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Nanoscale reference materials for environmental, health and safety measurements: needs, gaps and opportunities

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Abstract

The authors critically reviewed published lists of nano-objects and their physico-chemical properties deemed important for risk assessment and discussed metrological challenges associated with the development of nanoscale reference materials (RMs). Five lists were identified that contained 25 (classes of) nano-objects; only four (gold, silicon dioxide, silver, titanium dioxide) appeared on all lists. Twenty-three properties were identified for characterisation; only (specific) surface area appeared on all lists. The key themes that emerged from this review were: 1) various groups have prioritised nano-objects for development as "candidate RMs" with limited consensus; 2) a lack of harmonised terminology hinders accurate description of many nano-object properties; 3) many properties identified for characterisation are ill-defined or qualitative and hence are not metrologically traceable; 4) standardised protocols are critically needed for characterisation of nano-objects as delivered in relevant media and as administered to toxicological models; 5) the measurement processes being used to characterise a nano-object must be understood because instruments may measure a given sample in a different way; 6) appropriate RMs should be used for both accurate instrument calibration and for more general testing purposes (e.g., protocol validation); 7) there is a need to clarify that where RMs are not available, if "(representative) test materials" that lack reference or certified values may be useful for toxicology testing and 8) there is a need for consensus building within the nanotechnology and environmental, health and safety communities to prioritise RM needs and better define the required properties and (physical or chemical) forms of the candidate materials.

Vocabulary

Engineered nanomaterial: a material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale, and which is designed for a specific purpose or function (ISO 2010a).

Nanoscale: size range from approximately 1 to 100 nm (ASTM 2006; ISO 2010a).

Nano-object: a material with one, two or three external dimensions in the nanoscale (ISO 2010a).

Nanoparticle: a nano-object with all three external dimensions in the nanoscale (ISO 2008).

Reference material: material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process (ISO 1992).

Certified reference material: a reference material characterised by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability (ISO 1992).

Introduction

Engineered nanomaterials (ENMs) are a broad class of materials that includes nano-objects. A nano-object is a material with at least one dimension in the nanoscale

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(1–100 nm). Nano-objects can be further classified into nanoparticles that have all three orthogonal dimensions in the nanoscale, nanofibres that have two orthogonal dimensions in the nanoscale and nanoplates that have one dimension in the nanoscale (ISO 2008). While ENMs hold great promise for better performing products and improved quality of life, thorough and accurate characterisation of exposure and toxicity is needed for understanding and managing potential risks posed by exposure to workers, the public and the environment (Hassellöv et al. 2008; Murashov & Howard 2008; Schulte et al. 2008). Principally because of their size, nano-objects pose unique measurement challenges for characterisation (Weibel et al. 2005; Latham et al. 2006; Wang et al. 2007; Linsinger et al. 2011; Roebben et al. 2011a) and toxicity evaluations (Wörle-Knirsch et al. 2006; Balbus et al. 2007; Kroll et al. 2009; McNeil 2011).

Some material properties may change depending on the environment that the nano-object encounters. As such, there is a need to characterise not just the pristine (as received) nano-object, but also the nano-object as administered and after administration to an *in vitro* or *in vivo* system (Oberdörster et al. 2005; Dawson et al. 2012), as the latter will determine the bioavailable dose and the nature of the surface actually presented to the system (Naha et al. 2009; Walczyk et al. 2010). Unfortunately, few methods are available for characterisation of nano-object properties as administered (e.g., in air or cell culture media) and technical limitations preclude measurement of many properties after administration to a living organism. As a result, expedience and convenience frequently influence the type and completeness of characterisation performed (Dhawan et al. 2009). Hansen et al. (2007) reviewed over 400 published nanotoxicology studies and determined that the properties of nano-objects used in these investigations were often poorly described or not measured at all. Singh et al. (2009) reviewed genotoxicity studies and reported that almost 50% of published papers did not include any characterisation data and only 10% contained information on more than one nano-object physico-chemical property. Hence, incomplete characterisation of materials not only limits the reliability of conclusions that can be drawn from toxicity studies but also potentially impedes future research by introducing uncertainty in the interpretation of results (Hansen et al. 2007; Boczkowski & Hoet 2010; Gonzalez et al. 2008; Fubini et al. 2010). This uncertainty can impact commercialisation of nanotechnology by creating an uncertain safety and/or regulatory environment (NANO Risk Framework 2007).

Reference materials (RMs) are a means to assure or improve measurement quality. Generally, an RM is developed to address a specific measurement problem, for example, the verification of instruments for electrical resistivity measurements or the calibration of a guarded hot plate apparatus for thermal conductivity measurements. For nano-objects, there is broad interest in developing RMs to help assess risk (hazard and exposure potential), which may involve measurement of several properties. In the absence of nanoscale RMs for specific toxicity tests, undetected measurement error may contribute to substantial

uncertainties in estimates of nano-object hazard properties and therefore risk. It is generally recognised that one of the principal obstacles to obtaining adequate characterisation of nano-objects and their potential risk is the scarcity of reliable, that is, produced in a scientifically valid way, RMs for development and validation of exposure assessment tools (instruments, protocols, methods) and toxicological evaluations (materials, protocols) (The Royal Society 2004; Balbus et al. 2007; Handy & Shaw 2007; ICON 2008; NIST 2008; EFSA 2009; Kroll et al. 2009; Lynch et al. 2009; Riviere 2009; Fubini et al. 2010; Bouwmeester et al. 2011; Kumar et al. 2011). Nanoscale RMs can provide researchers with:

- suitable materials (including positive and negative controls) to develop harmonised protocols for *in vitro* and *in vivo* toxicity testing and elucidate mechanisms of toxicity resulting from nanoscale properties;
- materials to verify instrument or method performance and operator or laboratory proficiency thereby improving consistency in interpreting exposure and toxicity data.

It is worthwhile mentioning here that there often exist false expectations about RMs or inappropriate declaration of materials as RMs, resulting also in inappropriate use or reliance on RMs. It should be mentioned that production and certification of an RM is based on data measured on the candidate material. RMs therefore can only be generated when the state-of-the art of a particular scientific field has progressed to a sufficient level to make precise and reproducible measurements of the relevant physical, chemical and/or biological phenomena.

