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Grouping nanomaterials to predict their potential to induce pulmonary inflammation

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ABSTRACT

The rapidly expanding manufacturing, production and use of nanomaterials have raised concerns for both worker and consumer safety. Various studies have been published in which induction of pulmonary inflammation after inhalation exposure to nanomaterials has been described. Nanomaterials can vary in aspects such as size, shape, charge, crystallinity, chemical composition, and dissolution rate. Currently, efforts are made to increase the knowledge on the characteristics of nanomaterials that can be used to categorise them into hazard groups according to these characteristics. Grouping helps to gather information on nanomaterials in an efficient way with the aim to aid risk assessment. Here, we discuss different ways of grouping nanomaterials for their risk assessment after inhalation. Since the relation between single intrinsic particle characteristics and the severity of pulmonary inflammation is unknown, grouping of nanomaterials by their intrinsic characteristics alone is not sufficient to predict their risk after inhalation. The biokinetics of nanomaterials should be taken into account as that affects the dose present at a target site over time. The parameters determining the kinetic behaviour are not the same as the hazard-determining parameters. Furthermore, characteristics of nanomaterials change in the life-cycle, resulting in human exposure to different forms and doses of these nanomaterials. As information on the biokinetics and in situ characteristics of nanomaterials is essential but often lacking, efforts should be made to include these in testing strategies. Grouping nanomaterials will probably be of the most value to risk assessors when information on intrinsic characteristics, life-cycle, biokinetics and effects are all combined.

1. Introduction

In recent years, a large number of nanotechnology-enabled products have entered the global marketplace (Nanotechnologies, 2014). Exposure to nanomaterials is on the rise, and because of uncertainty regarding their toxic characteristics, concerns have arisen that such materials pose new health risks for consumers, workers, and the environment. Here, we focus on the potential of nanomaterials to induce pulmonary inflammation as inhalation is considered to be an important route of exposure to nanoparticles (Maynard and Kuempel, 2005; Christensen et al., 2010), especially in occupational settings. Many products, such as sprays, may likewise lead to inhalation by consumers (Oomen et al., 2011; Wijnhoven et al., 2010). Many studies report the induction of pulmonary inflammation after exposure to nanomaterials, largely indicated by an influx of neutrophils that can be observed in the bronchoalveolar lavage fluid in vivo and the induction of inflammatory cytokines in in vitro lung models (eg. Ji et al., 2007; Pauluhn, 2011; Sung et al., 2009; Yang et al., 2008; Oberdorster et al., 2000; Warheit et al., 2007a, 2007b; Pauluhn, 2009).

Nanomaterials are composed of primary and agglomerated particles that can vary in size, shape, charge, crystallinity, chemical composition, surface properties and other characteristics, and this variety will increase even further in the future ((SCENIHR) et al., 2010). All these characteristics have been suggested to affect the toxicity of nanomaterials, but not all existing and emerging types of nanomaterials can be tested separately in studies to evaluate their safety. Therefore, many efforts are made to increase the knowledge on the toxicity-determining characteristics of nanomaterials to categorize them into hazard groups according to these characteristics (Arts et al., 2015; Bolt, 2014; Braakhuis et al., 2014; Oomen et al., 2014; Sayes et al., 2013) in order to facilitate risk assessment.

In many cases, the grouping concept implies that information on physiochemical characteristics is available, and information on the hazard of a nanomaterial for one or more specific endpoint(s) can be derived from the respective bulk material, from molecule or ions of its constituents, or from similar nanomaterials with the aim to obtain sufficient and relevant data for risk assessment and avoid unnecessary new testing and making hazard assessment more efficient (Arts et al., 2014, 2015). In the present paper, we discuss different ways of grouping

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nanomaterials for their risk assessment after inhalation. We use an imaginary product, a deodorant containing silver nanoparticles, as an example to show how different ways of grouping can aid risk assessment.

