/s may more easily trigger an allergic response than the corresponding microscopic bulk materials of the same composition.

On the other hand, it has been suggested that fullerenes may play a leading role in the inhibition of the in vitro and in vivo IgE-mediated allergic response, thus blocking histamine release or reducing nickel uptake after the application of a cream containing fullerenes (Vermula et al., 2011).

Skin Diseases

There is only one case report of contact dermatitis (CD) and asthma in a woman exposed to nickel NOAAs (Journeay and Goldman, 2014). There are no other observational data related to workplace NOAA skin exposures and skin disease, even though the authors have witnessed numerous scenarios of extensive NOAA skin exposure.

Tattooing

Tattooing in humans is a relevant and interesting scenario to analyse, because tattoo inks contain engineered nanoparticles, and because injected ink is delivered in the dermis (Hogsberg et al., 2011, 2013a). In a recent study among young individuals tattooed with carbon black and organic pigments, 16% complained of mostly minor symptoms, including skin itching, skin elevation/nodules, inflammation and stinging, with over half of them being sun induced (Hogsberg et al., 2013b).

Factors involved in skin barrier function integrity

Mechanical action

Rouse et al. (2007) demonstrated that mechanical flexion can increase skin penetration of small fullerene (3.5 nm) that can be found in the intercellular spaces of stratum granulosum. On the contrary QDs applied to rat skin flexed for 60 min showed that larger nanoparticles QD655-COOH (18nm) and QD565-COOH (14nm) did not penetrate at 8 and 24h (Zhang et al., 2008).

Skin barrier disruption

Skin barrier disruption is a crucial aspect for NOAA skin penetration and permeation, so particular attention should be paid to workers who are at increased risk of irritant contact dermatitis or to atopic patients with an impaired skin barrier.

In certain occupations, such as construction, CD is prevalent and the disease causation in such settings is often multifactorial. The high market penetration by NOAA in this industry and potential for significant interactions of NOAA with damaged skin should be noted. Authors are not aware of any ongoing surveillance or epidemiological studies focusing on skin disease among cohorts of nanomanufacturing workers. They recommend the avoidance of skin contact with NOAA containing products and to undergo medical surveillance, with particular attention to skin conditions and skin diseases.

Occupational skin diseases are prevalent in most countries. More than 90% of occupational skin diseases are classified as CD (EU-OSHA, 2008). Acute irritant CD may occur as a result of exposure to strong irritants such as acids or alkalis, whereas chronic irritant CD can be caused by repeated exposure to mild irritants such as water (from wet work), soaps and detergents. Wet work is common amongst occupations such as hairdressers, food workers, cleaners and healthcare workers. Allergic CD is caused by an immunological reaction following exposure to an allergen or a sensitizer. In many cases, irritant CD can exacerbate the effects of skin sensitizers because of damage to the skin barrier (Elsner et al. 1994).

Skin permeability may increase 4 to 100 times in atopic subjects with damaged skin (Larese et al. 2009, 2011) and it is possible for the skin barrier to be compromised, although there are no visible signs (Kezic et al., 2009).

Frequent, repetitive exposure to water or other irritant chemicals results in disruption of the lipid bilayers in the stratum corneum, which can lead to chapping and fissuring of the skin (Chew and Maibach, 2003). In some work situations, there may be exposure to more than one irritant, for example, in addition to wet work, healthcare workers are likely to be exposed to cleansers, detergents and disinfectants.

Other hazards that may influence the integrity of the skin barrier include mechanical abrasion or friction caused by dusts or powders of the skin, cuts and punctures. Further, exposure to cold, heat, and pressure may lead to skin alteration and vibration can induce sklerodermal effects (EU-OSHA, 2008). Exposure to these physical agents may affect an individual's response to other chemical agents, allowing them to penetrate the skin more easily (CCOHS, Fluhr et al. 2002, 2008).

The commonest causes of dermatitis are wet work, soaps and cleaners, solvents, degreasing agents, metal working fluids, dusts/friction and low humidity (HSE, 2014; Pal et al., 2009; Cahill et al., 2012, Behroozy and Keegel, 2014). For example, Cahill et al. (2012) report the most common causes in patients with a primary diagnosis of irritant CD – water and wet work (37%), soap and detergents (33%), heat and sweating (16%), oils and coolants (14%), solvents (14%), dusts and fibres (10%), acids and alkalis (4%). Wet work includes activities where there is prolonged contact for more than two hours a day, frequent or intensive hand washing and where liquid-tight protective gloves are worn for extended periods (BAuA 2008). Other common agents where exposure increases the risk of dermatitis include hairdressing products, preservatives, rubber chemicals, cement, nickel, chromium and chromates, cobalt, resins and acrylics, cosmetics and fragrances, petroleum and products, disinfectants, degreasers and cutting oils and coolants (HSE, 2014; Carøe et al., 2013).

Overall consideration

Taking into consideration the limited penetration by NOAA through intact skin, and the easy release of metals or other impurities in nanoparticles by dissolution in the skin or skin contamination layer, it is reasonable to hypothesize that: i) skin exposure to NOAA in general may present more concerns where there is compromised skin integrity due to pre-existing disease or exposure to other factors (e.g. abrasion); ii) susceptible subpopulations may be particularly at risk for allergic skin disease, especially following dermal contact with nanoparticles containing sensitizing metals, and iii) although not the primary focus of this paper, in an accompanying paper we make the argument that skin exposure should be investigated as a potentially significant pathway for ingestion of NOAA (Cherrie et al., 2006, Christopher et al., 2007; Gorman et al., 2012, 2014).

RECOMMENDATIONS FOR HAZARD ASSESSMENT

Taking into account the literature reviewed in the previous sections, hazard assessment should consider the following steps:

1. Evaluation of NOAA, using the diagram reported in Figures 1, 2 and 3.
2. Evaluation of skin condition of exposed workers
3. Evaluation of jobs at high risk for occupational dermatitis (irritant and allergic CD)
4. Evaluation of jobs with use of NOAA

1. Evaluation of NOAA

If applicable, assessment of dermal exposure to NOAA should be incorporated in the general cycle of risk assessment in companies to control risks in the workplace. With respect to assessment of dermal exposure to NOAA in the workplace, a stepwise approach is proposed to assess the situation in the workplace in a systematic manner that focuses on determining the potential for exposure based on a potential for release and determining the potential for skin disruption. A stepwise approach is given, of which the first step is described in this paper, and the other steps are described in the accompanying paper of Brouwer et al. (2016). After each step, a decision should be made whether the situation at the workplace is considered to be safe based on the information that is gathered during that part of the assessment. If the situation is not considered to be safe, one should proceed to the following step of the assessment (Figure 3).