Worldwide, various groups (e.g., governments, treaty-based organisations, standards development organisations, research consortia) have more or less independently generated priority lists of nano-objects for potential development of RMs, and created lists of characterisation requirements for the RMs to better understand the results of exposure and toxicity assessments. It remains unclear whether these various groups share a common awareness or vision for the practical role to be served by nanoscale RMs, since they represent a diverse community of nanotechnology researchers, policy makers and regulators, each with different needs and perspectives. A substantial commitment in resources (both financial and time) is required to develop an RM and, more specifically, a certified RM (CRM), one that is accompanied by an appropriately determined measurement uncertainty and a detailed metrological traceability assessment, often produced by imprimatur of endorsement by formally recognised or government-mandated standards development bodies (Roebben et al. 2011a). In the case of nano-objects, progress towards development of nanoscale RMs is inhibited by: 1) the lack of user consensus on needs and applications; 2) the need for multiple-target measurements to enable safety testing as opposed to measurement of individual properties and 3) the fact that nanotechnology development has far outpaced toxicology and the development of scientifically sound, validated and widely adopted

Table I. Nano-objects of interest as nanoscale reference materials for risk assessment*.

NIST (2007)[†]	REFNANO[‡]	OECD (2010)[¶]	NanoImpactNet[§]	NanoValid[‖]
Dendrimers	Carbon black	Aluminium oxide	Polystyrene (f)	Copper oxide
Fullerenes	Combustion-derived	Cerium oxide	Silver	Fullerenes
Gold	Metals and their oxides (Cu, Fe)	Dendrimers	Titanium dioxide	Gold
Isotope labelled	MWCNT	Fullerenes	Combustion-derived	MWCNT
Metal oxides (Ce, Fe)	Polystyrene (f)	Gold	Copper/copper oxides, iron	Palladium
MWCNT	Silver	Iron	Fullerenes	Silicon dioxide
Quantum dots	SWCNT	MWCNT	Gold	Silver
Silicon dioxide (a, c)	Titanium dioxide	Nanoclays	Silicon dioxide	SWCNT
Silver (p, i)	Zinc oxide	Silicon dioxide		Titanium dioxide
SWCNT	Gold	Silver		Zinc oxide
Titanium dioxide	Cerium oxide	SWCNT		Calcium phosphate salt
	Silicon dioxide	Titanium dioxide		Cerium oxide
	Ceramics	Zinc oxide		Ceramics
	Rods, cubes, horns			Dendrimers
	Isotope labelled			Lead phosphate salt
	Nanoclays			Nanocellulose
				Nanoclays
				Quantum dots
				Polystyrene

*f = fluorescent; MWCNT = multi-walled carbon nanotubes; SWCNT = single-walled carbon nanotubes; a = amorphous; c = crystalline; p = particulate; i = ionic; [†]Nano-objects are listed without any implied priority; [‡]REFNANO: high priority materials are listed above the single horizontal line (listed alphabetically with no relative priority implied) and low priority materials are listed below the thick line in assigned order of priority (Aitken et al. 2008); [¶]Nano-objects are listed without any implied priority; [§]NanoImpactNet: Materials above single thick horizontal line were agreed upon by all three workshop discussion groups; materials between thick single horizontal line and thick double horizontal line were agreed upon by one or more but not all workshop groups; materials below thick double horizontal line were added based on perceived needs of ecotoxicology community. Within each grouping, materials are listed alphabetically with no relative priority implied (Stone et al. 2010); [‖]NanoValid: Priority 1 materials are listed above the single horizontal line and Priority 2 materials are listed below the line; within each grouping, materials are listed alphabetically with no relative priority implied (Reuther 2011).

characterisation methods needed to assign relevant measur- and values to RMs.

To ensure the development of maximally useful nano-scale RMs for risk assessment, there is a critical need for greater communication and coordination among those who utilise RMs and those capable and mandated to produce them. In this article, the authors reviewed existing lists of nano-objects and properties thought to be important for risk assessment and described the metrological considerations associated with developing nanoscale RMs for this purpose.

Lists of materials

Table I summarises the lists of nano-objects that have been identified by various groups as being important for risk assessment. With 25 individual nano-objects and/or classes of nano-objects on these lists, it is apparent that a wide variety of materials are under consideration by various groups around the world. The US national metrology insti- tute, the National Institute of Standards and Technology (NIST), hosted a workshop on "Standards for Environmental Health, and Safety Research Needs," which was attended primarily by government and academia, with some industry representation, and employed discussion groups to develop overall consensus recommendations of high priority nano-objects and material classes, (NIST 2007). The REFNANO project (funded by the UK) developed a list of nano-objects based on informed discussion and opinion-gathering of representatives from the toxicology, metrology and nano-object producer/user communities mainly in that country (Aitken et al. 2008). The Organization for Economic

Cooperation and Development (OECD), an international treaty-based organisation, through their Working Party on Manufactured Nanomaterials (WPMN) developed a list of "representative manufactured nanomaterials" and launched a sponsorship program for testing with the OECD acting as a clearinghouse for the program (OECD 2010). An objective of the NanoImpactNet project (funded by the European Commission) was to develop a priority list of candidate nano-objects for inclusion in a reference library for calibration and testing purposes. As part of this effort, Stone et al. (2010) reported a list of nano-objects from a workshop on ecotox- icology. Recently, Reuther (2011) reported on a new project called NanoValid (funded by the European Commission) which aims to develop reference methods using "test materials" based upon the OECD-WPMN list of nano-objects, as well as other standards bodies, for the purpose of early hazard identification, exposure assessment and risk identification.

Commonalities and differences among lists

A critical first step is to understand whether nano-objects overlap among lists or if there are great differences. As shown in Table I, only 4 of 25 nano-objects are common to all lists: gold, silicon dioxide, silver and titanium dioxide. Gold is available in aqueous suspensions (pH 7.2) from NIST as RMs 8011, 8012 and 8013 (each with a different technique-dependent reference values for size) to support biological testing of nano-objects. Sodium hydroxide stabilised amor- phous silica is available in suspension (pH 9.5) from the Institute for Reference Materials and Measurements (IRMM) as IRMM 304 with an information (non-certified) value for

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size and as ERM FD100 with several certified size values. The alkaline pH of the IRMM 304 and ERM FD100 RM suspensions may limit their applicability for biological assays. NIST is in production of PVP-capped silver nano-particles in two different sizes; the sizes and choice of capping agent align with materials chosen for the OECD-WPMN testing program on nano-silver. NIST and the Japanese National Institute of Advanced Industrial Science and Technology (AIST) are developing dry powder nano-scale titanium dioxide RMs; the NIST material now available as Standard Reference Material (SRM) 1898, is certified for specific surface area, but contains substantial informational values of interest to risk assessment (e.g., elemental com-position, purity, isoelectric point, primary crystallite size and detailed dispersion protocols for preparation in test media).