1.1. Grouping nanomaterials by their intrinsic physicochemical characteristics

In a previous published review, we used data of scientific papers to discuss the nanomaterial characteristics influencing the deposition, clearance, and interactions in the lungs that, in combination, ultimately determine whether pulmonary inflammation will occur and to what extent (Braakhuis et al., 2014). Lung deposition is mainly determined by the physical characteristics of the aerosol (size, density, shape, hygroscopicity) in relation to airflow and the anatomy of the respiratory system. Nanoparticles with a primary/agglomerate size of <100 nm, or an agglomerate size between 100 nm and 1 μm with a high density, will deposit efficiently in the alveolar region (Asgharian et al., 2009; Carvalho et al., 2011; Cassee et al., 2002; Donaldson et al., 2010; Geraets et al., 2012; Hinds, 1982; ICRP, 1994; Kreyling et al., 2009; Landsiedel et al., 2010; Noel et al., 2012; Oberdorster et al., 1994, 2000; Sanchez et al., 2012; Varghese and Gangamma, 2009). Non-hygroscopic nanoparticles will not grow in size by water uptake, resulting in a higher chance to reach the alveoli (Varghese and Gangamma, 2009). Clearance and translocation of nanoparticles are mainly determined by their geometry and surface characteristics: 1) particles/agglomerates of <100 nm are less efficiently phagocytised compared to microparticles (Bakand et al., 2012; Muhlfeld et al., 2008a; Phalen et al., 2010; Geiser, 2010; Geiser et al., 2008), 2) nanofibres and –platelets are less efficiently cleared compared to spheres (Donaldson et al., 2012; McClellan and Henderson, 1995; Schinwald et al., 2012a; Porter et al., 2012; Schinwald et al., 2012b), 3) chemical composition influences clearance rate (Landsiedel et al., 2010; Kreyling et al., 2009; Heinrich et al., 1995; Wang et al., 2010), 4) charged nanoparticles attract proteins and reduce their clearance (Choi et al., 2007, 2010; Gessner et al., 2002), and 5) none or slowly dissolving nanoparticles are less efficiently cleared compared to fast dissolving nanoparticles (Kreyling, 1992; Koch and Stober, 2001). After deposition of nanoparticles in the alveoli, different particle characteristics influence the induction of pulmonary inflammation. As some nanoparticles dissolve, they can release toxic ions that can damage the lung tissue, making dissolution rate an important characteristic that affects lung inflammation (Cho et al., 2011, 2012a, 2012b; Donaldson et al., 2013; Nel et al., 2009). Fibre-shaped materials are more toxic to the lungs compared to spherical shaped nanoparticles of the same chemical composition (Porter et al., 2013; Shvedova et al., 2005; Stoehr et al., 2011). In general, cationic nanoparticles are easily taken up by cells and more cytotoxic than neutral or anionic nanoparticles (Choi et al., 2010; Hornung et al., 2008; Yazdi et al., 2010; Zhang et al., 2011; Asati et al., 2010; Nagy et al., 2012). Finally, nanoparticles with a high surface reactivity can damage the lungs (van Ravenzwaay et al., 2009; Warheit et al., 2007a, 2007b; Moller et al., 2010). Nanoparticles have a larger percentage of surface molecules compared to their 'bulk' counterparts (Oberdorster et al., 2005; Donaldson et al., 2001). Surface reactivity is the potency of particles to react with the immediate environment by inducing reactive oxygen species (ROS) (Elsaesser and Howard, 2012; Nel et al., 2006; Cheng et al., 2013; Muhlfeld et al., 2008b), leakage of constituents, and other biochemical reactions. It depends on the chemical composition, shape, size, solubility, and surface area of particles (Knaapen et al., 2004; Moller et al., 2010). With all these characteristics affecting different stages of the events leading to pulmonary inflammation, no unifying dose metric could be identified to describe pulmonary inflammation for all nanomaterials. If one process is rate determining or dominant for other reasons, this might result in a suitable dose metric for the specific situation, as sometimes seems to be the case for surface reactivity.