Step 1. (Primary evaluation based on the NOAA composition) consists of a primary (desk) evaluation of the occurrence of possible health risks based on the composition / characteristics of NOAA. In Figure 1 and 3 a schematic overview of this evaluation and the further course of the overall assessment is given.

Attention should be given to:

- Metal NOAA, since the potential release of ions may induce local skin effects (e.g. irritation and CD) and absorption of toxic or sensitizing metals;
- NOAA with metal catalytic residue, since potential release of ions may induce local skin effects (e.g. irritation and CD) and absorption of toxic metals;
- Non-rigid or flexible NOAA, since due to their flexibility liposomes and micelles can penetrate and permeate the intact skin also at sizes >4 nm;
- Co-exposure to other toxic substances present in the workplace.

In the case of “high hazard” NOAA, dissolution of toxic or sensitizing substances in synthetic sweat should be evaluated under physiological relevant conditions (e.g. at 32°C to mimic the temperature of the hands). If the NOAA dissolve in synthetic sweat, in addition to continuing with the assessment, it is advised to also evaluate the level of contamination of surfaces (benches, tools etc.) in the workplace and to evaluate the internal exposure to these substances by means of biological monitoring (if available, e.g. As, Cr, Co, Ni in urine) for exposed workers. Health surveillance of workers potentially exposed to such NOAA is also advisable.

2. Evaluation of skin condition of exposed workers

As an impaired barrier function is a crucial aspect for NOAA skin penetration and permeation is import to evaluate this risk factor.

Various biophysical measurement methods that reflect the deterioration of barrier function are available. Routine workplace methods to assess skin integrity must be easy to use by those who are not dermatologists and sufficiently sensitive and reproducible to detect signs of very early degradation of skin barrier function, and to identify individuals at risk of increased uptake of nanoparticles.

Assessment of skin condition can be made by visual examination, which may include questionnaires or scoring systems. For example, the Nordic Occupational Skin Questionnaire Group has developed the Nordic Occupational Skin Questionnaire (NOSQ-2002) for surveys on work-related skin disease on the hands and forearms in relation to exposures to environmental factors (Susitaival et al., 2003).

Weistenhofer et al. (2010, 2011) reviewed the skin score tools available for quantifying hand eczema. Of the many scoring systems, only three have been validated: the Hand Eczema Severity Index (HECSI), the Manuscore and the Osnabrück Hand Eczema Severity Index (OHSI). They compared these three systems and concluded that both HECSI and OHSI were relevant in practice since the risk of observer bias was low. However, in an occupational setting damage to the skin is typically minimal which makes quantification of skin condition rather than skin disease difficult.

We suggest a modified Hand Eczema Severity Index (HECSI) to determine skin disruption.

The original questionnaire, suggested by Held et al. (2005) was modified considering only irritative aspects (fissures and scaling) and inserting 'dryness' as a clinical sign. Each hand is divided into five areas (fingertips, fingers (except the tips), palm of hand, back of hands, wrists. For each of these areas the intensity of the three clinical signs related to impairment of the skin (fissuring, scaling and dryness) are graded following original scale (1 - mild disease, 2 - Moderate, and 3 - Severe). For each locations (total of both hands) the affected area is given as score from 0 to 4 (0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%). The score obtained for the extent of each location is multiplied by the total sum of the intensity of each clinical feature, and the total sum was calculated as Skin Disruption Score Index, varying from 0 to 180 (Table S4).

There are also a number of biophysical parameters that can be used to objectively assess skin condition. The most commonly used ones are transepidermal water loss (TEWL) from the skin surface, skin hydration and quantitative measurement of skin colour. International guidelines for the in vivo assessment of skin properties in non-clinical settings, such as the workplace, have been published (duPlessis et al., 2013; Stefaniak et al., 2013) and cover pH, TEWL and skin hydration.

All of these biophysical assessment methods have the advantage that they are non-invasive, simple to use, provide quantitative data and may indicate sub-clinical damage to the skin barrier. However, they can be affected by environmental factors such as humidity and temperature, which may change rapidly. Biophysical measurements of skin barrier could be used to assess the potential for uptake of nanoparticles through compromised skin, but these tools are likely only to be useful in research studies or where there is particular concern about dermal exposure to nanomaterials.

3 Evaluation of jobs at high risk for occupational contact dermatitis (CD)

Since skin absorption of NOAA is relevant in condition where skin barrier is disrupted, it is crucial to evaluate skin barrier integrity in exposed workers. Typical industries where dermatitis occurs include agriculture, food industry (including catering), chemical industry, construction, health and electronics (HSE, 2014; Cahill et al, 2012; Pal et al, 2009; Zorba et al, 2013; Behroozy and Keegel, 2014).

Occupations with high rates of dermatitis are hairdressers and barbers, florists, cooks, beauticians, metal working machine workers, chemical, rubber, glass and ceramic process workers, dental practitioners, dental and other nurses and podiatrists (HSE, 2014). Other high risk jobs include cleaners, mechanics and vehicle assemblers (Royal College, 2011). Nano-enabled products have penetrated extensively most, if not all, of these professions (See accompanying paper by Brouwer et al 2016) , making assessment of skin integrity essential for these professions.

4. Evaluation of job title at high risk of dermatitis with use of NOAA

The accompanying paper by Brouwer et al (2016) links job titles with reported high incidence of skin diseases to reported use of nanomaterials or nano-enabled products or exposure to NOAA to flag potential high risk job titles with respect to dermal exposure: i.e. .nurses that can come in contact with nano drugs, dental workers that are using nanocomposites, hairdressers and beauticians using personal care products containing NOAA, construction workers using coatings, paints and mortars, cleaners using dirt repellent coating, and varnishes with NOAA.

Conclusions

Skin contact with certain nanoparticles and nano-enabled products that may release NOAA can cause adverse effects in the skin in particular circumstances. Moreover, some NOAA can release ions that can have local or systemic effects, if they are able to cross the skin barrier and to arrive into the skin or into blood circulation. For that reason it is necessary to consider factors that can cause nanoparticles skin penetration and permeation, metal and impurities released, contact conditions (surface involved, time of contact, sweating, other chemical enhancers as soaps) and skin conditions. Nanomaterial can be transported and stored in hair follicles from where they can release ions for periods of time. In conditions where skin barrier is impaired due to fissures or scaling, nanomaterial can pass directly through the stratum corneum reaching viable epidermis and derma, potentially causing adverse health effects-both locally and systemic. These concerns are most realistic for nanomaterials that are made of metal sensitizers or contain such impurities. NOAA made of sensitizer materials should be labelled for that hazard.

