Concern-driven integrated approaches for the grouping, testing and assessment of nanomaterials

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NM's potential to induce adverse effects in humans or the environment is being addressed in numerous research projects, and methods and tools for NM hazard identification and risk assessment are advancing. This article describes how integrated approaches for the testing and assessment of NMs can ensure the safety of nanomaterials, while adhering to the 3Rs principle.

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1. Introduction

Along with the increasing use of NMs, their potential to induce adverse effects in humans or the environment is being addressed in numerous research projects (Landsiedel et al., 2012a, 2014a,b; Stone et al., 2014). Several authors have expressed the opinion that a generally applicable paradigm for NM hazard identification and risk assessment is not yet available while others claim that the safety assessment of chemicals is generally applicable to NM, but needs to be complemented. (Hankin et al., 2011; Anzai et al., 2012; ECHA, 2012a, 2014). Considering the expected number of NMs and of their modifications regarding particle size, shape, or surface properties and different uses and life cycle stages, maximum regulatory information requirements, e.g. in accordance to the European chemicals regulation (Anon., 2006), for every single variant of a given NM would lead to an insurmountable amount of testing.

The present article outlines how integrated approaches for the testing and assessment (IATAs) of NMs can ensure the safety of nanomaterials, while reducing costs and expenditure for testing and replacing, reducing, and refining animal testing in accordance with the 3Rs principle (Russell and Burch, 1959) implemented in European legislation (Anon., 2010). IATAs streamline information requirements to data that are relevant for risk assessment. Grouping of NMs forms an intrinsic part of IATAs to determine and refine concerns until risks can be assessed (Oomen et al., 2014a,b).

2. Grouping of nanomaterials based on source-to-adverse-outcome pathways

As compared to classical read-across and grouping approaches correlating a substance's structural properties with its biological activity (ECHA, 2008, 2012b; 2013; Patlewicz et al., 2013; OECD, 2014), NM complexity requires developing more comprehensive approaches for the grouping of NMs (Kuempel et al., 2012; Scott-Fordsmand et al., 2014; Hristozov et al., 2014; Oomen et al. 2014a,b; Arts et al. 2014). Exposure to NMs does not occur to a distinct type of molecule, but to a diverse population of primary particles, aggregates, and agglomerates of various sizes and different surface coatings that further may change over the life cycle of the NM (Liu et al., 2012). Physico-chemical properties of NMs affect biokinetics and hazard potential. Various material properties may have an impact on site, extent and type of biological response, and this may confound the correlation between one single material property and an apical effect. So far, no single physical or chemical material property of NMs perfectly correlates with their observed biological effects (Thomas et al., 2011; Landsiedel et al., 2012b; Zuin et al., 2011; Nel et al., 2013a; Godwin et al. 2015).

Consequently, NM grouping should not be restricted to the determination of nanostructure-activity relationships, but take into account the substance’s entire source-to-adverse-outcome pathway (SAOP; Fig. 1). Adverse outcome pathways link molecular initiating events (the interactions of substances with biological target tissues resulting in cellular responses) to adverse outcomes
that are directly relevant for a given risk assessment context. Accordingly, SAOPs describe all steps of the evolvement of effects starting from a substance’s first release (Ankley et al., 2010; OECD, 2012a,b; Arts et al. 2014).

Taking into account the different steps of the SAOP, facilitates multi-perspective grouping, i.e. grouping according to (1) NM production, use and release over the entire NM life cycle; (2) NM physico-chemical characteristics, which can differ in different life cycle stages; (3) NM uptake, biodistribution and biopersistence in an organism taking into account the different physico-chemical characteristics of the NM in different tissues; (4) the NMs’ early and apical biological effects. Accordingly, one single NM can be assigned to a variety of different groups — or, possibly, no group at all (Fig. 2; Oomen et al., 2014a,b).

