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Research paper

Challenges on the toxicological predictions of engineered nanoparticles

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ABSTRACT

The perceived enormous potential of nanotechnology in contributing to sustainable innovation has led to the growth of investments into new industrial applications and consumer products. However, the lack of tools that are needed to generate early knowledge about the potential adverse effects, combined with the uncertainties regarding the health and safety risks of engineered nanoparticles (ENPs), are a potential threat to the acceptability by society of the nanotechnology innovations, due to the rising societal concerns that are based on generic worries. In order to tackle these issues, it has been necessary to adopt a more proactive approach into nanotechnology safety assessments. Multiple projects have been initiated around the world in order to understand how ENPs interact with living organisms, but the validation of most of the emerging knowledge may take years. This is while robust risk assessment results are urgently needed, in order to support timely regulatory decisions and risk management actions. The goal of this paper has been to review the present knowledge on the physicochemical characteristics of ENPs, focusing on titanium dioxide (TiO_2), gold (Au), copper oxide (CuO), and zinc oxide (ZnO), as well as on their biological interactions. In addition, the paper has been aimed at the identification of the main challenges on the current toxicological characterisation of these ENPs. Focus will also be given in this article to those ENPs that have been described by the Consumer Product Inventory as having prevalent nanomaterials present in consumer products, but also, with those having therapeutic and diagnostic applications, due to their physical (ex: confined plasmon resonances) and biological (biocompatibility and antimicrobial) properties.

Abbreviations: ENPs, Engineered Nanoparticles; TiO_2, titanium dioxide; Au, gold; CuO, copper oxide; ZnO, zinc oxide; MNM, manufactured nanomaterials; GHS, globally harmonized system; 3Rs, three Rs principles; QSAR, quantitative structure–activity relationship; DLS, dynamic light scattering; NTA, Nanoparticle Tracking Analysis; FFF, Flow Field-Flow Fractionation Analysis; GLC-TEM, Graphene Liquid Cells - Transmission Electron Microscopy; EELS, electron energy-loss spectroscopy; Cu^{2+}, copper ions; Zn^{2+}, zinc ions; ICP-MS, Inductively Coupled Plasma Mass Spectrometry; IARC, International Agency for Research on Cancer; FDA, Food and Drug Administration; FP, fine particles; NIOSH, National Institute for Occupational Safety and Health; NEDO, New Energy and Industrial Technology Development Organization; ROS, reactive oxygen species; OECD, Organisation for Economic Co-operation and Development; SCCS, Scientific Committee on Consumer Safety; A549, Lung Carcinoma Cell Line; THP-1, Human Monocytic Cell Line; MAPKs, activated protein kinases; ERK, Extracellular Signal–Regulated Kinases; JNK, c-Jun N-terminal Protein Kinase; hESCs, Human Embryonic Stem Cells; SOPs, standard operating procedures; GLP, good laboratory practice; PLGA, poly (lactic-*co*-glycolic acid; MTT, water-soluble tetrazolium salts (WST-1), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; XTT, 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H–tetrazolium-5-carboxanilide; ELISA, Enzyme-linked Immunosorbent Assay; AgNPs, silver nanoparticles; TNFα, tumour necrosis factor alpha; HTS, high throughput screening; HCA, High Content Analysis; RMs, proper reference materials

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1. Introduction

Humans and other living organisms are continuously exposed to nanometer-sized materials (Buzea et al., 2007; Oberdörster, 2010; Aschberger et al., 2011). Modern science has learned how to synthesise tailored nanomaterials by manipulating matter at the atomic scale, in order to have well-defined properties for specific purposes. These so called engineered nanoparticles (ENPs) are commonly used in therapeutics, cosmetics, sporting goods, tyres, stain-resistant clothing, sunscreens, toothpaste, and food additives, among many others (Buzea et al., 2007; Oberdörster, 2010; Becker et al., 2011). In fact, intentionally produced nanometer-sized particles are inhaled every day. They are absorbed through the skin (when using consumer care products) and/or they are consumed in processed food and beverages (Buzea et al., 2007; Aschberger et al., 2011; Chen et al., 2016; Monteiro-Riviere et al., 2011; Smijs and Pavel, 2011; Jeon et al., 2016).

Most of these nanoparticles are expected to cause little or no effects on human health and be unnoticed. But in some cases, they might cause appreciable harm to organisms (Buzea et al., 2007; Becker et al., 2011; Martirosyan and Schneider, 2014). The amount of man-made nanomaterials ranges from several million tons/year (*e.g.* carbon black for car tyres) to microgram quantities for fluorescent quantum dot markers for biological imaging (Schulte et al., 2013; Bogart et al., 2014). As a consequence, workers and consumers are exposed to potentially hazardous substances when they are involved in activities such as research, development, synthesis, and the usage of ENPs or ENP-containing products (Buzea et al., 2007; Bogart et al., 2014; Bitounis et al., 2015). The lungs, the gastrointestinal tract, as well as human skin, are the most likely points of entry for ENPs into the human body. Injections (*e.g.* ENPs for drug delivery) and biomedical implants (ENPs generated by surface degradation) are other feasible routes of exposure to these engineered materials (Margarethe et al., 2015).