NOAA that contain them as impurities above the appropriate concentration limits, as determined in contact sensitization documents or patch testing recommendations, also should carry similar notations

Furthermore, we identify important knowledge gaps that need to be addressed experimentally, including NOAA dissolution potential, impurities released, the presence of toxics substances as well as allergic metals released, that must be considered together with skin condition for exposed workers. More data on metal release from NOAA are urgently needed for hazard assessment. The systematic stepwise approach presented here and in the accompanying paper should be linked to observations of the actual occupational use of nanoparticles and nano-enabled products to help occupational health practitioners in risk assessment and management.

Figure 1: Skin absorption of NOAA considered available knowledge

Figure 2: Overview of stepwise approach for assessment of dermal exposure to NOAA

Figure 3: Schematic overview of primary evaluation based on composition of NOAA and following steps.

Conflict of interest statement

The authors have no conflict of interests to disclose.

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References

Adachi K., Yamada N., Yoshida Y., Yamamoto O., 2013. Subchronic exposure of titanium dioxide nanoparticles to hairless rat skin. Exp. Derm. 22, 278-283.

Aitken R.J., Chaudhry M.Q., Boxall A.B.A., Hull M., 2006. Manufacture and use of nanomaterials: current status in the UK and global trends. Occup. Med. 56,300-306.

Aitken R.J., Creely K.S., Tran C.L., 2004.Nanoparticles: An Occupational Hygiene Review. HSE Research Report 274. London: HSE Books.

Aoshima H., Saitoh Y., Ito S., Yamana S., Miwa N., 2009. Safety evaluation of highly purified fullerenes (hpfs): Based on screening of eye and skin damage. J. toxicol. sciences 34,555-562.

Baroli B., Ennas M.G., Loffredo F., Isola M., Pinna R., Lopez-Quintela A., 2007. Penetration of metallic nanoparticles in human full-thickness skin. J. Invest. Dermatol. 127, 1701–1712.

BauA, 2008. Risk resulting from skin contact – identification, assessment, measures. http://www.baua.de/en/Topics-from-A-to-Z/Hazardous-Substances/TRGS/pdf/TRGS-401.pdf%3F__blob=publicationFile%26v=4 (accessed 15.03.16).

Behroozy A., Keegel T.G., 2014. Wet-work Exposure: A Main Risk Factor for Occupational Hand Dermatitis. Saf. Health Work 5,175-80.

Bello D., Hsieh S.F., Schmidt D., Rogers E.J., 2009. Nanomaterials properties vs. biological oxidant damage: Implications for toxicity screening and exposure assessment. Nanotoxicology 3,249–261.

Cahill J., Williams J., Matheson M., Palmer A., Burgess J., Dharmage S., Nixon R., 2012.

Occupational contact dermatitis: a review of 18 years of data from an occupational dermatology clinic. Report for Safe Work Australia, Australia www.safeworkaustralia.gov.au (accessed 15.03.16).

Carøe T.K., Ebbehøj N.E., Wulf H.C., Agner T., 2013. Occupational skin cancer may be underreported. Dan. Med. J. 60,A4624.

CCOHS, http://www.ccohs.ca/oshanswers/diseases/dermatitis.html (accessed 12.12.15).

Chew A.L., Maibach H.I., 2003. Occupational issues of irritant contact dermatitis Int Arch Occup Environ Health 76,339-46.

Cherrie J.W., Semple S., Christopher Y., Saleem A., Hughson G.W., Philips A., 2006. How important is inadvertent ingestion of hazardous substances at work? Ann. Occup. Hyg. 50, 693-704.

Christopher Y., Semple S., Hughson G.W., van Tongeren M., Cherrie J.W., 2007. Inadvertant ingestion exposure in the workplace. HSE Books (Research project R551).

Chu M., Wu Q., Wang J., Hou S., Miao Y., Peng J., 2007. In vitro and in vivo transdermal delivery capacity of quantum dots through mouse skin. Nanotechnology 18,455-460.

Cohen D.M. and Moore M.M., 2007. Occupational Skin Disease. Chapter 38, pg. 617-639. In: Rom W. and Markowitz SB editors. Environmental and Occupational Medicine, 4th edition. Wolters Kluwer, Lippincot Williams & Wilkins health, New York.

Crosera M., Bovenzi M., Maina G., Adami G., Zanette C., Florio C., Larese Filon F., 2009. Nanoparticle dermal absorption and toxicity: A review of the literature. Int. Arch. Occup. Environ. Health 82,1043-1055.

Donaldson K., Aitken R., Tran L., Stone V., Duffin R., Forrest G., 2006. Carbon nanotubes: A review of their properties in relation to pulmonary toxicology and workplace safety. Toxicol. Sci. 92,5–22.

Eedy D.J., 1996. Carbon-fibre-induced airborne irritant contact dermatitis. Contact Dermatitis 35,362-363.

Egawa M., Hirao T., Takahashi M., 2007. In vivo estimation of stratum corneum thickness from water concentration profiles obtained with Raman spectroscopy. Acta Derm. Venereol. 87,4-8.

Elsner P., 1994. Irritant dermatitis in the workplace. Dermatol. Clin. 12,461–467.

Ema M., Matsuda A., Kobayashi N., Naya M., Nakanishi J., 2011. Evaluation of dermal and eye irritation and skin sensitization due to carbon nanotubes. Reg. toxicol. Pharmacol. 61,276-281.

Ema M., Matsuda A., Kobayashi N., Naya M., Nakanishi J., 2013. Dermal and ocular irritation and skin sensitization studies of fullerene C60 nanoparticles. Cutan. Ocul. Toxicol. 32,128-34.

EU-OSHA European Agency for Safety and Health at Work., 2008. Occupational skin diseases and dermal exposure in the European Union (EU-25): policy and practice overview. 2008. https://osha.europa.eu/en/node/6875/file_view (accessed 14.12.2015)

Fluhr J. W., Dickel H., Kuss O., Weyher I., Diepgen T.L., Berardesca E., 2002. Impact of anatomical location on barrier recovery, surface ph and stratum corneum hydration after acute barrier disruption. Br. J. Dermat. 146,770-776.

Fluhr JW, Darlenski R, Angelova-Fischer I, Tsankow N, Basketter D. 2008. Skin irritation and sensitization: mechanisms and new approaches for risk assessment. Skin Pharmacol Physiol 21: 124-135.

Forte G., Petrucci F., Cristaudo A., Bocca B., 2009. Market survey on toxic metals contained in tattoo inks. Science Total Environ. 407,5997-6002.

