Regulatory Toxicology and Pharmacology 76 (2016) 234–261

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Case studies putting the decision-making framework for the grouping and testing of nanomaterials (DF4nanoGrouping) into practice

Josje H.E. Arts [a], Muhammad-Adeel Irfan [b], Athena M. Keene [c], Reinhard Kreiling [d], Delina Lyon [e], Monika Maier [f], Karin Michel [g], Nicole Neubauer [b], Thomas Petry [h], Ursula G. Sauer [i], David Warheit [j], Karin Wiench [b], Wendel Wohlleben [b], Robert Landsiedel [b, *]

[a] AkzoNobel NV, Arnhem, The Netherlands
[b] BASF SE, Ludwigshafen, Germany
[c] Afton Chemical, Richmond, VA, USA
[d] Clariant Produkte GmbH, Sulzbach, Germany
[e] Shell Health, Shell Oil Company, Houston TX, USA
[f] Evonik Resource Efficiency GmbH, Hanau, Germany
[g] Henkel AG & Co. KGaA, Düsseldorf, Germany
[h] Toxminds BVBA, Brussels, Belgium
[i] Scientific Consultancy – Animal Welfare, Neubiberg, Germany
[j] The Chemours Company, Wilmington DE, USA

ARTICLE INFO

Article history:
Received 24 November 2015
Accepted 30 November 2015
Available online 10 December 2015

Keywords:
Carbonaceous nanomaterials
Metal oxide and metal sulphate nanomaterials
Amorphous silica nanomaterials
Organic pigments
Grouping
Read-across
Intrinsic material and system-dependent properties

ABSTRACT

Case studies covering carbonaceous nanomaterials, metal oxide and metal sulphate nanomaterials, amorphous silica and organic pigments were performed to assess the *Decision-making framework for the grouping and testing of nanomaterials* (DF4nanoGrouping). The usefulness of the DF4nanoGrouping for nanomaterial hazard assessment was confirmed. In two tiers that rely exclusively on non-animal test methods followed by a third tier, if necessary, in which data from rat short-term inhalation studies are evaluated, nanomaterials are assigned to one of four main groups (MGs). The DF4nanoGrouping proved efficient in sorting out nanomaterials that could undergo hazard assessment without further testing. These are soluble nanomaterials (MG1) whose further hazard assessment should rely on read-across to the dissolved materials, high aspect-ratio nanomaterials (MG2) which could be assessed according to their potential fibre toxicity and passive nanomaterials (MG3) that only elicit effects under pulmonary overload conditions. Thereby, the DF4nanoGrouping allows identifying active nanomaterials (MG4) that merit in-depth investigations, and it provides a solid rationale for their sub-grouping to specify the further information needs. Finally, the evaluated case study materials may be used as source

Abbreviations: AA, Atomic adsorption; AAN, Average agglomerate number; ALF, Artificial lysosomal fluid; AMA, (*in vitro*) Alveolar macrophage assay; AOP, Adverse outcome pathway; AUC, Analytical ultracentrifugation; BAuA, *German Federal Institute for Occupational Safety and Health*; BET, (method of) Brunauer–Emmett–Teller; Cat, Category; CPH, Centrophenoxine; DF4nanoGrouping, Decision-making framework for the grouping of nanomaterials; DLS, Dynamic light scattering; DMEM, Dulbecco's modified Eagle medium; dnp, Determination not possible for technical reasons; DOPG, 1,2- Dioleolyl-sn-glycero-3-phosphocholin; DPP, Diketopyrrololpyrrol; DPPG, 1,2-Dipalmitoyl-sn-glycero-3-phosphatidylcholin; ECETOC, European Centre for the Ecotoxicology and Toxicology of Chemicals; ECHA, European Chemicals Agency; EDAX, Energy dispersive analysis of x-rays; EPA, Environmental Protection Agency; ESR, Electron spin resonance; FCS, Foetal calf serum; FFF, Field-flow-fractionation; FPG, Formamidopyrimidine DNA glycosylase; FRAS, Ferric reducing ability of serum; FTIR, Fourier-transformed infrared; GBP, Respirable granular biodurable particles; GHS, Globally harmonized system; HAR NM, High aspect ratio nanomaterial; HPRT, Hypoxanthine-guanine phosphoribosyltransferase; IATA, Integrated approach for testing and assessment; ICP-AES, inductively coupled plasma – atomic emission spectrometry; ICP-MS, Inductively coupled plasma – mass spectrometry; IEP, Iso-electric point; JRC, Joint Research Centre; LDH, Lactate dehydrogenase; LMM, Low molar mass; LO(A)EL, Lowest observed (adverse) effect level; MEM, Minimum essential medium; MG, Main group; MNvit, *In vitro* micronucleus test; MNviv, *In vivo* micronucleus test; MPS, Mononuclear phagocyte system; MTT, C,N-diphenyl-N′-4,5-dimethyl thiazol-2-yl tetrazolium bromide; MWCNT, Multi-walled carbon nanotube; N/A, Not available; NAA, Neutron activation analysis; NM, Nanomaterial; NMR, Nuclear magnetic resonance; NOAEC, No observed adverse effect concentration; OEL, Occupational exposure limit; PBS, Phosphate buffered saline; PEG, Polyethylene glycol; PSF, Phagolysosomal simulant fluid; REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals; RIVM, Netherlands National Institute for Public Health and the Environment; SEM, Scanning electron microscopy; SIMS, Secondary ion mass spectrometry; SSA, Specific surface area; STIS, Short-term inhalation study; TEM, Transmission electron microscopy; TG, Test guideline; UBA, *German Environmental Protection Agency*; wt%, Weight percentage; XPS, X-ray photoelectron spectroscopy; XRD, X-ray diffraction.