The lack of communication by stakeholders, as well as issues in the regulatory robustness of data (exposure and toxicological studies), together with that is generated by unsuitable methods, are factors that are potentially increasing the risk perceptions by consumers, and at the same time, decreasing perceptions of the benefits (Grobe et al., 2012). This is while a lack of robust knowledge contributes to regulators' insecurity, such that this too, has the potentiality to nurture public fear, in the light of nano-related media-driven accidents, hence, restraining the economic development of manufactured nanomaterials (MNM).

As referred to above, there are already a reasonable number of products in the marketplace, as reported in Woodrow Wilson's Database/Consumer Product Inventory (http://www.nanotechproject.org/cpi/), as well as in the periodic reporting of the French Registry of Nanomaterials (https://www.r-nano.fr/?locale=en). Among the 1814 products that are listed in the Consumer Product Inventory, 47% of them advertise the composition of at least one nanomaterial component (Vance et al., 2015). Titanium dioxide, zinc oxide, gold, and copper oxide are considered by the Consumer Product Inventory to be the most prevalent nanomaterials present in consumer products (Vance et al., 2015). Nevertheless, currently, the available industry-derived data regarding ENPs is limited (Becker et al., 2011; Vance et al., 2015). Essential information is not being incorporated on Safety Data Sheets. However, it is also not clear how nanomaterials should be classified and labelled, in order to follow the globally harmonized system (GHS) (Schulte et al., 2013; Hodson et al., 2009).

Some precautionary guidelines and recommendations for the safe handling of ENPs have been produced by organisations and agencies around the world, in order to protect their workers (Schulte et al., 2013; Hodson et al., 2009). The current regulatory frameworks for risk assessment (RA) are in principle applicable to ENPs, but adjustments are considered necessary, at least in terms of the testing guidelines (Schulte et al., 2013; Hristozov and Malsch, 2009; Hristozov et al., 2012, 2014; Seaton et al., 2010; Landsiedel et al., 2010; Steiling et al., 2014; Petersen et al., 2015; Kühnel et al., 2016; Schwirn et al., 2014). The principles of chemical risk assessments do not reflect some important properties of ENPs (size, specific surface area, reactivity) that are considered to be determinants of their toxicity (Schwirn et al., 2014). The risk assessments of ENPs are a massive task, because the regulatory frameworks require a case-by-case approach (Hodson et al., 2009; Hristozov and Malsch, 2009). Due to the huge number of existing and emerging ENPs, RAs are time and money consuming, conflicting with the three R principles (3Rs) of to replace, reduce and refine animal testing (Oomen et al., 2000). Significant developments overcoming these limitations (*e.g.* intelligent testing and grouping strategies), in favour of effective regulatory control, are under evaluation (Stone et al., 2014; Arts et al., 2015).

The scientific community is working hard in order to develop methods and tools that regulators can apply to a wide array of nanomaterials (overcoming the need of case-by-case assessments). The development of standardised methods and new risk assessment tools, such as foresight approaches, tiered schemes, grouping schemes, quantitative structure–activity relationship models (QSAR models), safe-by-design approaches, high throughput and high content methods, are some of the present strategies. These methods are now being followed-up by technical and scientific communities (Schwirn et al., 2014; Stone et al., 2014). In addition, there is a clear trend for the development of decision supporting frameworks that are based upon iterative dialogues, the engagement of all stakeholders, as well as considerations for the socio-economic, cultural and political contexts (Oomen et al., 2000). These complementary approaches can also serve as research prioritisation tools, which can help industry in identifying the relevant sources of risk in ENP life-cycles and pinpoint the areas of knowledge deficits (Stone et al., 2014; Arts et al., 2015).

This review has aimed to: (1) highlight the important aspects of the physicochemical characteristics of ENPs, focusing on titanium dioxide (TiO_2), gold (Au), copper oxide (CuO), and zinc oxide (ZnO) and their biological interactions; and (2) identify the main challenges on the current toxicological characterisation of these ENPs. Two of the NPs that have been reviewed in this work are inert (TiO_2 and Au), while the other two (CuO and ZnO) are known to release metal ions, resulting in a Trojan-horse mechanism of toxicity.

2. Nanoparticle physicochemical characteristics and their biological relationships

The field of nanotoxicology aims to establish the relationships between nanoparticle physicochemical properties and their toxic potentials. In fact, nanoparticle toxicity depends upon various physicochemical characteristics, such as size, number, mass, aggregation, composition, crystallinity, surface functionalisation, among many others (Pettitt and Lead, 2013). Some of the physicochemical properties that are relevant for toxicological studies are reported in Table 1. However, it is still a challenge to identify the physicochemical parameters which are most relevant for eventual adverse health effects. In the last few years, different publications have come out regarding the nanoparticle characterisation required, in order to evaluate human health hazards from ENPs. In some of them, there is some overlap on the proposed parameters that are being considered as essential or desirable (Oberdörster et al., 2005; Emond et al., 2013). The biological effects of ENPs are affected by their physicochemical properties, such as size, surface area, solubility, shape, crystalline structure, surface charge, catalytic activity, and chemistry, as well as by their number. Most probably, it will not be single parameters, but various combinations that need to be considered, in order to be decisive on their ENP toxicities.

Systematic studies concerning which physicochemical properties are the most relevant for hazard assessments have revealed the following rankings (Orts-Gil et al., 2013): surface area (100%), elemental composition (96%), surface chemistry (89%), particle size (86%), particle size distribution (86%), surface charge (86%), agglomeration state