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Review article

Reliability of methods and data for regulatory assessment of nanomaterial risks

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ABSTRACT

Within the EU funded project Prosafe a review on the regulatory relevance of the results of several EU and US funded nanosafety research projects was conducted. The objective was to identify those methods, data and protocols that are appropriate for regulatory risk assessment of manufactured nanomaterials. A task force with nine experienced experts was established which examined approximately 1000 publications. They looked at reliability and regulatory relevance as the main criteria to identify which research products are most useful to regulators. The results of this review are published in this Special Issue. This article summarizes the main recommendations, identifies the most relevant knowledge gaps and draws some general conclusions. The review demonstrates that a number of new tools to enable regulatory risk assessment of nanomaterials are now available or near completion.

1. Introduction

According to the National Academy of Sciences (NAS) risk assessment of chemical substances includes four steps: hazard identification, hazard characterization, exposure assessment and risk characterization (National Research Council, 1983). Risk can be determined by comparing hazard and exposure. While the traditional risk assessment paradigm holds for nanomaterials, many of the test guidelines and guidance documents for assessment of physico-chemical properties, fate, exposure and effects used for conventional chemicals need to be modified when applied to nanomaterials (OECD, 2010, 2011). Other test guidelines and guidances – taking into account the differing characteristics of nanomaterials – may require more extensive modifications or replacements to address relevant regulatory endpoints. There is also a lack of adequate, reproducible data to validate risk assessment strategies for manufactured nanomaterials (MNs) and develop a science-based understanding of how to quantify and predict the potential risks of many nanomaterials. A complication when testing and assessing nanomaterials is that the characteristics, fate and effects may change from one nanoform to another slight variation of the same nanoform, thus affecting evaluations throughout the life cycle of the nanomaterial.

Since not every individual variation in the characteristics of a nanomaterial can be tested sufficiently for risk assessment, approaches for tiered testing schemes, read-across and grouping, as well as modelling approaches and *in silico* methods are urgently needed. The rapid development of nanotechnology makes it essential to develop risk assessment methods which are reliable, relevant, easy to perform, and cost-effective for regulatory purposes, in order to keep pace with rapid technological developments and guaranteeing, as much as possible, their safe use.

The EU funded ProSafe project supported the aims of EU Member States in their EU and international efforts (OECD; http://www.oecd.org/science/nanosafety/, and EU-US CORs; http://us-eu.org/communities-of-research/) regarding risk assessment, management and governance focussing on regulatory oriented toxicology testing of nanomaterials, exposure monitoring, life cycle assessment, and disposal and treatment of waste nanomaterials. Within the project the regulatory relevance of the outcomes of 16 European projects, funded under FP7 and H2020 schemes, one German project and available results from the OECD have been reviewed. First, a detailed set of regulatory related questions were identified and referred to as the Roadmap (Sayre et al., 2017).

A task force of nine international experts was established to carry out this study, who looked firstly at the reliability, and secondly the regulatory relevance of the outcomes from the selected projects. The experts were supported by scientists with specialized expertise. The task force experts were nominated for nine areas of concern which were identified: (i) physicochemical characterization, (ii) exposure through the lifecycle, (iii) fate – persistence – bioaccumulation; (iv) modelling of environmental fate and exposure, (v) ecological effects and biokinetics,

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Table 1
Summary of key physicochemical properties, their preferred measurement metrics, and strengths/weaknesses.