Five nano-objects (cerium oxide, fullerenes, iron and iron oxide, multi-wall carbon nanotubes (MWCNT) and single-wall carbon nanotubes (SWCNT)) were common to four lists. Both the National Research Council of Canada (NRC) and NIST are developing SWCNT RMs. NIST is developing three forms of SWCNTs, all from the same material batch. These RMs will include length-sorted (three sizes) SWCNT materi-als in suspension, "Bucky" paper which is a thin sheet of aggregated nanotubes, and the raw SWCNT soot in dry powder form. The currently available SWCNT soot carries the NIST designation SRM 2483 and has certified values for mass content of 10 metals and reference values for 7 other metals (Zeisler et al. 2011) along with information on oxi-dation temperature and the G/D Raman spectroscopy peak ratio. The SWCNT material being characterised by NRC Canada was made by a laser ablation process and therefore is catalyst-free, which is an important attribute for many toxicology tests (Wörle-Knirsch et al. 2006).

By contrast, 60% (16/25) of nano-objects appear on three or fewer lists: copper and copper oxides, dendrimers, nano-clays, zinc oxide, carbon black, combustion-derived materi-als, isotope-labelled materials, ceramics, quantum dots, aluminium oxide, rods and cubes and horns, nanocellulose, palladium, polystyrene, calcium phosphate salt and lead phosphate salt. NRC Canada is characterising a nanocrystal-line cellulose material in powder form and in suspension. Among currently available nanoscale RMs, polystyrene is most prevalent and is sold by the Chinese Institute for Metrology (NIM), AIST, ThermoScientific (previously Duke Scientific) and NIST. The NIM polystyrene RM is designated GBW-12011. AIST maintains traceability for polystyrene RMs (which are sold by JSR Corp.) in seven different sizes des-ignated SC-0030, -0040, -0050, -0060, -0070, -0080, -0090 and -0100. NIST has two polystyrene SRMs designated SRM 1963a and 1964. The polystyrene RMs from JSR Corp. and NIST have certified values for size. None of the available polystyrene RMs is fluorescent labelled.

A key message from Table I is that worldwide, groups are independently prioritising nano-objects for development as "candidate RMs" with limited consensus. Note that many of these groups classified nano-objects in variable ways, which highlights the need for improved communication and agree-ment regarding terminology. For example, "ceramics" cov-ers a very wide range of refractory materials including metal

oxides, nitrides and carbides, whereas "rods and cubes and horns" refers to shapes and/morphologies and does not define a specific material or composition. For additional information, readers are referred to the compilation of nanoscale RMs available worldwide (http://www.nano-refmat.bam.de/en/) created by the German Federal Institute for Materials Research and Testing (BAM). Of the 74 RMs on this list, 16 are potentially relevant to risk assessment appli-cations (though not all are suitable for biological applica-tions). An additional source of information on available nanoscale CRMs is available from the COMAR database.

Prioritisation of nano-objects on lists

The nano-objects identified by the NIST (2007) workshop participants and OECD (2010) were developed without any relative priority. The OECD list of nano-objects was derived based on needs of its 31 member countries in North America, Europe and the Asia and Pacific region. The selection was based on the need to assess risk from various nominal chemical compositions of nano-objects in com-merce at the time the list was developed, or materials soon to enter into commerce. Additional criteria were consid-ered, including production volume, likely availability of a material for testing, and existing available information on a material. The *REFNANO* list (Aitken et al. 2008) was devel-oped using criteria such as scale of material production and likelihood of exposure (i.e., high production volume); how the physico-chemical properties of the material are expected to interact with the living system and will be useful in answering particular (eco)toxicology questions and how the materials could be used in the context of standardised comparative studies. The list of nano-objects identified by the *REFNANO* report was divided into high and low priority materials (Table I). The high priority materials were identified to meet the needs of the toxicology, ecotoxicology and metrology communities and the order in which they are listed does not imply any relative priority. By contrast, the low priority materials are listed in an assigned order (from most to least) of relative priority (Aitken et al. 2008).

The *NanoImpactNet* workshop started with the 16 nano-objects on the *REFNANO* list and utilised discussion groups to adapt the list to ecotoxicology issues. Within the ecotox-icology community, there is disagreement over the role of RMs in risk assessment. Some investigators cite the lack of RMs as a constraint for performing risk assessments (Kumar et al. 2011), whereas others question the ability to select nano-objects based upon current knowledge and whether RMs are needed for testing (Crane et al. 2008; Handy et al. 2008a,b). This controversy within the ecotox-icology community is evident from Table I, which notes that the *NanoImpactNet* workshop participants reached little consensus on their list of nano-objects (Stone et al. 2010).

The *NanoValid* project divided candidate nano-objects into two priority groups. Selection and classification of nano-objects into groups was based on the OECD list, input from standards organisations and in consultation with other European Union nanotechnology research projects (Reuther 2011). The alignment with the OECD-WPMN list

Table II. Properties of interest for nano-object characterisation during risk assessment collected from published literature.