Although the review of Braakhuis et al. identifies many physicochemical characteristics of nanomaterials that affect their lung deposition, clearance, and pulmonary response that, in combination, ultimately determine whether pulmonary inflammation will occur and to what extent, the relation between single characteristics and the severity of pulmonary inflammation remains unknown. When using a deodorant contacting silver nanoparticles, the physicochemical characteristics of the silver nanoparticles that were added to the product are not sufficient to predict their potential risk during use of the deodorant. In addition, the physicochemical characteristics of nanomaterials change during their life-cycle, for example due to agglomeration or aggregation, corona formation or dissolution during production and use (Braakhuis et al., 2014; Oomen et al., 2014). Humans can thus be exposed to different forms of a nanomaterial. The silver nanoparticles in the deodorant might be completely dissolved during or after production, or consumers can be exposed to large agglomerates that do not reach the alveoli. The interdependence of intrinsic material characteristics and biological systems leading to a toxic effect are not yet understood, at least in part by the changes in nanomaterial characteristics throughout their lifetime and their biokinetic behaviour (Arts et al., 2015; Oomen et al., 2014). Therefore, the grouping of nanomaterials should also consider changes in physicochemical characteristics in the life-cycle, and not only concentrate on their intrinsic physicochemical characteristics to predict if they might induce pulmonary inflammation (Arts et al., 2015).

1.2. Grouping nanomaterials by their in situ characteristics

To unravel the relation between specific nanoparticle characteristics and an adverse effect, information on the biokinetics of nanoparticles is essential. The uptake/adsorption/deposition, distribution, corona formation and elimination (ADCE) of nanomaterials determine the form and dose of the nanomaterials at the site of action. A complicating factor is that these processes are driven by other physicochemical characteristics or other combinations of physicochemical characteristics of nanoparticles than hazard, as mentioned in the section above. In order to group nanomaterials on their potential to induce pulmonary inflammation according to their characteristics, the characteristics that influence their ADCE should be identified and used to determine the characteristics and dose of the nanomaterials at the site of toxicity (in situ characteristics). For the deodorant containing silver nanoparticles, geometry and surface characteristics (particle size, shape, surface charge and dissolution) of the nanoparticles in the product determine the ADCE of the silver nanoparticles. However, the in situ characteristics are different from the characteristics during product use as during inhalation the silver nanoparticles can agglomerate, dissolve or a protein corona is formed. It is difficult to characterize nanomaterials in situ (Card and Magnuson, 2010; Oomen et al., 2014), efforts should be made to improve detection techniques that can characterize nanomaterials and their surface molecules in tissues and biological fluids. These techniques are not widely available yet and therefore nanomaterials could be characterized in the appropriate biological media to mimic the in situ characteristics.

Knowledge on the intrinsic physicochemical characteristics of nanomaterials, their ADCE (biokinetics), and the characteristics at the site of toxicity help to group nanomaterials by toxicity-determining characteristics. The biokinetics relate the intrinsic nanomaterial characteristics with the level of nanomaterials and their characteristics at the site of toxicity in time. For the silver nanoparticles in deodorant, the characteristics of the nanoparticles in the product could be related to the characteristics at the site of toxicity using biokinetics information (Fig. 1). Once the principles behind these relationships are revealed, computer models can be developed that can predict the characteristics of nanomaterials at the site of toxicity from their intrinsic characteristics. Then, nanomaterials can be grouped by their toxicity-determining characteristics. Oomen et al. proposed a targeted (concern-driven) testing strategy that includes biopersistence, fate and biokinetics as well as