Gopee N.V., Roberts D.W., Wepp P., Cozart C.R., Sitonen P.H., Laterdresse G.R., 2009. Quantitative determination of skin penetration of PEG-coated CdSe quantum dots in dermoabraded but not intact SKH-I hairless mouse skin. Toxicol. Sci. 111, 37-48.

Gorman Ng M., Semple S., Cherrie J.W., Christopher Y., Northage C., Tielemans E., Veroughstraete V., van Tongeren M., 2012. The relationship between inadvertent ingestion and dermal exposure pathways: a new integrated conceptual model and a database of dermal and oral transfer efficiencies. Ann. Occup. hygiene 56,1000-1012.

Gulson B., McCall M., Korsch M., Gomez L., Casey P., Oytam Y., Taylor A., McCulloch M., Trotter J., Kinsley L., Greenoak G., 2010. Small amounts of zinc from zinc oxide particles in sunscreens applied outdoors are absorbed through human skin. Toxicol. Sci. 118,140-149.

Guo L., Morris D.G., Liu X., Vaslet C., Hurt R.H., Kane A.B., 2007. Iron bioavailability and redox activity in diverse carbon nanotube samples. Chem. Materials 19,3472–3478.

Held E., Skoet R., Johansen J.D., Agner T., 2005. The hand eczema severity index (HECSI): a scoring system for clinical assessment of hand eczema. A study of inter- and intraobserver reliability. Br. J. Dermatol.152,302-7.

Hogsberg T., Loeschner K., Lof D., Serup J., 2011. Tattoo inks in general usage contain nanoparticles. British j. dermatol. 165,1210-1218.

Hogsberg T., Jacobsen N.R., Clausen P.A., Serup J., 2013a. Black tattoo inks induce reactive oxygen species production correlating with aggregation of pigment nanoparticles and product brand but not with the polycyclic aromatic hydrocarbon content. Exp. Dermatol. 22,464-469.

Hogsberg T., Hutton Carlsen K., Serup J., 2013b. High prevalence of minor symptoms in tattoos among a young population tattooed with carbon black and organic pigments. J. Eur.Acad..Dermat.Venereol. JEADV 27.846-852.

Holbrook K.A., Odland G.F., 1974. Regional differences in the thickness (cell layers) of the human stratum corneum: an ultrastructural analysis. J. Invest. Dermatol. 62,415-22.

Honnert B. and Gryzebyk M., 2014. Manufactured Nano-objects: An Occupational Survey in Five Industries in France. Ann Occup Hyg 58, 121-135.

HSE, Health and Safety Executive, 2014. Work related skin disease in Great Britain 2014. http://www.hse.gov.uk/statistics/causdis/dermatitis/skin.pdf (accessed 12.12.15).

Hsieh S.F., Bello D., Schmidt D.F., Pal A.K., Rogers E.J., 2012. Biological oxidative damage by carbon nanotubes: fingerprint or footprint? Nanotoxicology 12,61-76.

Hsieh S.F., Bello D., Schmidt D.F., Pal A.K., Stella A., Isaacs J., Rogers E.J., 2013. Mapping the Biological Oxidative Damage of Engineered Nanomaterials. Small 27,1853-65.

Huzaira M., Rius F., Rajadhyaksha M., Anderson R.R., González S., 2001. Topographic variations in normal skin, as viewed by in vivo reflectance confocal microscopy. J. Invest. Dermatol. 116,846-52.

ISO/TR 14294:2011, Workplace atmospheres - Measurement of dermal exposure - Principles and methods.

Journeay W.S., Goldman RH., 2014. Occupational handling of nickel nanoparticles: a case report. Am. J. Ind. Med. 57,1073-6.

Kezic S., Visser M.J., Verbeek M.M., 2009. Individual susceptibility to occupational contact dermatitis. Indust. Health 47, 469-478.

Kishore A.S., Surekha P., Murthy P. B., 2009. Assessment of the dermal and ocular irritation potential of multi-walled carbon nanotubes by using in vitro and in vivo methods. Toxicol. Lett. 191, 268-274.

Labouta H. and Schneider M., 2013. Interaction of inorganic nanoparticles with the skin barrier: current status and critical review. Nanomedicine 9,49-54.

Lademann J., Patzelt A., Richter H., Antoniou C., Sterry W., Knorr F., 2009. Determination of the cuticula thickness of human and porcine hairs and their potential influence on the penetration of nanoparticles into the hair follicles. J. Biomed. Opt. 14,021014.

Larese Filon F., D'Agostin F., Bovenzi M., Crosera M., Adami G., Romano C., Maina G., 2009. Human skin penetration of silver nanoparticles through intact and damaged skin. Toxicol 255, 33-37.

Larese Filon F., Crosera M., Adami G., Bovenzi M., Rossi F., Maina G., 2011. Human skin penetration of gold nanoparticles through intact and damaged skin. Nanotoxicology 5,493-501.

Larese Filon F., Crosera M., Timeus E., Adami G., Bovenzi M., Ponti J., Maina G., 2013. Human skin penetration of cobalt nanoparticles through intact and damaged skin. Toxicol In Vitro 27,121-7.

Larese Filon F., Mauro M., Adami G., Bovenzi M., Crosera M., 2015. Nanoparticles skin absorption: New aspects for a safety profile evaluation. Reg. Toxicol. Pharmacol. 72,310-22.

Lee S.E., Choi K.J., Menon G.K., Kim H.J., Choi E.H., Ahn S.K., Lee S.H., 2010.

Penetration pathways induced by low-frequency sonophoresis with physical and chemical enhancers: iron oxide nanoparticles versus lanthanum nitrates. J. Invest. Dermatol 130, 1063–1072.

Liu W., Zhang S., Wang L., Qu C., Zhang C., Hong L., Yuan L., Huang Z., Wang Z., Liu S., Jiang G., 2011. CdSe quantum dot (QD)-induced morphological and functional impairments to liver in mice. PLoS One 6,e24406.

Mauro M., Crosera C., Bianco C., Adami G., Montini T., Fornasiero P., Bovenzi M., Larese F., 2015 Human skin penetration of platinum and rhodium nanoparticles through intact and damaged skin. J Nanoparticles Res. 17, 253-262.

Monteiro-Riviere N. and Larese Filon F., 2012. Nanomaterial interaction with skin in Adverse effects of engineered nanomaterials. Fadell, Pietroiusti, Shvedova ed. Elsevier London 185-208.

Monteiro-Riviere N.A. and Riviere J.E., 2009. Interaction of nanomaterials with skin: Aspects of absorption and biodistribution. Nanotoxicology 3:188-193.