Property	SI units	Frequency(#/28 lists)	List source*
Surface area(specific)	m^2 (m^2/kg, m^2/m^3)	28	a – bb
Elemental/molecular composition (bulk)	mol/mol, kg/kg[†]	27	a – j, l – bb
Surface chemistry	mol/m^2	25	a – f, h, k – bb
Particle size	m	24	a, b, d – l, n – p, s – bb
Particle size distribution	–[‡]	24	b – d, f – w, z – bb
Morphology/shape/form	–[¶,§]	24	a – i, k, l, n – x, z, aa
Surface charge	–[‖]	24	a – h, k – t, v – aa
Agglomeration/aggregation state	-	20	a – g, j, l, m, o – s, w, y, z – bb
Crystal structure	–[&]	17	a, d – f, j, l, n, o, q – t, v, w, z – bb
Surface reactivity	–[#]	16	a – f, l, n – p, r, t, v, w, x, bb
Solubility (water)	mol/l, kg/kg, kg/m^3	14	a, e, h, l – n, p, r, t, v, w, x, z, aa
Dispersibility (dry/wet)	–[∫]	13	a, d, e, g, h, l – n, r, t, w, y, aa
Particle concentration[∞]	particles/m^3	8	a, g, h, l, m, p, q, z
Solubility (biological)	-	8	a – c, h, l, n, y, aa
Porosity (specific)	m^3/m^3(m^3/kg)	7	l, n, q – t, z
Stability	–[⌡]	6	c, h, m, p, t, v
Density	kg/m^3	4	n, r, t, v
Surface morphology/structure	-	4	e, l, t, w
Conductivity	–′	3	l, t, v
Defect density[+]	Defects/m^3, defects/kg	2	l, w
Hardness	-	2	t, v
Magnetic properties	-	2	e, v
Optical properties (refractive index)	1[	2	t, v

*a = Aitken et al. 2008; b = Balbus et al. 2007; c = Bouwmeester et al. 2011; d = Boverhof & David 2010 (adapted from the MINChar initiative, http://www. characterizationmatters.org); e = Bucher et al. 2004; f = Card & Magnuson 2009; g = Crane et al. 2008; h = EFSA 2009; i = Fubini et al. 2010; j = Gonzalez et al. 2008; k = Hankin et al. 2011; l = ICON 2007; m = Klaine et al. 2008; n = NANO Risk Framework 2007; o = NIST 2007; p = NIST 2008; q = Oberdörster et al. 2005; r = OECD 2010; s = Park et al. 2007; t = Powers et al. 2006; u = Sayes & Warheit 2009; v = SCENIHR 2007; w = SCENIHR 2009; x = Šimon & Joner 2008; y = Stone et al. 2010; z = Tiede et al. 2008; aa = Warheit et al. 2007; bb = Warheit 2008; [†]Heterogeneity, monomer chemistry, oxidation state, valency and speciation are qualitative properties and no unit can be assigned; [‡]It is assumed here that a particle size distribution is represented either by $dN(D_p)/dD_p$ or $dM(D_p)/dD_p$, where $N(D_p)$ and $M(D_p)$ denote cumulative number and mass concentrations, respectively, of particles smaller than size D_p. Particle size distribution may also be determined using a frequency histogram in which the relative amount of particles in a size range is plotted versus the mid-point size of the range. While there are many different ways to present size distribution data, generally none have SI units; [¶]Aspect ratio has non-dimensional units; [§]- = ill-defined or qualitative property and no unit can be assigned; [‖]Includes the terms "surface charge density" with SI units of C/m^2 and "zeta potential" with SI units of V; [&]Includes the terms "crystallite size" with SI units of m and physico-chemical structure which is a qualitative property and no unit can be assigned; [#]Redox potential has SI units of V and surface energy has SI units of J/m^2; [∫]Partition coefficient has non-dimensional units; [∞]The term "particles" is not an SI unit, but is used here for the sake of clarity. Note that various types of particle concentration exist: volume concentration (m^3/m^3), surface area concentration (m^2/m^3) and mass concentration (kg/m^3, mol/m^3, kg/kg); [⌡]Melting point has SI units of K; ′Includes the terms "Conductivity" with SI units of S/m and "electrochemical potential" with SI units of J/m; [+]The term "defects" is not an SI unit, but is used here for the sake of clarity; [1 = non-dimensional units.

is particularly evident for many of their Priority 1 group materials.

Lists of physico-chemical properties

Several lists of physico-chemical properties deemed important for nano-object characterisation as part of the risk assessment process (e.g., human toxicity, ecotoxicity, food safety, etc.) have been developed by various groups. These lists reflect the widely held belief that information on nano-object properties is needed to improve measurement quality (e.g., testing protocols, method development), facilitate inter-laboratory comparison of results and evaluate property-hazard correlations. Such information is needed for a wide range of purposes, including product stewardship, regulatory oversight (including defining nanomaterials), compliance verification, etc.

Compilation and organisation of these lists of properties for nano-object characterisation highlights the critical need for harmonised terminology in nanotechnology. Confusion exists because some terms are used interchangeably in many fields. The same terms have specific and often opposite meanings in certain fields (e.g., surface charge vs. zeta potential). In some publications as reviewed by the

authors, distinctions were made between related terms whereas in others terms were used interchangeably. Hence, for the purposes of this review, when applicable, related properties were grouped under broader concepts as follows:

- Elemental/molecular composition (bulk) was used to denote the related terms "purity/impurity", "heterogeneity", "monomer chemistry", "oxidation state", "speciation" and "valency".
- Surface chemistry denotes terms such as "adsorbed species" and "surface contamination".
- Morphology/shape/form includes "aspect ratio" and "physical form" of the nano-object.
- Agglomeration/aggregation state: in nanotechnology, "agglomeration" has been used to infer reversibility with weak physical forces dominating, while "aggregation" infers irreversibility with strong and rigid connections between the constituent particles (e.g., fused crystallites) (ISO 2008). Since many lists did not specify the intended property, aggregation and agglomeration were grouped together.
- Surface charge denotes the terms "charge", "surface charge" and "zeta potential". In many publications,

these terms are used interchangeably, despite differences in these properties. Charge and surface charge were assumed to refer to the charge that arises from the adsorption or desorption of protons on hydroxylated sites on a nano-object surface. Zeta potential refers to shear-plane charge near the surface of a nano-object in suspension, and is used as an indicator of colloidal stability.

