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# Towards health-based nano reference values (HNRVs) for occupational exposure: Recommendations from an expert panel

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## Abstract

Unique physicochemical characteristics of engineered nanomaterials (ENMs) suggest the need for nanomaterial-specific occupational exposure limits (OELs). Setting these limits remains a challenge. Therefore, the aim of this study was to set out a framework to evaluate the feasibility of deriving advisory health-based occupational limit values for groups of ENMs, based on scientific knowledge.

We have used an expert panel approach to address three questions: 1) What ENM-categories should be distinguished to derive advisory health-based occupational limit values (or health-based Nano Reference Values, HNRVs) for groups of ENMs? 2) What evidence would be needed to define values for these categories? And 3) How much effort would it take to achieve this?

The panel experts distinguished six possible categories of HNRVs: A) WHO-fiber-like high aspect ratio ENMs (HARNs), B) Non-WHO-fiber-like HARNs and other non-spheroidal ENMs, C) readily soluble spheroidal ENMs, D) biopersistent spheroidal ENMs with unknown toxicity, E)



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Declaration of Competing Interest

The authors have no competing financial interests that could influence the study. The findings and conclusions in this paper are those of the author(s) and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention. This paper contents, including any opinions and/or conclusions expressed, are those of the author(s) alone and do not necessarily reflect HSE policy.

biopersistent spheroidal ENMs with substance-specific toxicity and F) biopersistent spheroidal ENMs with relatively low substance-specific toxicity. For category A, the WHO-fiber-like HARNs, agreement was reached on criteria defining this category and the approach of using health-based risk estimates for asbestos to derive the HNRV. For category B, a quite heterogeneous category, more toxicity data are needed to set an HNRV. For category C, readily soluble spheroidal ENMs, using the OEL of their molecular or ionic counterpart would be a good starting point. For the biopersistent ENMs with unknown toxicity, HNRVs cannot be applied as case-by-case testing is required. For the other biopersistent ENMs in category E and F, we make several recommendations that can facilitate the derivation of these HNRVs. The proposed categories and recommendations as outlined by this expert panel can serve as a reference point for derivation of HNRVs when health-based OELs for ENMs are not yet available.

**Keywords**

Engineered nanomaterials; Health based; Nano reference values; Occupational exposure limit; Expert panel

## 1. Introduction

Nanotechnology has moved from the focused research environment to wider application in the workplace, and engineered nanomaterials (ENMs) have been incorporated into novel products and technological solutions in the past decade or more. However, uncertainty remains about possible adverse human health effects from exposure to ENMs (Halappanavar et al., 2020; Oberdorster et al., 2005; Riediker et al., 2019; Stone et al., 2017). A unique hazard potency cannot be attributed to all ENMs since their toxicity can change considerably based on their physical and chemical characteristics.

Since nanomaterial production is increasing worldwide, a growing number of workers are potentially exposed (Kaluza et al., 2009; Schmid et al., 2010). Workplace exposure assessments have shown that the release and exposure to ENMs depends on the type of nanomaterial and the working process (Debia et al., 2016; Ding et al., 2017a; Kuhlbusch et al., 2011).

Different national and international bodies have developed approaches and protocols for managing the occupational health and safety considerations of ENMs (BSI, 2007; EU., 2019; IFA., 2019; WHO, 2017). For several nanomaterials, occupational exposure limit values have been suggested by international organizations or in scientific literature (ANSES, 2020; Jacobsen et al., 2018; Mihalache et al., 2017; NIOSH, 2011; NIOSH, 2013; NIOSH, 2021; Poulsen et al., 2018). These values are usually derived from data from one or more health hazard studies (mostly performed in rodents) and applying safety assessment factors. However, to our knowledge, no legally binding occupational exposure limits (OELs) for ENMs are available (Mihalache et al., 2017). Apart from chemical composition, the adverse human health effects of ENMs are determined by several other physicochemical characteristics, such as particle size, shape, surface, agglomeration state, charge, and solubility. Therefore, setting OELs for ENMs can be challenging (Guseva Canu et al.,2018; Mihalache et al., 2017; Riediker et al., 2012).