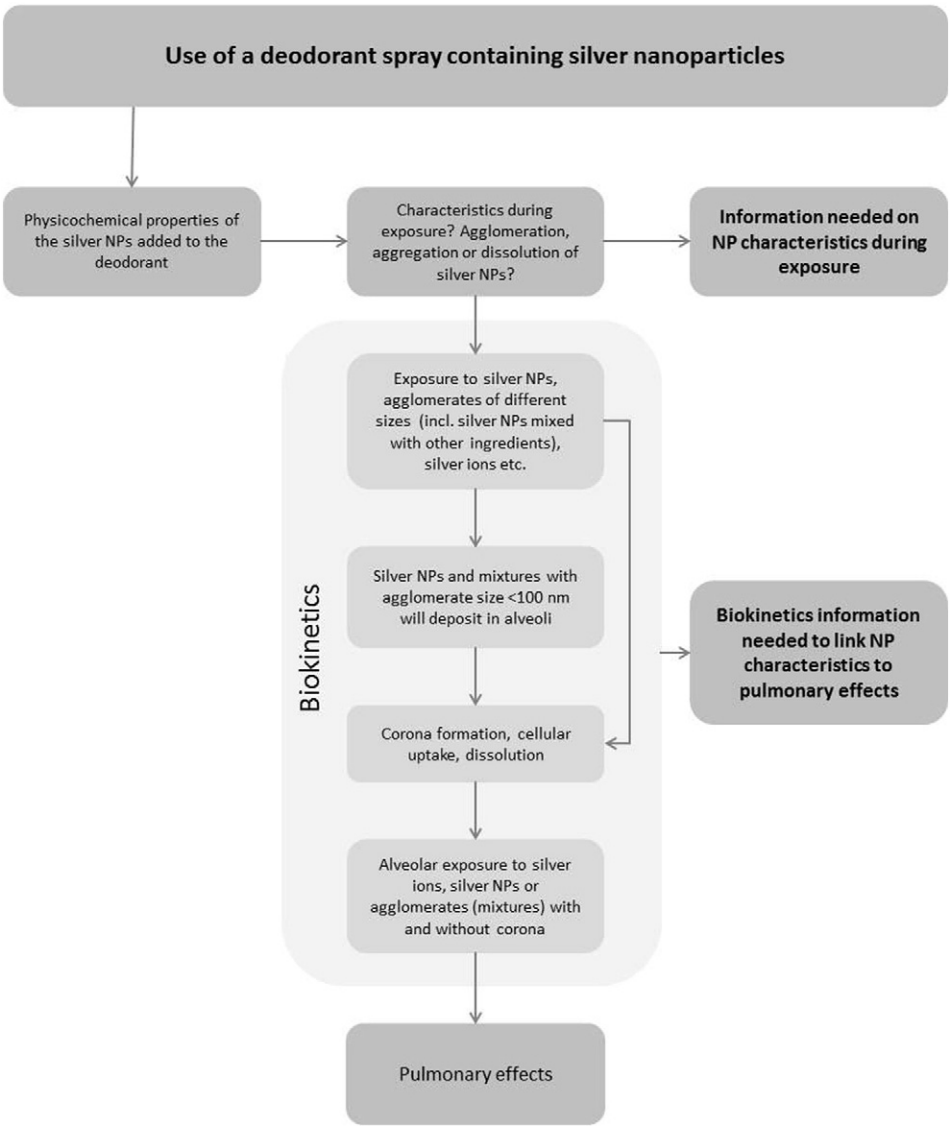

Fig. 1. Information needed for the grouping of nanomaterials, using silver nanoparticles in a deodorant spray as an example. In order to link physicochemical characteristics of nanomaterials to an effect, information on their biokinetic behaviour is needed.

effects of nanomaterials, that can be used as guidance for grouping of nanomaterials (Oomen et al., 2014).