- Crystal structure includes the terms "crystallite size" and "physico-chemical structure".
- Solubility (biological): terms such as "dissolution", "biodurability", "biodegradability", "persistence" and "solubility" were also seemingly used interchangeably in several studies. However, dissolution refers to the rate and degree to which a material dissolves in a liquid. If that liquid is a biological fluid or a surrogate, the rate of dissolution is referred to as biodurability (Oberdörster 2002). Persistence (or bio-persistence) is the rate at which a material is physically cleared, for example, from the lung by mucocilliary action, cell-mediated action and dissolution. In the absence of specific information, these terms were grouped together.
- Dispersibility (dry/wet) refers to similar terms such as "hydrophobicity", "hydrophilicity", "lipophilicity", "amphiphilic character", "partition coefficient" and "dustiness".
- Surface reactivity is a grouping for terms such as "surface tension", "surface reactivity", "reactive oxygen species generation", "photocatalytic activity", "redox potential" and "catalytic activity".
- Surface morphology/structure is distinguished from the bulk nano-object and includes "surface morphology", "surface structure" and "surface roughness".
- Stability refers to resistance to destabilisation via thermal, photochemical and biodegradation mechanisms but is distinguished from "solubility (water)", which is a chemical equilibrium phenomenon and "solubility (biological)" as described above.
- Conductivity includes both "electrical conductivity" and "electrochemical potential".

NanoImpactNet has produced a freely available terminology guide, based on the ISO nomenclature and contributions from the scientific community, which is periodically updated and aims to reduce such confusion in terminology (Clift et al. 2010). Additionally, ISO nomenclature can be directly consulted through the publically available ISO Concepts Database (http://cdb.iso.org).

Table II summarises 23 different physico-chemical material properties that were identified as important for nano-object characterisation as part of the risk assessment process. These properties were compiled from 28 different lists. In addition to these material properties, other information deemed pertinent included synthesis method, suspension medium and storage conditions. Some properties listed in Table II such as hardness, melting point and refractive index are relevant for understanding material properties but may not have toxicological significance. Although not listed

in Table II, a Nanomaterial Registry (Beta version: www.nanomaterialregistry.org) has recently been launched to provide a web-based registry of curated information necessary to convey biological or environmental interactions of well-characterised nano-objects. The project is funded by the National Institute of Biomedical Imaging and Bioengineering, the National Institute of Environmental Health Sciences and the US National Cancer Institute under a contract to RTI International. A principal component of this effort is the development of minimum information about nanomaterials (MIAN) that goes beyond simply creating a list of physico-chemical properties and characteristics; it also includes information related to the techniques, instruments and protocols used to determine those characteristics, and assigns a quality value to the curated data based on the provided ancillary information (thus promoting the use of standards, protocols and RMs).

Commonalities and differences among lists

The only property that appeared on all 28 lists was surface area (or specific surface area) (Table II). The next most cited property was elemental/molecular composition (bulk) (27/28 lists) followed by surface chemistry (25/28 lists); size, size distribution, morphology/shape/form, surface charge (24/28 lists); agglomeration/aggregation state (20/28 lists); crystal structure (17/28 lists); surface reactivity (16/28 lists) and solubility (water) (14/28 lists). The remaining properties appeared on less than half of these lists. Whether this divergence in perceived needs for nano-object characterisation is a reflection of differences in opinion or specific requirements of a scientific discipline is unclear. In an attempt to elucidate the source of disagreement, these lists were grouped by discipline (toxicology, ecotoxicology, food safety and risk assessment) and the properties ranked by frequency. Among the 17 lists developed for toxicology testing (Bucher et al. 2004; Oberdörster et al. 2005; Powers et al. 2006; Balbus et al. 2007; ICON 2007; NIST 2007; Warheit et al. 2007; Aitken et al. 2008; Gonzalez et al. 2008; NIST 2008; Warheit 2008; Hackley et al. 2009; Sayes & Warheit 2009; Boverhof & David 2010; Fubini et al. 2010; OECD 2010; Bouwmeester et al. 2011; Hankin et al. 2011), full consensus was reached only for surface area and elemental/molecular composition (bulk). For ecotoxicology testing (Park et al. 2007; Warheit et al. 2007; Crane et al. 2008; Klaine et al. 2008; Stone et al. 2010), full consensus was reached on 4 of 15 properties identified on these lists (surface area, elemental/molecular composition (bulk), agglomeration/aggregation state, surface charge). Among the lists developed for food safety testing (EFSA 2009; Šimon & Joner 2008; Tiede et al. 2008; Card & Magnuson 2009), there was consensus on six of 16 nano-object properties identified on these lists (surface area, elemental/molecular composition (bulk), particle size, surface chemistry, morphology/shape/form and surface charge). Among the 23 properties identified on the general risk assessment lists (NANO Risk Framework 2007; SCENIHR 2007, 2009), there was consensus on the same six nano-object properties for food safety plus particle

Table III. "Priority" properties of interest for nano-object characterisation during toxicological assessment.

Property	Frequency (#/18 lists)	List source*
Elemental/molecular composition (bulk)	16	a – i, k, l, n – p
Surface area (specific)	14	a – c, e, f – i, k – p
Particle size	13	a, c, e – j, l – p
Morphology/shape/form	13	a – g, i – l, o
Particle size distribution	12	b, c, e – i, k, m, o, p
Surface chemistry	12	a – c, e, h – n, p
Agglomeration/aggregation state	11	b, c, e, f, h, j, k, m, n, p
Crystal structure	10	a, c – e, h, i, k, l, p
Surface charge	9	b, c, e, f, i – k, n
Dispersability (dry/wet)	7	c, f, i, j, l – n
Surface reactivity	6	b, c, e, i, m, p
Particle concentration	4	a, f, k
Solubility (biological)	4	b, i, n, o
Solubility (water)	3	i, j, m
Density	1	i
Porosity (specific)	1	i
Stability	1	b
Surface morphology/structure	1	d

*a = Aitken et al. 2008; b = Bouwmeester et al. 2011; c = Boverhof & David 2010 (adapted from the MINChar initiative, http://www.characterizationmatters.org); d = Bucher et al. 2004; e = Card & Magnuson 2009; f = Crane et al. 2008; g = Fubini et al. 2010; h = Gonzalez et al. 2008; i = NANO Risk Framework 2007; j = NIST 2008; k = Oberdörster et al. 2005 (three separate lists); l = Powers et al. 2006; m = SCENIHR 2009; n = Stone et al. 2010; o = Warheit et al. 2007; p = Warheit 2008.

size distribution, crystal structure and solubility (water). Hence, within certain disciplines (food safety, general risk assessment) there appears to be some consensus on which nano-object properties are important for characterisation.