Oberdörster G., Oberdörster E., Oberdörster J., 2005. Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles. Environ. Health. Perspect. 113,823-839.

Otberg N., Richter H., Schaefer H., Blume-Peytavi U., Sterry W., Lademann J., 2004. Variations of hair follicle size and distribution in different body sites. J. Invest. Dermatol. 122,14-9.

Ovissipour M., Sablani S.S., Rasco B., 2013. Engineered nanoparticle adhesion and removal from tomato surfaces. J. Agric. Food Chemistry 61,10183-10190.

Pal T.M., de Wilde N.S., van Beurden M.M., Coenraads P.J., Bruynzeel D.P.. 2009. Notification of occupational skin diseases by dermatologists in the Netherlands. Occup. Med. 59, 38-43.

Park Y.H., Jeong S.H., Yi S.M., Choi B.H., Kim Y.R., Kim I.K., 2011. Analysis for the potential of polystyrene and TiO2 nanoparticles to induce skin irritation, phototoxicity, and sensitization. Toxicol.in Vitro 25,1863-1869.

Plata D.L., Gschwend P.M., Reddy C.M., 2008. Industrially synthesized single-walled carbon nanotubes: Compositional data for users, environmental risk assessments, and source apportionment. Nanotechnology 19,185706.

Plessis J.D., Stefaniak A., Eloff F., John S., Agner T., Chou T.C., Nixon R., Steiner M., Franken A., Kudla I., Holness L., 2013. International guidelines for the in vivo assessment of skin properties in non-clinical settings: Part 2. Transepidermal water loss and skin hydration. Skin Research Technol. 19, 265–278.

Poland C.A., Read S.A.K., Varet J., Carse G., Christensen F.M., Hankin S.M., 2013. Dermal Absorption of Nanomaterials Part of the ”Better control of nano” initiative 2012-2015. The Danish Environmental Protection Agency.

Rancan F., Gao Q., Graf C., Troppens S., Hadam S., Vogt A., 2012. Skin penetration and cellular uptake of amorphous silica nanoparticles with variable size, surface functionalization and colloidal stability. ACS Nano 8, 6829-6842.

Rice R.H. & Mauro T.M., 2008. Toxic responses of the skin. Chapter 19, pg. 741-759. In: Curtis D. Klaassen editor, Casarett & Doull's Toxicology, The basic science of poison, 7th edition. 2008. McGraw-Hill Medical Publishing Division, New York.

Robertson K., Rees J.L., 2010. Variation in epidermal morphology in human skin at different body sites as measured by reflectance confocal microscopy. Acta Derm. Venereol. 90,368-73.

Rouse J.G., Yang J., Ryman-Rasmussen J.P., Barron A.R., Monteiro-Riviere N.A., 2007. Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin. Nano Lett. 7, 155-160.

Royal College of Physicians. 2011. Concise guidance: diagnosis, management and prevention of contact dermatitis.

Ryman-Rasmussen J.P., Riviere J.E., Monteiro-Riviere N.A., 2006. Penetration of intact skin by quantum dots with diverse physicochemical properties. Toxicol Sci 91,159–165.

SCCP – Scientific Committee on Consumer Products, 2007. Preliminary opinion on safety of nanomaterials in cosmetic products. European Commission, Brussels, Belgium. http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_123.pdf (accessed 12.12.2015).

Scheuplein R.J., 1965. Mechanism of percutaneous absorption. I. Routes of penetration and the influence of solubility. J. Invest. Dermatol. 45, 334-46.

Scheuplein R.J., 1967. Mechanism of percutaneous absorption. II. Transient diffusion and the relative importance of various routes of skin penetration. J. Invest. Dermatol. 48,79-88.

Sonavane G., Tomoda K., Sano A., Ohshima H., Terada H., Makino K., 2008. In vitro permeation of gold nanoparticles through rat skin and rat intestine: Effect of particle size. Colloids Surf. B. 65,1-10.

Stefaniak A.B., Plessis J., John S.M., Eloff F., Agner T., Chou T.C., Nixon R., Steiner M.F. C., Kudla I., Holness L.D., 2013. International guidelines for the in vivo assessment of skin properties in non-clinical settings: part 1. pH. Skin Res.Technol. 19, 59–68.

Susitaival P., Flyvholm M.A., Meding B., Kanerva L., Lindberg M., Svesson A., Olafsson J.H., 2003. Nordic Occupational Skin Questionnaire (NOSQ-2002): a new tool for surveying occupational skin diseases and exposure. Contact Dermatitis 49, 7-76.

Vermula P.K., Anderson R.R., Karp J.M., 2011. Nanoparticles reduce nickel allergy by capturing metal ions. Nat Nanotechnol 5,291-5.

Watkinson A.C., Bunge A.L., Hadgraft J., Lane M.E., 2013. Nanoparticles do not penetrate human skin--a theoretical perspective. Pharm. Res. 30,1943-6.

Weistenhöfer W., Baumeister T., Drexler H., Kütting B., 2011. How to quantify skin impairment in primary and secondary prevention? HEROS: a proposal of a hand eczema score for occupational screenings. Brit. J. Dermatol. 164, 807–813.

Weistenhöfer W., Baumeister T., Drexler H., Kütting B., 2010. An overview of skin scores used for quantifying hand eczema: a critical update according to the criteria of evidence-based medicine. Brit. J. Dermatol. 162, 239-250.

Wu J., Liu W., Xue C., Zhou S., Lan F., Bi L., 2009. Toxicity and penetration of tio2 nanoparticles in hairless mice and porcine skin after subchronic dermal exposure. Toxicol. letters 191,1-8.

Zorba E., Karpouzis A., Zorba A., Bazas T., Zorbas S., Alexopoulos E., Zorbas I., Louskoukis K., Konstandinidis T., 2013.Occupational dermatoses by type of work in Greece. Safety and Health at Work 4, 142-148.