In conventional risk assessment for non-nano substances, substance-specific toxicity data are preferably used for OEL derivation. However, the amount of toxicity data needed to achieve substance-specific risk assessment for each type of ENMs seems unfeasibly large. In addition, the reliability and validity of existing ENM data in public literature was found to be poor for many ENMs (Krug, 2018), and workers may be exposed to aggregated nanoparticles or to process-released nanoparticles that may differ from the pristine particles tested in toxicity studies.

New ENMs are introduced into the market at a rapid pace (Foss Hansen et al., 2008; Nanodatabase, W. T, 2013; StatNano, W, 2010). Given the many differences in shape, composition and sizes of ENMs, legislation is unable to keep up with the pace of their development. As an alternative, several initiatives to set benchmark levels to reduce the exposure of workers to ENMs have previously been undertaken (Table 1). As an example, in 2011, the Nano Reference Values (NRVs) were accepted in the Netherlands as an elaboration of the precautionary principle based on acceptable worst-case scenarios for workplace exposure assessment of ENMs (SER, 2012). The current NRVs are based on the Institute for Occupational Safety and Health of the German Social Accident Insurance (IFA) approach and are not health-based (IFA, 2008). These NRVs have been accepted in the Netherlands and several other countries as benchmark exposure values when health-based OELs are not (yet) available for ENMs.

Benchmark values such as the NRVs require little ENM-specific toxicity data, but the uncertainty in their level of protection is relatively high as compared to substance-specific OELs. Fig. 1 summarizes visually the effort to obtain toxicity data that are expected to be needed to reduce the uncertainty in the derivation of OELs for ENMs.

A recent comparison of OELs available in the literature for ENMs with the Dutch NRVs showed that for some ENMs such as those of silver and titanium dioxide, recommended exposure limit values were lower than the corresponding NRV. As the comparison required calculation of mass-based OEL values to particle numbers, which results in some uncertainty, these results are to be interpreted with caution. Nevertheless, these findings indicate that the NRVs, which have been derived as precautionary values, may not be adequate for some EMNs (Buist and Oosterwijk, 2017). However, current scientific knowledge about the toxicity and grouping of ENMs has grown, indicating that it may now be possible to develop advisory health-based occupational limit values for groups of ENMs (Arts et al., 2015; Giusti et al., 2019; Lamon et al., 2019; Mihalache et al., 2017; Stone et al., 2020). Such values would provide more certainty in the level of protection of workers than the current NRVs, while the amount of toxicity data needed is not as high as would be required to determine substance-specific OELs for all ENMs. Ideally, these health-based reference values for groups of ENMs would also be applicable to new ENMs, that are introduced into the market. In Fig. 1, these group values would be depicted at the “state of the art” level, in between the “current NRVs” and “deriving OELs for every ENM”.

We have explored the feasibility and current initiatives that are undertaken to arrive at the “state of the art” situation by organizing discussions within an international panel of experts. We focus on ENMs that are currently on the market, the so-called first generation

ENMs or “passive nanostructures” (Oomen et al., 2018; Teunenbroek et al., 2017). The main questions the panel addressed were: 1) What ENM- categories should be distinguished to derive advisory health-based occupational limit values for groups of ENMs, here referred to as health-based Nano Reference Values (HNRVs)? 2) What evidence would be needed to define values for these categories? and 3) How much effort would it take to achieve this?

This paper sets out a process that could be used to derive HNRVs for ENMs from a health sciences point of view. How these limits can be set and implemented by national regulators was outside the scope of this study.

## 2. Methods

This paper presents the results of an Expert Panel Approach, which is particularly appropriate for highly complex issues requiring specific technical knowledge and the synthesis of expertise from many different disciplines (Slocum, 2003). The main task of the expert panel was to provide a vision and recommendations for future directions for the derivation of HNRVs.