Prioritisation of properties

It is generally accepted that complete characterisation of all nano-object properties is not feasible nor would it be necessary to meet all study goals. As such, many groups have designated certain "priority" properties (also called "base" or "minimum" or "essential" properties) for characterisation as part of any study. Eighteen of the 28 lists in Table II contain priority properties of nano-object characterisation. Only one of these 18 lists, that by Warheit (2008), ranked the minimum properties in order of relative priority (from highest to lowest): size and size distribution and surface area > crystallinity > aggregation state > composition and surface coatings > surface reactivity > method of synthesis > purity. No rationale was provided for this ordering and it could be argued for different priorities based on the findings of more recently published studies (Lundqvist et al. 2008; Adler & Leong 2010). Table III summarises the 18 lists of nano-object properties that have been deemed as priorities for characterisation. Note that Oberdörster et al. (2005) developed three lists of priority nano-object properties, one for human exposure studies and two for in vitro and in vivo toxicology studies (as-received and as-administered nano-objects). Prioritisation reduced the number of properties from 23 to 18. No single property appeared on all priority lists, the most cited property was elemental/molecular composition (bulk) (14/18 lists), followed by surface area

(14/18 lists); particle size and morphology/shape/form (13/18 lists), particle size distribution and surface chemistry (12/18 lists), agglomeration/aggregation state (11/18 lists) and crystal structure (10/18 lists). The reason for this level of consensus is most likely because these properties are applicable to all nano-objects, whereas some other properties (density, porosity, etc.) are applicable only to specific nano-objects. The properties most frequently occurring on these priority lists were in agreement with those occurring in Table II; however, the order in which they appear differs. Hence, differences exist with respect to perceived needs for the "minimum" properties needed for nano-object characterisation for toxicity testing. In addition to the lists of minimum characteristics summarised in Table III, ISO Technical Committee 229 recently approved a similar document (ISO TR 13014) developed in its Working Group 3, to develop international consensus. Finally, it is noteworthy that few publications have been able to provide information on all of these minimum characteristics requirements. This short coming may, in part, be due to practical limitations (e.g., availability of instrumentation), but may also reflect current technical measurement limitations for some properties, especially with regard to the material as administered or after administration to an in vitro or in vivo system. For example, surface area of a pristine nano-object powder and aerosolized powder collected on a filter in an exposure chamber can be determined using gas adsorption (LeBouf et al. 2011); however, no method exists for determination of nano-object surface area after administration to an in vivo model. Electron microscopy is useful for determination of particle size of pristine material, aerosolised or suspended material and after administration to an in vivo system, for example, tissues sections or sealable capsules with electron transparent windows that permit imaging of live cells. Zucker & Daniel (2012) recently reported an optical microscopy method for detection of nanoparticles in cells. Note that careful collection and preparation of samples is needed to avoid artefacts and standardised protocols are critically needed to facilitate reproducible results.

Metrological considerations for nanoscale RMs

A third aspect of the RM development process, which has to be considered in parallel with material selection and property choice, are the metrological considerations. For a more detailed discussion of the nanoscale RM development process and additional considerations, readers are referred to the article by Linsinger et al. (2011).

Generally, RMs are produced as CRMs or as non-CRMs. According to ISO Guide 34, the generic term reference material stands for a material that is sufficiently homogeneous and stable with respect to one (or more) properties, which has been established to be acceptable for its intended measurement use (ISO 2009). These materials can be, for example, benchmark materials for inter- and intra-laboratory comparison studies of new test methods or protocols, or to monitor proper instrument performance, as in control chart testing. A sub-set of RMs are CRMs, for example, for calibration purposes, or for method testing. For these

applications, the certified property values have to be metrologically traceable, that is, traceable to a specified reference system and accompanied by an uncertainty value derived from an uncertainty budget covering all significant uncertainty contributions (ISO 1992). It is critical to note that only values of well-defined properties can be metrologically traceable. Many of the properties listed in Table II are only broadly defined or qualitative (e.g., surface chemistry). Without clear specification of the property, certified reference values cannot be assigned.

Because of their size and complexity, characterisation of a nano-object property for RM (or CRM) development presents many unique challenges. For example, Zeisler et al. (2011) described the characterisation of the elemental composition of a SWCNT material that is distributed by NIST as SRM 2483: Carbon Nanotube Soot. This material presented many measurement challenges because it contained carbon species, catalyst contamination and other trace element contaminants. By using a suite of analytical techniques, certified and reference values could be assigned for mass concentrations of several elements.

Methods of divergence and modelling

Linsinger et al. (2011) recently described the metrological challenges associated with the development of nanoscale RMs for particle size, a property deemed important for environmental, health and safety testing. Measurement of nanoparticle size is method dependent. Hence, while most lists of nano-object properties included particle "size", few define what is meant by size. The dependency of a property on the chosen analytical method can be illustrated with NIST RMs 8011 (10 nm), 8012 (30 nm) and 8013 (60 nm), which are gold nanoparticles in aqueous suspension. For these RMs, NIST assigned different values of "size" for atomic force microscopy, scanning electron microscopy, transmission electron microscopy, differential mobility analysis, dynamic light scattering (DLS) and small-angle X-ray scattering (SAXS) techniques. As an example, for RM 8011 assigned size values ranged from (8.5 ± 0.3) nm (atomic force microscopy) to (13.5 ± 0.1) nm (DLS). It is worth noting that the spread between the assigned reference values for particle diameter strongly converges as the nominal size of the RM increases. Another example is ERM FD100, a CRM produced by IRMM, consisting of colloidal silica (near-spherical amorphous silica nanoparticles in aqueous suspension), for which the certified values for equivalent spherical particle diameter ranged from (19.0 ± 0.6) nm (intensity-weighted harmonic mean diameter, as obtained with DLS, cumulants method) to (21.8 ± 0.7) nm (intensity-weighted mean diameter as obtained by SAXS).

From Tables II and III, not only is size of interest, but so is the distribution or dispersion in sizes; however, significant challenges remain in the development of reliable estimates of measurement uncertainties for nanoparticle size distributions. Full size distributions have been certified for coarse particulate materials, for example, for quartz powder BCR-66, in the size range 350–3500 nm (Wilson et al. 1980). For particulate nano-objects, however, most existing RMs only carry information about one or more mean diameters. Moreover, most of these RMs currently come with indicative reference values (e.g., NIST 8011, 8012 and 8013), and less often with certified values (e.g., ERM-FD100) (Braun et al. 2011), while measured size distributions are provided only for informational purposes, for example, for NIST 8011, 8012 and 8013.