1.3. Grouping nanomaterials by their mode of action

Nanomaterials have specific physicochemical characteristics because of their small size. However, none of the in vivo and in vitro studies with nanomaterials found 'nanospecific' mechanisms of action. Observed effects from nanomaterials versus their bulk counterparts can mostly be explained by a quantitative difference due to differences in exposure, biokinetics (mainly related to absorption and distribution) and toxicity (Gebel et al., 2014). As mentioned above, the exact correlation of intrinsic material characteristics and observed toxic effects is not yet established. Therefore, Gebel et al. proposes to use the 'functionality' of nanomaterials for grouping rather than relying on intrinsic material characteristics alone. The functionality of nanomaterials refers to their mode of action. More specifically, it is proposed to group nanomaterials into 3 groups according to the route of exposure and mode of action. In the first category, nanomaterials are included for which toxicity is mediated by the specific chemical characteristics of its components, such as released ions or functional groups on the surface. Nanomaterials in this category have to be evaluated on a case-by-case basis, depending on their chemical identity. The second category focuses on rigid biopersistent respirable fibrous nanomaterials of >5 μm length, <3 μm diameter and a high aspect ratio of $>3{:}1$. For these fibres, hazard assessment can be based on the experiences with asbestos. The third category focuses on respirable granular biodurable nanoparticles (GBP). Their toxicity is not mediated by specific chemical surface groups and they are not fibrous, but they are poorly soluble and persistent. After inhalation, GBP may cause inflammation and secondary mutagenicity that may finally lead to lung cancer (Gebel et al., 2014). Silver nanoparticles in deodorant would belong in the first category as they can release silver ions that might induce pulmonary inflammation. Some nanomaterials can be assigned to more than one category for example by surface coatings on GBP nanomaterials. These categories help to structure the large amount of nanomaterials. The link, however, between the categories and the induction of adverse effects like pulmonary inflammation is not clear. Within the categories, experiments are needed to investigate the biokinetics and effects of nanomaterials to predict their potential to induce pulmonary inflammation.

The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) 'Nano Task Force' also proposes the grouping of nanomaterials by their specific mode-of-action that results in a toxic effect (Arts et al., 2015). They recently published a decision-making

framework for the grouping and testing of nanomaterials (DF4nanoGrouping), taking into account previously published papers on grouping of nanomaterials. The framework consists of 3 tiers to assign nanomaterials to 4 main groups. These groups are 1) soluble nanomaterials, 2) biopersistent high aspect ratio nanomaterials, 3) passive nanomaterials, and 4) active nanomaterials. The framework covers intrinsic material characteristics and aspects of a nanomaterial's life cycle like system-dependent characteristics, biopersistence, uptake and biodistribution, and effects. By including the groups in a decision-making framework, this could be used in practise (Arts et al., 2015).

This kind of grouping will most likely and for most cases not be sufficient to perform a risk assessment and fully demonstrate safe use. Yet, the more is known about relationships between physicochemical characteristics, kinetic behaviour and toxicity, the more targeted potented risks can be studied. In addition, these relationships can be used to substantiate read-across of nanomaterials, as suggested by Oomen et al. (2015). It may be possible to study the relative hazard potential of a series of nanomaterials by in vitro assays that are aimed at a specific mode of action. This information should be linked to the biokinetic information, as a very hazardous nanomaterial may not be able to reach the target side and vice versa.

1.4. Conclusions on grouping nanomaterials

There are no specific regulatory frameworks or guidance documents for the grouping of nanomaterials (yet). Although some studies showed a link between surface reactivity and the induction of pulmonary inflammation, the relation between single characteristics and the severity of pulmonary inflammation remains unknown. In addition, the recent advances in the field of nanotoxicology and the recently published papers on grouping of nanomaterials all suggest that using the intrinsic nanomaterial characteristics alone is not sufficient (Arts et al., 2015; Gebel et al., 2014; Oomen et al., 2014). The life-cycle of nanomaterials should be taken into account as their characteristics change over time during their lifetime resulting in human exposure to different forms and doses of these nanomaterials, as well as their biokinetics. However, there is a lack of information on the biokinetic behaviour of nanomaterials and their in situ characteristics at the site of toxicity and more experiments are needed to elucidate these aspects. Grouping approaches that combine information on intrinsic characteristics, life-cycle, biokinetics and effects of nanomaterials are helpful for risk assessment. In addition, grouping can prevent unnecessary testing by gathering and combining information in a structured way.

Competing interests

The authors declare that they have no competing interests.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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