The panel members did not represent organizations or interest groups, but all contributed as recognized experts in their own field. Since our focus was to evaluate the feasibility of deriving HNRVs for groups of engineered nanomaterials (ENMs) from a health sciences point of view, we limited our panel to experts from the fields of toxicology, epidemiology, occupational health and exposure sciences. A request for interest in this approach via 33 EU-OSHA focal points was used as a starting point. Then the request to join the panel was extended via the network of EU-OSHA identified contact persons in the first phase of the project (see Fig. 2). A core panel and a review panel were assembled based on indicated availability and extent of the personal contribution. The core panel consisted of nine members in total (the main authors of this paper, including three technical writers) that indicated they were available for participating in the digital discussion meetings. The review panel consisted of 24 members (see acknowledgements) and provided written comments on the project proposal, the discussion topics, and the draft version(s) of the scientific publication. The core panel undertook the same work as the review panel and also participated in the (teleconference) discussions, commented on the meeting notes of these discussions, and provided written input in their area of expertise for the peer-reviewed publication. The technical writers undertook the same work as the core panel and prepared the draft project proposal, the discussion topics, documents and meeting notes and the draft publication.

The discussion took place over four rounds of online discussion meetings held in 2020 and each discussion round was centered around one topic. Before every panel discussion round, an overview of relevant literature available at that time (December 2020)together with questions to be discussed were distributed as background information to the core panel. The following topics were discussed at the four meeting rounds:

- Topic I: Groups or categories of ENMs
- Topic II: Use of scientific knowledge on health effects for individual ENMs

- Topic III: Use of scientific knowledge on particle toxicity
- Topic IV: Reaching agreement on the conclusions and approach for deriving HNRVs

The expert panel strived for agreement, but not at the expense of overly simplifying the analyses and results. When the experts disagreed, this was recorded as an essential aspect of the process.

A schematic view of the different process steps is included in Fig. 2.

Although a quick screening of the literature was performed before each discussion meeting, a systematic literature review was not performed. Rather, this paper presents the results of the expert panel discussions, supported by relevant literature.

## 3. Results and discussion

### 3.1. Categorization of ENMs in health-based NRVs (HNRVs)

In the current published methodologies to derive advisory health-based occupational limit values, grouping approaches have been helpful to differentiate ENMs into different categories based on physicochemical properties, availability of a viable testing strategy and associated biological effects (Mihalache et al. (2017), Landvik et al. (2018) and Giusti et al. (2019) (Table 1). These approaches distinguish several main groups of ENMs: high-aspect ratio nanomaterials (HARNs) or fibrous ENMs versus non-fibrous forms, biopersistent spheroidal ENMs versus non-biopersistent ENMs, non-soluble versus highly soluble ENMs, chemically reactive versus chemically non-reactive materials and ENMs with high toxicity versus low toxicity. Some of the previously developed approaches have split the group of biopersistent ENMs into two subgroups, based on classification of the chemical components of the nanoparticles, their chemical reactivity, or their density.

The experts' opinion was that these main groups are a good starting point for grouping of ENMs for HNRVs. Please note that biopersistence of ENMs can refer to poor lung clearance as well as poor solubility. The term 'biopersistent ENMs' is not synonymous with 'poorly soluble ENMs'. However, for the grouping of ENMs for HNRVs we have chosen to put biopersistent ENMs and poorly soluble ENMs in the same category, because the factors to be considered in deriving an HNRV are similar.

We discern a specific subgroup of HARNs, that may have effects similar to asbestos. This subgroup of HARNs are elongated shapes with two similar external dimensions and a significantly larger third dimension (aspect ratio larger than or equal to 3:1) (ECHA, 2019) with a length larger than 5 μm and that are biopersistent. The dimension criteria of this subgroup follow the WHO definition for counting airborne fibers (WHO, 1997). We have categorized these as WHO-fiber-like HARNs (Category A in Fig. 3). Although this manuscript focuses on ENMs, which are a subfraction of WHO-fiber-like HARNs, Category A considerations could apply as well to larger fibers. Therefore, a strict threshold based on the nano dimensions is unnecessary in this context. ENMs that do not fulfil the WHO fiber

criteria but are also not sphere-like in shape, for example, elongated ENMs that are shorter than 5 μm or platelets are placed in a separate category (Category B in Fig. 3).

We have placed readily soluble spheroidal ENMs in a separate category that can be recognized by their fast dissolution in lung lining fluid (Category C). Furthermore, we consider a further subdivision of biopersistent spheroidal ENMs according to their toxicity (Category D – F in Fig. 3). The term spheroidal is adopted from the ECHA guidance document "Appendix for nanoforms applicable to the Guidance on Registration and substance identification"(ECHA, 2019). It includes "particles with an aspect ratio up to 3:1. This is a category for approximately 'equiaxial' particles and encompasses shapes such as spheres, but also non-spherical ones such as cubes and prisms".