The first aspect of the SAOP addresses how the respective NM has been incorporated into the given product, e.g. if it is embedded in a matrix or prevails on the surface of the product, and how these properties change during the life cycle of the product (Fig. 1). The subsequent aspect of the SAOP (‘dispersion’ in Fig. 1) relates to the ‘external exposure’ to the NM, i.e. the level and physico-chemical form of the NM exposure outside the body. The external exposure determines how the NM can be taken up into the organism, and it is dependent upon the various life cycle stages, use and releases of the NM (Landsiedel et al., 2010, 2012b; Hristozov et al., 2013). For many NMs, inhalation is the most important route of occupational exposure (Landsiedel et al., 2012a, 2014a). However oral, dermal, and, for specific medical use scenarios, injection routes may also be relevant (Oberdorster, 2010; Landsiedel et al., 2012b).

NMs may be assigned to different ‘exposure, release, and use groups’. Potential release of the NM from the product is a decisive parameter for further (sub-)grouping. For instance, NMs introduced into cement are firmly and irrevocably embedded in a solid matrix irrespective of subsequent mechanical machining or wear (Brau et al., 2012; Wohlleben et al., 2011, 2013). Regardless of chemical

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composition, such NMs are assigned to a common group of NMs that are not released from their products. NMs use in car tyres may be released in traffic (UJA, 2014), just as NMs introduced into varnish and paints might be released during weathering and abrasion (Wohlleben et al., 2011). NMs used as cosmetic ingredients are assigned to a ‘use group’ of high consumer proximity (Anzai et al., 2012), whereas their assignment to a ‘release group’ depends on the type of the final product, e.g. if it is an aerosol, gel, or suspension. ‘Uptake’ and ‘internal exposure’ of an NM refers to its concentration and physico-chemical form at the site of action in the organism. NMs can change their surface properties, e.g. by interacting with proteins and phospholipids from the surrounding biological fluids, whereby a ‘corona’ forms on the NM surface (Nel et al., 2009; Lundqvist et al., 2011; Wohlleben, 2012; Schieh et al., 2013). Depending on their physico-chemical characteristics (e.g. dispersibility, hydrophobicity), NMs might penetrate external barriers (skin, lung, gastrointestinal tract) or internal barriers (blood–brain, blood–testis, placenta). Systemic uptake and effects of NM are generally low (Moreno-Horn and Gebel, 2014) and if taken up systemically, NMs tend to disappear rapidly from the blood, mainly by being taken up into tissues containing phagocytic cells. Once taken up, NMs are hardly eliminated from macrophages, or the body (Lankveld et al., 2010; Dan et al., 2012). On the other hand, NMs used in sunscreen formulations are effectively blocked from entering the body by intact (Gamer et al., 2006) or sunburned skin (Montero-Riviere et al., 2011).

As regards grouping of NMs by early biological effects, ion shedding metal and metal oxide NMs can be grouped by their common property of dissolving toxic ions. Poorly soluble metal oxide NMs can be grouped by their capacity to generate reactive oxygen species, to activate pro-inflammatory reactions, or to damage cell membranes. Persistent long-aspect-ratio NMs, such as carbon nanotubes (CNTs), can be grouped by their potential to cause sustained inflammations which might progress to pulmonary fibrosis (Xia et al., 2008; Meng et al., 2009; Damaoiseaux et al., 2011; Wang et al., 2011; Zhang et al., 2012; Castranova et al., 2013; Nel et al., 2013a,b; Landsiedel et al., 2014 Sauer et al., 2014a). One type of NM may be able to induce more than one early biological effect. To date, no biological effects of NM have been reported which, per se, had not yet been observed with non-nanoparticle substances (Landsiedel et al., 2008, 2010, 2012a; Godwin et al., 2015).

Finally, NMs can be grouped by their potential to induce apical effects. A broad spectrum of metal oxide and carbon-based NMs has been successfully grouped by no-observed-adverse-effect-levels determined in rat short-term inhalation studies (STIS). The STIS study protocol encompasses a 5-day exposure and 3-week post-exposure observation period and provides information on NM local and systemic hazard potency and the progression or reversibility of effects (Ma-Hock et al., 2009; Klein et al., 2012; Landsiedel et al., 2014a). BaSO4, ZrO2, and surface-functionalized SiO2 NMs were ranked as being of very low respiratory toxicity, whereas MWCNTs were ranked as being of higher respiratory toxicity, and ZnO, TiO2 and CeO2 NMs were ranked in between (Landsiedel et al., 2014a). A battery of assays to measure bio–physical interactions and cellular effects may be adequate for grouping nanomaterials without the need for in vivo studies (Arts et al., 2015; Godwin et al., 2015).