The apparent differences in size values obtained by different techniques for the aforementioned RMs result from the fact that (a) not all primary nanoparticles in a population are identical (i.e., with few exceptions, there is always a dispersion with respect to dimensions and sometimes shape), (b) most nanoparticle populations contain some agglomerates (which will impact the mean size and potentially skew results) and (c) measurement techniques vary with respect to the way in which they "sense" the dimensional properties of particles and under what conditions these measurements are conducted. For example, microscope-based methods and other counting methods generally produce number-weighted distributions, while light scattering techniques generate intensity-weighted distributions (comparable with the particle volume squared). Furthermore, methods may differ with respect to whether they produce an ensemble average or single particle measurements, or they may simply measure different aspects of the nanoparticle dimensions (e.g., height above a flat substrate, equivalent spherical diameter, radius of gyration or hydrodynamic size). The different values may in fact reflect real differences in the "size" of the nanoparticle measured, for example, electron dense core of a dry particle on a grid (transmission electron microscopy) versus the effective hydrodynamic size of a particle in solution with physically sorbed solvent molecules and possibly an organic corona. Sample preparation can also introduce biases into the assessment of size, and such artefacts are difficult to separate in many cases (Glover et al. 2011). Other potential sources for variance between methods can be attributed to measurement artefacts or technical limitations. For example, in optical microscopy, illumination source and diffraction are fundamental factors that must be considered; electron beam/sample interactions, signal origination and collection provide measurement limitations in particle beam microscopy (electron and ion beams), and tip/probe/sample interactions are limiting factors in probe microscopy (e.g., atomic force microscopy). Hence, it is critical that the investigator understands the characteristics and the measurement processes being used, because instruments may measure a given sample in a different way (sometimes referred to as "methods divergence").

A clear understanding of the numerous factors that comprise and contribute to imaging and measurement uncertainty in a scanning electron microscope or any measurement tool is essential, and true dimensional accuracy can only be achieved through modelling of the entire measurement process (Postek and Vladar 2011). Physics-based models are being developed for optical, scanning probe and scanning particle beam instrumentation, but have generally not been fully applied to nano-object characterisation. While modelling may be too involved or

unnecessary for some applications, in order to claim accuracy for any dimensional measurement it is essential to account for all significant methods divergence.

Finally, it can be noted here that the particle size distribution of a nanoparticle material is a rather dynamic property that can be severely influenced by sample preparation steps (including the overall time taken from sample preparation to measurement) in any measurement procedure. Therefore, the comparability of particle size distribution results relies on the agreement of common or equivalent sample preparation protocols (Taurozzi et al. 2012).

RMs for performance check and instrument calibration

In addition to nanoscale RMs for risk assessment, RMs are needed for performance control and instrument calibration. A detailed review of this topic is outside the scope of this paper and is discussed by Roebben et al. (2011a); however, the topic is worth mentioning and a limited discussion is presented herein using dimensional analysis by scanning electron microscopy as an example. Accurate characterisation of nano-objects requires high-quality measurement instrumentation, often operating at the upper limit of their performance range. This high level of performance must be documented, tested and periodically monitored to assure consistent data. So-called "round robin" testing between instruments within the same laboratory, as well as, inter-laboratory studies should be periodically conducted on known samples in order to adequately compare data and develop confidence in the comparative measurements being made. Hence, use of RMs for both accurate instrument calibration and testing for nano-objects is a critical component of any measurement strategy (Roebben et al. 2011b).

The capabilities of any instrument must be understood before accurate measurements can be made. An instrument may have a high degree of precision (i.e., results are very repeatable), but its results may have a systematic bias (poor trueness). For example, Postek et al. (1993) using a prototype RM demonstrated that accurate scanning electron microscope magnification calibration and error analysis were major problems confronting measurements with this instrument. The work demonstrated that (at the time), instrument miscalibration varied between 10% and 60% among those instruments tested. Additionally, the calibration of the "X" scan to the "Y" scan was not 1:1 so a round particle under those circumstances would be recorded as distorted or elongated. In that same study, multiple instruments from the same laboratory were tested and major calibration differences were observed between instruments. Interestingly, the high-end operating range of the instruments in this older study was about 100,000–125,000$\times$ magnification, whereas with new instruments, a factor of 10 times that range is routinely possible. As such, if one were to only consider a potential 10% error in a newer instrument's magnification calibration, that error is greater than the entire useful range of most of the earlier studied instruments. Therefore, it is incumbent upon the instrument user to verify proper performance (e.g., establish control charts demonstrating the instrument stability

over time). Characterisation and dimensional metrology of nanomaterials requires accurate measurement not only in two dimensions, but also in three dimensions at very high resolution, hence full, accurate calibration to known reference artefacts is essential.

Opportunities

This review has summarised efforts to date to identify nano-objects of interest for development into RMs and to determine the physico-chemical properties that should be characterised for proper identification of the materials going into risk assessment. Though it is tempting to search for a relationship between the lists of nano-objects identified for development into nanoscale RMs and the lists of priority measurement parameters, such a relationship is not straightforward. As described in the following sections, this apparent discrepancy provides an opportunity to clarify the relationship between nanomaterial characterisation requirements and the concept of RMs. Additionally, several opportunities to improve communication and collaboration between the metrology and environmental, health and safety communities are described for nanoscale RM development.