Figure 1
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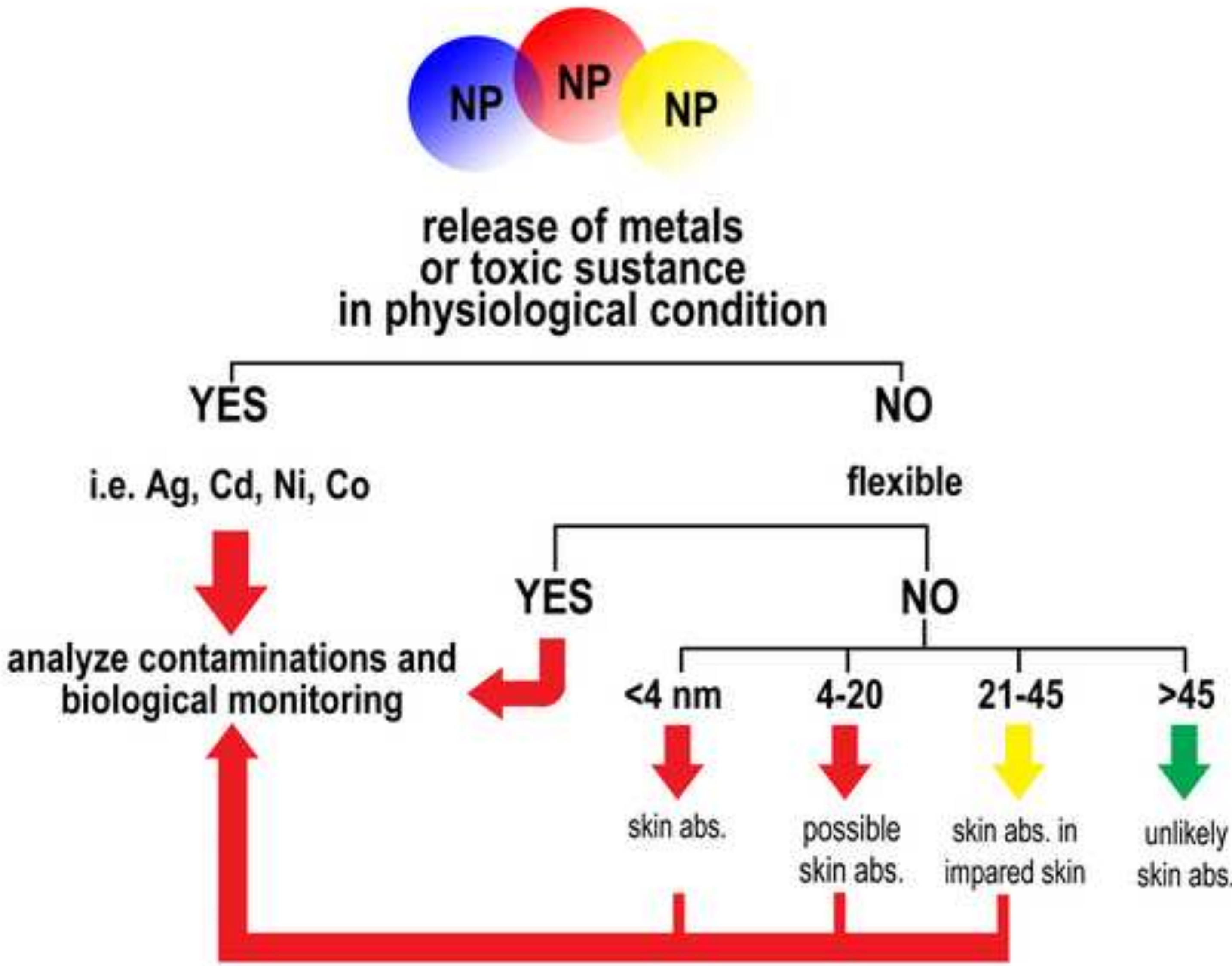

Figure 2

Figure 2: Overview of stepwise approach for assessment of dermal exposure to NOAA

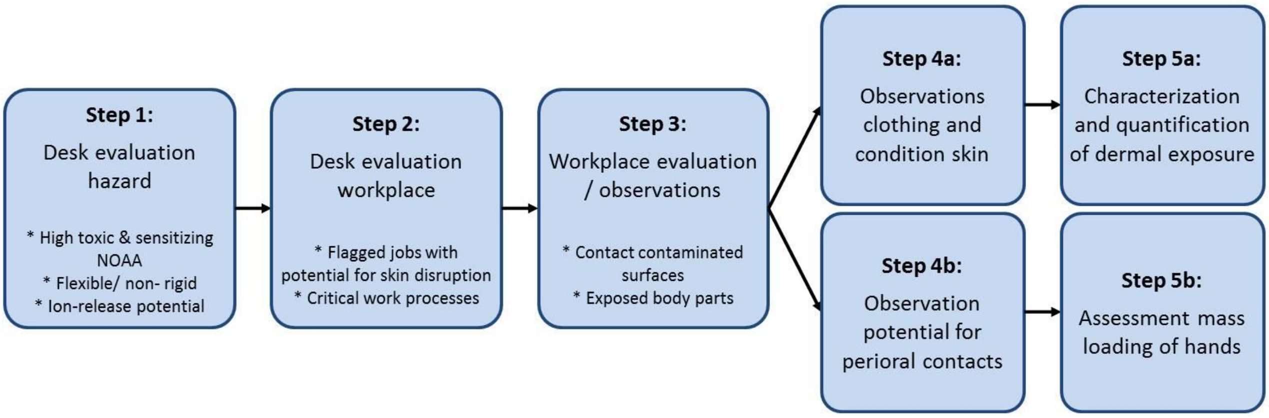

Figure 3

Figure 3: Schematic overview of primary evaluation based on composition of NOAA and following steps.