3. Integrated approaches for the testing and assessment of nanomaterials

Grouping of NMs by all aspects subsumed in the SAOP directly serves to guide NM testing and assessment. In fact grouping and testing strategies are often guided by the very same decision criteria. The first step of an IATA aims at identifying ‘NMs of concern’. Concerns should be based upon realistic exposure and use scenarios (Oomen et al., 2014a,b; Schilter et al., 2014). Many NMs will not raise specific concerns, but will, e.g., readily dissolve (before internalization and intracellular release of relevant amounts of cytotoxic ions), whereas poorly soluble NMs might possess properties raising concerns for toxic effects.

Based upon identified concerns, crucial human health and environmental endpoints are determined for investigation in focused studies. In the early tiers of the IATA, existing information is collected for a preliminary assessment of relevant concerns. As necessary, the IATA proceeds through further tiers during which first basic and then increasingly complex information on exposure, biokinetics and hazard of the NM is obtained. Determining the biokinetic behaviour of NM forms an essential part of IATAs and should involve addressing NM absorption/uptake, distribution, corona formation, and elimination/deposition. By contrast, for most insoluble NMs, metabolism appears to play a negligible role (Landsiedel et al., 2012b).

Accordingly, testing, if necessary, begins with basic tests followed by increasingly specific tests, using in vitro test systems addressing relevant toxicity pathways, up until short-term and long-term in vivo studies. If inhalation is a relevant route of exposure, the STIS can provide comprehensive early-tier hazard and biokinetic information (Landsiedel et al., 2014a); for the oral route of exposure a short-term oral study (STOS) protocol was recently introduced (SUN, 2015). Exposure, hazard and risk assessment should be performed for the pristine material and, in situ, at all critical life cycle stages of the NM (ECHA, 2014). Overall, standardized methods should be applied for testing, just as for physicochemical characterization. Appropriate NM dispersion protocols should be used for test substance preparation (Sager et al., 2007; Schulze et al., 2008; Wang et al., 2010; Sauer et al., 2015); this includes in situ characterization and considerations on the nanoparticle material as tested compared to the nanomaterial as seen in real life exposure. In vitro studies should be performed with relevant test substance concentrations reflecting effective dosages applied in vivo (Teeguarden et al., 2007; Oberdörster, 2010; Rushton et al., 2010). Besides test item preparation and dosage, dose-rates should be considered in designing a toxicological study on nanomaterials (e.g. instillation bolus versus inhalation; cf. Brain et al., 1976).

After each tier, risk assessment is performed, i.e. all information on exposure, hazard, and biokinetics obtained so far is combined to characterize the risk. The decision to proceed to subsequent tiers does not only take into account the acceptability or unacceptability of the assessed risk, but also the associated uncertainty level. Hazard assessment can stop at any given tier as soon as there is sufficient certainty on the conclusion to be drawn. Recently different groups have suggested grouping and testing approaches based on the principles outlined here (Gebel et al., 2014; Arts et al., 2015; Godwin et al., 2015).

4. Conclusion

Grouping of NMs by exposure, use and release should be applied to determine NMs of concern and can be complemented by data on NM’s biopersistence and biokinetics. This information should be used to streamline testing to investigations that are relevant for NM risk assessment. Grouping of NMs by their biophysical interactions and early biological should form an intrinsic part of all subsequent tiers of the IATA with the aim of refining concerns until risk assessment can be performed; in vivo studies for apical toxic effects could thus be minimized and restricted to nanomaterials serving as
reference for a group or those nanomaterials which could not be assigned to a group. This approach to NM risk assessment serves to ensure product safety of a large variety of NM in different modi-

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