Selection of candidate reference materials

The nano-objects listed in Table I were selected for risk assessment, based in part, on their availability and market relevance; however, such prioritisation criteria may not be appropriate in all situations. It is logical, but not necessary, that RMs with corresponding nominal chemical composition are chosen for RM development to address a specific chemical, physical or biological measurement problem. The number and kind of RMs needed is determined by the number and kind of measurement problems to be solved. To illustrate this difference in approaches, goethite (α-FeOOH) is used as a standard for electrophoretic mobility (NIST SRM 1980) because it is suitable for this purpose. By contrast, when conducting a risk assessment of a nano-object, for example, titanium dioxide, it may be of interest to measure several physico-chemical properties, one of which might be electrophoretic mobility (used to calculate zeta potential). Hence, while a CRM for electrophoretic mobility fulfils a need for instrument calibration and/or performance verification, a risk assessor may be more interested in comparing values of electrophoretic mobility for titanium dioxide that were obtained by several different laboratories using the same material but different protocols. This example of solving a specific nanoscale measurement problem versus understanding potential toxicity or exposure risk of a nano-object makes it apparent that the two types of measurement needs may not benefit from the availability of the same RM of a certain nominal composition. Though it may theoretically be possible to produce a limited set of RMs, each of which should be used to tackle multiple measurement problems, even if this were possible it would likely not be efficient or cost-effective. As such, an opportunity exists for the metrology and risk assessment communities to clarify whether RMs

are truly needed or whether "test materials", based, for example, on the nano-objects listed in Table I, that carry no reference or certified values may be useful for hypothesis testing or to facilitate the inter-laboratory reproducibility aspect of method validation studies, for instance. In addition to the use of test materials for comparative studies, such materials can also fill existing gaps for nanoscale control materials (positive or negative) for toxicity assays or as references for specific (eco-) toxicological end points such as genotoxicity, oxidative stress or reproductive toxicity. For example, as a first attempt to address these gaps, the European Union *QNano* Research Infrastructure project is focusing on development of positive and negative control nanomaterials for four biological end points (apoptosis, cell cycle regulation, oxidative stress and genotoxicity). Similarly, the European Union *NanoLyse* project (www.nanolyse.eu) is developing methods for detecting and quantifying nanoparticles, using tailor-made RMs, which comprise both suspensions of nanoparticles as well as nanoparticles in food matrices.

Selection of relevant properties

Table III summarises lists of "minimum" properties deemed important for the characterisation of nano-objects during risk assessment. It is important not to confuse these lists of properties with a list of minimum characteristics for a nano-object RM. A CRM, strictly speaking, only needs to be characterised in terms of the property for which it is used as a reference. For example, there is no need to characterise the composition of an RM that is used solely for quality assurance of particle size analysis instruments. These lists of minimum properties have been created by various entities with the hope that nano-objects with similar values for these minimum properties would behave similarly when subjected to biological or clinical testing. The minimum characterisation of a nano-object for risk assessment is a very useful exercise. For example, different results obtained on previously nominally similar materials would not be misinterpreted as "conflicting" or "contradictory" as currently occurs in some toxicity tests, but could be interpreted in terms of the differences in other fundamental material properties. This type of testing would be facilitated by use of inter-laboratory studies to develop and refine measurement protocols.

The production of an RM requires, as a start, consensus on the property to be characterised. If this property is an intrinsic property (to the material), then it should be possible to measure it with different methods; however, as noted above in the examples of NIST RM 8011 or ERM FD100, most nano-object properties are method-defined, that is, the measured value depends on the method used to assess the property. RMs for use in the assessment of method-defined properties rely on the consensus of an agreed upon measurement method. However, as an emerging technology area, there are few fully agreed upon, validated, consensus measurement methods for nano-object characterisation. This lack of methods implies that it is currently not possible to produce useful nanoscale RMs for many properties that may be related to hazard.

The work on measurement methods for which there is no suitable RM available yet, benefits from the availability of the aforementioned "test materials" (also referred to as common test materials or study materials) that are well identified and, where possible, characterised in terms of the properties that are believed to be indicative for the investigated property. In view of the importance of this type of test material, and to better distinguish it from materials that do meet the requirements of an RM, a separate term such as "representative test material" (RTM) might be more appropriate. Examples of these RTMs are the materials developed for the OECD-WPMN sponsorship program. This relatively new concept may help bridge apparent communication issues between developers of RMs and those working in the nanotoxicology field. Semantically, an RM that is used outside the scope of its reference status is being utilised as an RTM; for example, a size RM that is used for the assessment of properties, such as specific hazards, for which its homogeneity and stability have not been explicitly verified nor quantified.

Coordination and communication

Among the many opportunities presented by the current state of nanoscale RM availability is greater coordination and collaboration between RM producers and risk assessors to better understand the perceived needs of these communities. Such communication and collaboration may allow joint development of nanoscale RMs (if truly needed) or test materials, if a mutually agreeable approach can be worked out to deal with issues such as value assignment (reference, certified), packaging and distribution. A second opportunity for communication that needs to be explored is whether reasonable values for many of these material properties would be sufficient for most risk assessment evaluations. As noted above, one approach is the development of RTMs. Another approach is to include, with RMs, values that do not meet the requirement for reference or certified values. One can refer to such values as "informational" values (approach followed by NIST) or as "additional material information" (approach followed by IRMM). An example is the previously mentioned NIST RM 8011. In addition to reference values for size, NIST provides informational values for properties such as specific element or ionic concentrations, pH, zeta potential and particle size histograms for several techniques. A third opportunity is the need to develop standard methods or protocols. One example of this approach is ISO 10801: Nanotechnologies – Generation of metal nanoparticles for inhalation toxicity testing using the evaporation/condensation method (ISO, 2010b). Coordination of the use of acceptable RMs to standard protocols will be essential for validation. Perhaps the greatest opportunity at this point in time is the prospect of consensus building within the nanotechnology and environmental, health and safety communities to prioritise RM needs and better define the required properties (including value assignment) and physical or chemical forms of the candidate materials. The authors hope that this process will accelerate and lead to broader consensus to properly inform RM producers.

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Summary

The authors compiled and reviewed known published lists of materials of interest for creating RMs and various nano-objects properties that have been identified for characterisation as part of the risk assessment process. By compiling these lists, it allows the international risk assessment and metrology communities to identify areas of agreement and areas where resolution is needed to clarify needs. By identifying critical needs facing the nanotoxicology and metrology communities, the authors hope that this review identifies future directions of research and helps to eliminate areas of confusion. From this review, eight major themes were identified that need international attention to resolve:

- consensus on prioritisation of RM needs,
- harmonisation of terminology,
- poorly defined or qualitative measurands lack metrological traceability,
- reproducible characterisation of nano-objects before and after administration in test media or animal models,
- better understanding of measurement processes will inform their use and avoid artefacts or misinterpretation,
- RMs may be useful for both traditional (e.g., calibration) and more general testing purposes (e.g., protocol development and validation),
- when RMs are not available, clarify whether "(representative) test materials" that carry no reference or certified values may be useful for hypothesis testing and interlaboratory studies and
- consensus building across disciplines to define RM and property measurement needs.

Acknowledgements

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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