* Corresponding author. Experimental Toxicology and Ecology, BASF SE, 67056 Ludwigshafen, Germany.
E-mail address: robert.landsiedel@basf.com (R. Landsiedel).

http://dx.doi.org/10.1016/j.yrtph.2015.11.020

Biopersistence and biodistribution
Cellular effects
Apical toxic effects

nanomaterials in future read-across applications. Overall, the DF4nanoGrouping is a hazard assessment strategy that strictly uses animals as a last resort.

Information box: definitions of terms

Benchmark material: A (nano-)material, which has been tested and evaluated according to standard criteria and to which new materials may reliably be compared for grouping purposes (Kuempel et al., 2012).

(Certified) reference material: A material that has undergone a process for validation or round robin assessment as 'reference material', thereby having fulfilled specific predefined requirements for, e.g., its homogeneity and stability (Stefaniak et al., 2013).

Functionality: A (nano)material's activity affecting its environment, such as dissolution rate in biological media, surface reactivity, and dispersibility (*cf.* system-dependent properties).

Intrinsic (material) properties: Characteristics of the material that are determined independently of the biological environment or test system. Accordingly, intrinsic material properties include chemical composition and impurities, primary particle size, surface area, water solubility and shape or aspect ratio.

Mode-of-action: Mechanisms by which materials may elicit cellular or apical toxic effects. To date, only a limited number of such mechanisms have been discerned for nanomaterials (*cf.* Arts et al. (2015) for further information on different modes-of action).

Nanoform: As defined by the EU Commission's NANO SUPPORT Project (2012), the term 'nanoform' is used for REACH registration dossiers *that (seem to) also address other forms (e.g. bulk). Thus, a nanoform registered 'alone' (not along with non-nanoforms) would be a nanomaterial.*

Nanomaterial: In line with the EU definition (EU Commission, 2011), 'nanomaterial' is an overarching term to describe materials containing particles with external dimensions in the size range 1–100 nm.

Substance: The EU Regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH; EP and Council of the EU, 2006) defines a substance *a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition.* Accordingly, in the present article, 'substance' is used as an overarching term encompassing nanosized and non-nanosized substances in all forms regardless of their state of dissolution.

System-dependent properties: Characteristics that are linked to the material's functionality in its environment, such as surface reactivity, dissolution in biological media, and dispersibility. The outcome of measurements of system-dependent properties is affected by the given surroundings, i.e. the choice of the test system (culture media, supplements, dispersing agents, etc.) or of the product application. System-dependent properties constitute biophysical interactions of the particles with their environment. Accordingly, 'systems' may be, e.g., matrices in which a nanomaterial is embedded in a product, exposure media (aerosols, suspensions, etc.), or biological systems that the nanomaterial comes into contact with.

1. Introduction

In the context of the EU chemicals regulation REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals; EP and Council of the EU, 2006), grouping is defined as the process of uniting substances into a common group *if they are structurally similar with physico-chemical, toxicological, ecotoxicological and/or environmental fate properties that are likely to be similar or to follow a regular pattern* (ECHA, 2013). Within a group, each individual substance may not need to be tested. Instead, endpoint-specific effects of an unknown substance may be derived from the endpoint-specific effects of further substances within the group (ECHA, 2013). For substances in general, technical guidance documents on grouping are available, e.g. from the European Chemicals Agency (ECHA, 2008, 2012a, 2012b, 2013, 2014) or from the Organization for Economic Cooperation and Development (OECD, 2014). By contrast, to date there are no specific regulatory frameworks for the grouping of nanomaterials (NMs; *cf.* Information box for definitions of key terms). However, this topic is addressed in different publications, and preliminary guidance is provided in the context of substance-related legislation or the occupational setting (Arts et al., 2014).

The *International Standardisation Organisation* (ISO) suggests addressing the following questions in determining the potential hazard of a NM: Does its water solubility exceed 100 mg/L; does it contain biopersistent fibres or fibre-like structures; are there hazard indications for the NM, or is there a hazard band for the bulk material or an analogous material (ISO, 2014)? The *United States Environmental Protection Agency* (EPA) has proposed to exclude NMs which dissociate completely in water from the foreseen rule on the reporting and recordkeeping of nanoscale materials under the Toxic Substances Control Act (EPA, 2015). The *German Environmental Protection Agency* (UBA; Umweltbundesamt) suggests assigning nanotubes into a distinct group and proposes a preliminary long-term lowest-observed-effect-level (LOEL) of 0.1 mg/m^3 to distinguish 'inert' NMs from NMs with specific toxicity (UBA, 2014). Walser and Studer (2015) from the *Swiss Federal Office for Public Health* call for the establishment of predefined test strategies for different groups of NMs based upon their specific modes-of-action, which may lead via specific adverse outcome pathways (AOPs) to apical toxic effects. A report from the *Dutch National Institute for Public Health and the Environment* (RIVM; Sellers et al., 2015) highlights the scientific relevance to perform NM testing in tiers of increasing complexity of the endpoints addressed. As proposed in the RIVM report, Tier 1 serves to obtain additional physico-chemical data to fulfil REACH endpoints (exceeding the basic data that should be available by default) or to support grouping or read-across. In Tier 2, the behaviour of the NM is