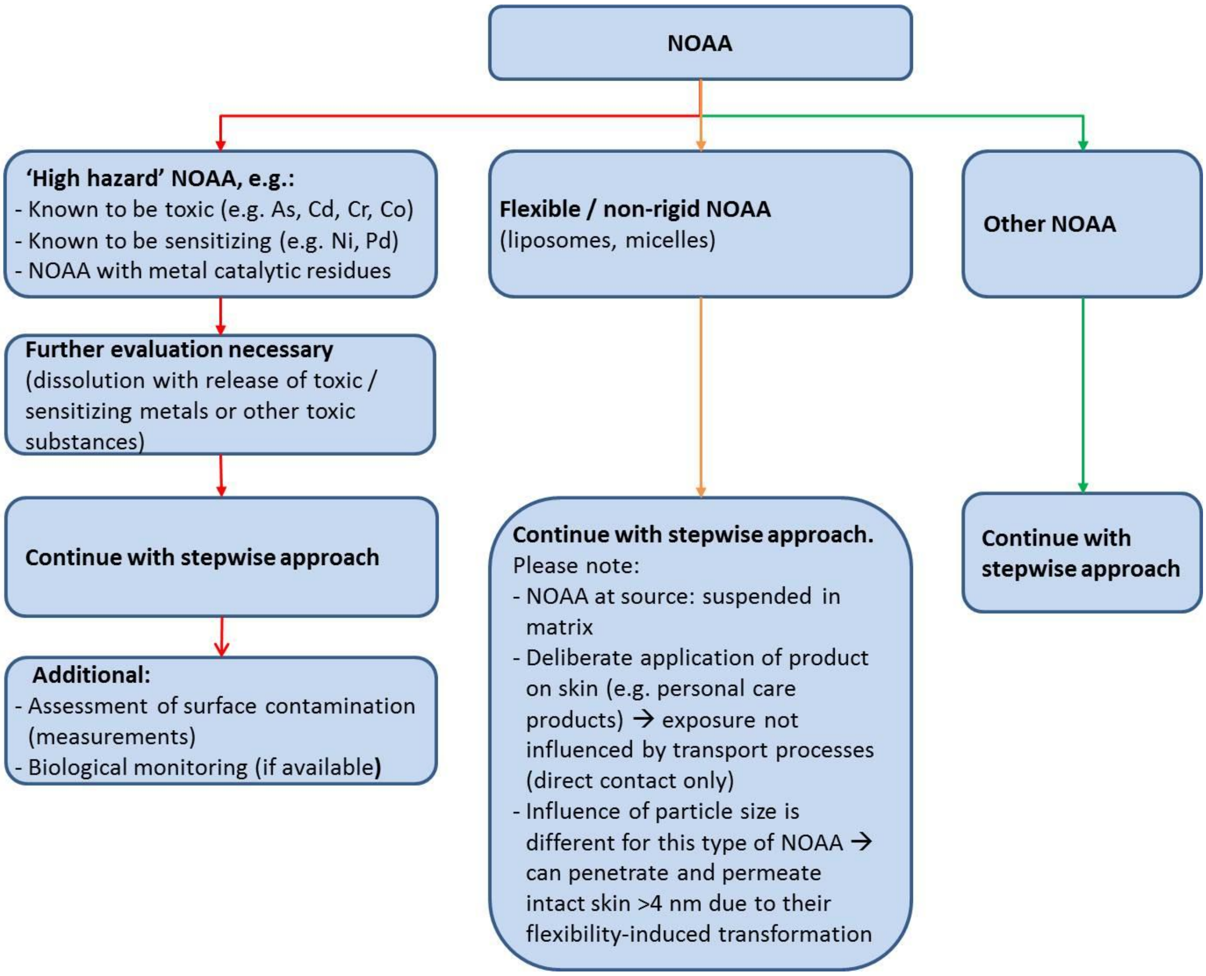

Table 1

Table 1: Some examples of relevant data on effect and penetration/permeation of NOAA

Nanomaterials	Examples	Critical size (nm)	Comment	Ref.
Carbon nanotubes		Not specified in the paper	Possible only irritation effects	Eedy 1996
Non-metal NPs	Fullerene	3.5	Penetration and permeation in flexures	Rouse 2007
	Silica	42	Penetration and permeation possible in damaged skin through follicles	Rancan 2012
Quantum dots	CdSe	4-12	Penetration and penetration possible and ions release	Chu 2007
Metal-oxides	TiO_2 ZnO	-	No penetration or permeation in vitro. One paper reports systemic absorption in vivo for ZnO containing cream (Gulson 2010)	Labouta 2011 (review)
	Fe_3 O	6-10	Possible permeation with blade incision (10 nm) – Penetration in intact skin (6 nm)	Lee 2010 Baroli 2007
Metal NPs	Fe, Ag, Co, Ni, Pd	12-25	They can release ions so permeation can be related to dissolution. They can cause sensitization (except for Fe)	Baroli 2007, Larese 2009-2015
	Au, Rh, Pt	12	They can't release ions in physiological conditions. Possible penetration.	Sonovane 2008, Larese 2011 Mauro 2015

Table 1S

Table S1. Polycyclic aromatic hydrocarbon (PAH) analysis of 18 diverse ENM using the US EPA Method 3546-microwave extraction coupled with method 8270 GC-MS SVOC analysis. The '< x' symbol indicates that the analyte was not positively identified in the sample, and the amount represents the maximum estimated concentration of particular analyte. All results are expressed in ng/g (ppb). The more abundant analytes (>0.5 µg/g) are highlighted in bold for easier observation. CB, carbon black

Sample [1]	Fluorene	Phenanthrene	Anthacenene	Fluoranthene	Pyrene	Benzo (a) anthracene	Chrysene	Benzo (b) fluoanthene	Benzo (k) fluoranthene	Benzo (a) pyrene	Indeno (1,2,3-cd) pyrene	Dibenz (a,h) anthracene	Benzo (ghi) perylene
CB N110	2	21	< 17	4	22	< 17	< 17	< 43	< 17	< 17	< 58	< 72	< 29
CB, N550	< 13	**846**	<13	**834**	**5659**	< 13	< 13	< 33	< 13	< 13	< 44	< 55	46
CB, N990	< 56	323	< 56	**1447**	**4765**	119	141	**1269**	**727**	**5252**	**13843**	< 234	**29531**
Fullerene, soot	2	17	< 24	< 24	< 24	< 24	< 24	< 60	< 24	< 24	< 80	< 100	< 40
Fullerene, refined	**4133**	**4693**	< 34	457	**743**	49	39	< 86	< 34	5	< 115	< 143	< 57
Fullerene, purified	39	73	< 78	18	46	< 78	< 78	< 195	< 78	< 78	< 260	< 325	< 130
SWCNT_L	95	189	< 37	< 37	< 37	< 37	< 37	< 93	< 37	< 37	< 124	< 155	< 62
SWCNT_S	21	88	< 25	< 25	< 25	< 25	< 25	< 63	< 25	< 25	< 84	< 105	< 42
MWCNT_S	20	59	< 13	< 13	< 13	< 13	< 13	< 32	< 13	< 13	< 43	< 53	< 21
MWCNT_I	61	**677**	< 33	95	147	< 33	< 33	< 84	< 33	< 33	< 111	< 139	< 56
MWCNT_L	34	136	< 37	19	22	< 38	< 38	< 94	< 38	< 38	< 125	< 156	< 63
SWCNH-ox	17	83	< 37	< 37	< 37	< 37	< 37	< 93	< 37	< 37	< 123	< 154	< 62
$nTiO_2$, Anatase	< 48	1	< 48	< 48	< 48	< 48	< 48	< 121	< 48	< 48	< 161	< 202	< 81
nAl_2O_3	< 33	14	< 33	< 32	< 33	< 33	< 33	< 81	< 33	< 33	< 108	< 136	< 54

[1] The source and physicochemical properties of this set of ENM has been described in detail in an earlier publication by Bello et al 2009. This set of PAH data has not been published previously. [Bello D, Hsieh SF, Schmidt D, Rogers EJ. 2009. Nanomaterials properties vs. biological oxidant damage: Implications for toxicity screening and exposure assessment. Nanotoxicology 3:249–261.] CB, carbon black; N110 (15 nm), N550 (44 nm), N990 (>200 nm). SW- (Single wall) and MW- (multi wall) CNT (carbon nanotubes), L long, S, short; I, industrial grade; nTiO2, nano titanium dioxide, nAl2O3, nano alumina.