In total, 6 different categories, (A - F) are distinguished:

- **A.** WHO-fiber-like HARNs
- **B.** Non-WHO-fiber-like HARNs and other non-spheroidal ENMs
- **C.** Readily soluble spheroidal ENMs
- **D.** Biopersistent spheroidal ENMs with unknown toxicity
- **E.** Biopersistent spheroidal ENMs with substance-specific toxicity
- **F.** Biopersistent spheroidal ENMs with relatively low substance-specific toxicity

Fig. 3 summarizes definitions for the categories, some examples of ENMs that could fit in these categories, as well as recommendations and considerations for deriving a health-based occupational indicative limit value. The recommendations and considerations from the expert panel are discussed in more detail in the rest of this chapter.

## 3.2. High-aspect ratio ENMs (HARNs) and other non-spheroidal ENMs (category A and B)

**3.2.1. Rationale behind these categories—**The similarity in characteristics such as length, diameter and biopersistence of some HARNs with asbestos fibers has raised concerns that they may cause similar toxicity. In some of the current approaches (Table 1), HARNs are defined as 'fiber-like ENMs, for which asbestos-like effects cannot be excluded'. Unless evidence is provided by the manufacturer or supplier that the nanomaterial does not cause mesothelioma or lung cancer, all HARNs high aspect ratio biopersistent fiber-like ENMs are precautionarily assumed as causing these asbestos-like effects. The panel concluded that defining clear criteria for fiber-like ENMs with asbestos-like effects would be preferable over the precautionary stance of assuming that all fiber-like ENMs behave as asbestos, unless proven otherwise by the producer.

There is a large variety in fiber-like ENMs and other non-spheroidal ENMs, including nanorods or nanowires. The ability of these type of ENMs to cause adverse effects via inhalation exposure is partly dependent on their aerodynamic properties, biopersistence in the lung and the fiber length and rigidity. Fibers are more toxic when they are thin enough to enter the alveolar region of the lungs, are resilient to degradation and are too large to be phagocytized by macrophages, the so- called fiber paradigm (Poland et al., 2008).

The expert panel agreed that in the absence of information to the contrary, it is reasonable to assume that fiber-like structures complying with the WHO fiber criteria should be treated as WHO-fiber-like HARNs. However, it is unclear if non-WHO-fiber-like HARNS or other non-spheroidal ENMs may lead to asbestos-like effects in the lung or effects in other organ systems. There are many unknowns in understanding how specific physicochemical properties of non-spheroidal ENMs such as plate-like ENMs link to specific adverse effects (Donaldson et al., 2011; Fadeel et al., 2018). For example, graphene nanoplatelets have been shown to be respirable due to their unusual aerodynamic behavior. Nanoplatelets up to 25 μm in diameter were found to deposit beyond the ciliated airways following inhalation (Schinwald et al., 2012). They can have high aspect ratios, making it difficult for macrophages to engulf them. Graphene-based materials can be functionalized in different ways, changing their properties and interaction with biological systems. These materials are also not necessarily biopersistent after inhalation, as it has been shown that graphene oxide sheets of differing lateral dimensions can be digested by neutrophils (Mukherjee et al., 2018).

Therefore, the experts agreed to distinguish between WHO-fiber-like HARNs (category A in Fig. 3), and other non-spheroidal ENMs that do not fulfil the WHO definition (i.e., fiber-like materials, flakes and platelets shorter than 5 μm or not rigid) (category B in Fig. 3).