Categories of physical chemical property metrics	Key/preferred measurement methods	Strengths/limitations/knowledge gaps
Intrinsic properties:		
Particle size distribution (number average)	1. Electron Microscopy (for equivalent diameter) 2. DLS/FFF/centrifuge (for hydrodynamic diameter	1. EM is the only method that can distinguish primary particles in aggregates. This is likely the best measurement of PSD to fit with current EC definition. But sample preparation can skew results. Quantitative estimates of PSD can be achieved with automated EM image processing; method is partially validated. 2. DLS has data interpretation problems, especially for polydispersity samples and oddly-shaped structures, but could serve as a first tier assessment for monodisperse particles.
Particle shape (*e.g.* aspect ratio)	Electron microscopy	1. 2-D projection of 3-D images can complicate interpretations 2. Dispersion protocol standardization needed. 3. High aspect ratio materials are difficult to determine due to limited field of view.
Surface area	1. Specific surface area by gas sorption 2. VSSA calculated from specific surface area	1. Specific surface area by gas sorption is a reliable method for powders, but interpretation of data requires assumptions about pore geometry. Also, aggregation and coatings can affect surface area measurements. 2. VSSA is not valid for polydispersity particle systems; values are model-dependent.
Redox potential/band gap	1. Band gap measured by UV–vis-NIR absorption measurements of the dry powder of the material; probe force microscopy; soft X-ray methods	1. Band gap measurements are influenced by size, charge, the presence of an adsorbed layer, and the solution properties. 2. Probe force microscopy and soft X-ray methods can be more accurate than UV–vis-NIR absorption methods, but are more laborious. 3. Band gap measurements for nanomaterials have not yet been validated.
Crystalline phase(s)	1. X-ray diffraction 2. EM, with electron diffraction	1. Both methods are reasonably reliable. 2. Both methods only applicable to crystalline powders. 3. Both methods lose reliability for particles < 10 nm 4. XRD sensitivity to phases is generally ~ 1 wt% or greater. 5. Electron diffraction is slower than XRD, more expensive, and can be more difficult to interpret.
Hydrophobicity	1. Sorption of a probe molecule 2. Contact angle measurement 3. K_{ow} 4. Hydrophobic interaction chromatography	1. No validated methods exist for hydrophobicity/data are scarce. Sorption of a dye and contact angle measurements are more feasible. 2. Methods correlate with each other and with expected relative hydrophobicity a. Absolute measurement is not validated. b. The K_{ow} method has limited applicability to nanomaterials due to difficulty or impossibility to reach an equilibrium state.
Chemical composition (impurities, surface chemistry)	• ICP-MS for inorganic composition • TGA for organic coatings • EM and XPS for inorganic shell	1. All 3 Methods are considered robust and reliable 2. ICP-MS gives no spatial information 3. Most methods provide information only on presence/absence • Quantification and nanomaterial structural information requires more expensive and complicated methods, *e.g.* XPS or TGA-MS, and often requires interpretation of multiple lines of evidence.
Rigidity	1. Young's Modulus estimation 2. AFM force-displacement estimation	1. Both methods have limited data to judge their applicability to MNs and reliability.
Extrinsic properties ("Where they go; persistence"):		
Biodurability	1. *In vivo* and *in vitro* methods applicable to mammalian fluids/tissues/cell cultures 2. Acellular dissolution in physiological fluids 3. Environmental dissolution 4. Environmental biodegradation	1. Methods for mammalian biodurability estimation *(in vivo* and *in vitro)* exist, but still need validation 2. Acellular dissolution tests with physiological fluids exist (see 'Dissolution rate' below) 3. Environmental dissolution test guideline is under development within the OECD: relevant, and reliability assessment ongoing. 4. Environmental biodegradation for carbonaceous materials may be assessed *via* adaptation of existing OECD biodegradation test guidelines.
Zeta potential	Electrophoretic Methods	1. Electrophoretic methods are: • Reliable • Good to $\sim \pm 2$ mV or 10% (whichever is greater) • Affected by pH, Ionic strength, coatings 2. Necessary to report EPM and associated metadata to make zeta potential measurements scientifically useful 3. Determination and reporting of a nanomaterial's isoelectric point (pH_{iep}) may be more comparable across materials 4. Limitations: • Organic coatings complicate calculation of zeta potential • Interferences due to media-induced agglomeration or electrode blackening • Lack of clear reporting guidelines
Density (including effects of milieu)	1. Gas pycnometry for powders 2. Analytical centrifugation for MNs in water	1. Gas pycnometry is reliable, but requires a large sample size for analysis 2. Analytical centrifugation is reliable, but expensive and not commonly available. 3. A new benchtop centrifugation method appears reliable, based on a small data set, and is less cost- and time-intensive and more readily

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