Table 2S

Table S2. Organic carbon content for select ENM stratified by ENM type. Note the unit is µg/mg (parts per thousand). The OC% represents percent of the total carbon.

ENM Class	N	OC (µg/mg)				OC%
		GM	*GSD*	*Min*	*Max*	*Range*
MWCNT	19	192	2.1	26.4	510	2.2 - 54.3
SWCNT	6	192	1.3	111	258	9.2 - 26.8
Graphenes	5	138	1.3	103	189	10.1 - 32.6
CB	7	62	1.3	38	90	3.4 - 8.4
TiO_2	2	9.0	2.1	5.4	15.1	99.7 - 100
CeO_2	1	6.4	-	6.4	-	100
ZnO	1	3.3	-	-	-	100
ZrO_2	1	1.3	-	-	-	100

Table S3

Table 3. Summary of total and water-soluble metal impurities for select contact sensitizer and irritants by ENM type. Analysis was conducted by ICP-MC (microwave assisted acid digestion for total metal) and extraction with D.I. water (for 90 min at 37 ^{0}C). Units are in (µg/g, ppm). The minimum is the method limit of detection for each element (routinely in ng/g, ppb). All samples were above the limit of detection for Ni, Cr, Co, Pd, and As (except for ZnO). Zr was not detected in any of the carbonaceous samples.

ENM Class [1]	N	Type	Ni		Cr		Co		Pd		Zr		As	
			GM (GSD)	*Max*	*GM (GSD)*	*Max*	*GM (GSD)*	*Max*	*GM (GSD)*	*Max*	*GM (GSD)*	*Max*	*GM (GSD)*	*Max*
MWCNT	21	*Total*	816 (15)	11757	19 (3.7)	138	67 (14.8)	5656	0.05 (17)	4.8	-	-	0.1 (2.2)	0.7
		WS	1.3 (18)	208	0.04 (6.6)	2	0.2 (8.3)	11	0.001 (5)	0.1	-	-	0.008 (2.5)	0.03
SWCNT	7	*Total*	80 (1.8)	111	759 (1.6)	1931	2238 (1.3)	3225	0.08 (10)	2.5	-	-	0.18 (2.4)	0.7
		WS	0.6 (6.9)	3.4	0.4 (3.0)	3.1	7.0 (2.8)	28	0.001 (5.7)	0.01	-	-	0.006 (4)	0.03
Graphenes	6	*Total*	14 (14)	230	6.9 (20.7)	249	5.2 (02.8)	24	0.04 (4.4)	0.8	-	-	0.12 (1.9)	0.4
		WS	0.7 (99)	37.9	0.14 (50)	23	0.08 (27)	3	0.001 (4.1)	0.004	-	-	0.013 (2.5)	0.05
CB	8	*Total*	2 (1.3)	3	0.7 (2.4)	4.5	0.08 (2.0)	0.3	0.04 (2.8)	0.01	-	-	0.1 (1.8)	0.3
		WS	0.04 (2.2)	0.14	0.005 (2.5)	0.03	0.004 (4.0)	0.03	0.000 (3.1)	0.001	-	-	0.01 (1.8)	0.02
TiO_2	7	*Total*	0.7 (3.5)	3	0.3 (10)	4	0.09 (2.3)	0.2	0.06 (4.2)	0.2	21 (8.8)	166	4.3 (6.7)	51
		WS	0.09 (4.2)	0.4	0.7 (3.8)	1.8	0.008 (3.2)	0.04	0.002 (8)	0.01	0.1 (5.9)	3.4	0.6 (94)	14
CeO_2	2	*Total*	0.9 (1.1)	1	0.9 (2.3)	1.5	10.04 (1.9)	0.06	0.12 (1.2)	0.15	1664 (8.6)	5866	0.4 (1.3)	0.5
		WS	0.04 (4.3)	0.1	0.02 (1.7)	0.02	0.005 (-)	0.1	0.007 (1.3)	0.01	0.03 (2.0)	0.05	0.03 (1.1)	0.04
ZnO	2	*Total*	0.4 (35)	5	0.7 (3.8)	1.8	0.04 (5.1)	0.1	0.06 (1.5)	0.1	90 (59)	1600	1.2 (-)	1.2
		WS	0.1 (2.9)	0.2	0.01 (2.1)	0.02	0.03 (2.7)	0.06	0.001 (4.1)	0.04	0.067 (1.1)	0.072	-	-

1. The source, physicochemical properties and other descriptions have been published in a previous publication (Hsieh et al 2013 Small, 27;9(9-10):1853-65. doi: 10.1002/smll.201201995]. MWCNT, multi wall carbon nanotubes; SWCNT, single wall carbon nanotubes; CB, carbon blacks of different primary particle sizes (15 nm to >200nm), TiO_2, nanoscale titania of different phases and primary sizes. CeO_2, cerium oxide; ZnO, zinc oxide.

Table 4S

OCCUPATIONAL DERMAL EXPOSURE TO NANOOBJECTS, AND THEIR AGGLOMERATES AND AGGREGATES: Part I - Factors affecting skin absorption

Supplemental material

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Table S4. Scoring of skin condition to obtain skin disruption score index (SDSI) , showing a worked example for a worker with severe fissures on the whole back of both hands and moderate scaling and dryness on the whole palm of the right hand (resulting in a SDSI of 20 out of 180).

Clinica signs	Fingertips	Finger (exept tips)	Palm of hands	Back of hands	Wristes
Fissures (F)	0	0	0	3	0
Scaling (S)	0	0	2	0	0
Dryness (D)	0	0	2	0	0
SUM (F+S+D)	0	0	4	3	0
Extent (Ex)	0	0	2	4	0
Area Score (AS) (SUM*Ex)	$AS_{fingertips}$ =0	$AS_{fingers}$ = 0	AS_{palms} = 8	AS_{backs} = 12	$AS_{writsts}$ = 0
Total Disruption Score	$AS_{fingertips} + AS_{fingers} + AS_{palms} + AS_{backs} + AS_{wrists} = 0 + 0 + 8 + 12 + 0 = 20$				