**3.2.2. Definition of WHO-fiber-like HARNs (category A)—**For the HNRVs, an ENM is defined as a WHO-fiber-like HARN when it has a length of >5 μm, an aspect ratio ≥ 3:1 length to width, and if it can be considered biopersistent based on the solubility of the fiber in the lung and the ability of the lung to clear the fiber (note that the criterion of biopersistence is not part of the WHO definition) (WHO, 1997). The length threshold is based on the observed increase in carcinogenic potency of longer fibers in rats and mice, and the inability of macrophages to effectively phagocytose fibers longer than 5 μm (in the pleural space) and 10–20 μm (in the lungs, depending on the species) (Lippmann, 2014; Murphy et al., 2021). Practice has shown that the length of many CNTs measured with Electron Microscopy (EM) after dispersion in a solvent that allows visualization of individual fibers, may differ from what is stated on their safety data sheet (SDS). The heterogeneity within and between different batches of CNTs may be quite large. There are different opinions on how to deal with the large variety in the size distributions. Because there is no clear threshold dose related to mesothelioma development, it is not possible to define a proportion of fibers >5 μm that differentiates between a hazardous and non-hazardous HARN (Murphy et al., 2021). Moreover, the lengths of HARNs dispersed for EM (in solvent and/or treated by ultrasonication) do not necessarily reflect the lengths of the HARN when aerosolized (Murphy et al., 2021). This argues for counting aerosolized fibers in exposure assessment instead of using the proportion of fibers >5 μm in a material as reported by the manufacturer.

Rigidity is another criterion to characterize HARNs with asbestos-like effects (Duke and Bonner, 2018; Kane et al., 2018). A recent publication presents a method using dynamic scanning electron microscopy (DySEM) to measure flexural rigidity of carbon nanotubes, related to frustrated phagocytosis by macrophages (Fortini et al., 2020). The results indicated that flexural rigidity can indeed be used to assess potential fiber hazards. However,

general cut-off values for flexural rigidity have not been set up yet. Some studies suggest using the diameter as a proxy for rigidity (Broßell et al., 2020). However, the threshold diameter to distinguish between rigid and non-rigid HARNs depends also on the material, and has up to now only been verified experimentally for carbon-based nanotubes or -fibers. Broßell et al. (Broßell et al., 2020) and Murphy et al. (Murphy et al., 2021) have reported a threshold diameter for the rigidity of CNTs to be above ~30 to 40 nm, while in the German Federal Institute for Occupational Safety and Health (BAuA) measurement strategy for nanosized fibers a cut-off diameter of 20 nm is used (Meyer-Plath et al., 2020). This figure is based on data on MWCNTs, but includes a safety margin of about a factor of 16 for nanofibers for which no experimental data on their toxicity or rigidity is available (Meyer-Plath et al., 2020). However, the experts' opinion was that there is not enough evidence yet to recommend the use of diameter as a proxy for rigidity. Please note that rigidity is not part of the WHO fiber definition (WHO, 1997), but has been added based on other evidence as recently summarized by Murphy et al. (Murphy et al., 2021). Therefore, rigidity was not explicitly included as a criterion to assign fibers to HNRV category A.

Biopersistence of HARNs can be related to the solubility of the fiber in the lung and the ability of the lung to clear the fiber. If in vivo data are available, the biopersistence of HARNs can be related to the pulmonary clearance half-life derived from lung burden measurements. If no in vivo data are available, the durability of the HARNs in relevant physiological media has been proposed as a predictor of in vivo biopersistence (Murphy et al., 2021). The GRACIOUS project suggests to use dissolution in lung lining fluid or lysosomal fluid (e.g. (ISO., 2017), because pH and the presence of salts and proteins will influence the dissolution rate (see also Chapter 3.3, paragraph 3.3.2). In the GRACIOUS project, a threshold of a half-life of >60 days or dissolution rate < 1 mg/cm$^2$/h in both lung lining and lysosomal fluid was proposed as values for grouping HARNs with the potential to cause mesothelioma, based on literature from durability and biopersistence studies of mineral fibers and metal oxide nanofibers in rodents (Murphy et al., 2021).

At the time of the panel discussions, the recommendations from the GRACIOUS project were not published yet and therefore were not discussed. Consequently, no clear cut-off values for biopersistence is included in the recommended definition of WHO-fiber-like HARNs (category A in Fig. 3).

**3.2.3. How to categorize agglomerated WHO-fiber-like HARNs?—**Several factors can influence the process of agglomeration of fibers in the air, such as particle size, shape, electrostatic surface charge and ambient humidity. ENMs have a high tendency to clump together. Most ENMs in the air, once agglomerated, will not easily dis-agglomerate. Outside the body, agglomeration of ENMs can influence the probability and location of deposition within the respiratory tract. Respirable particles have some probability of depositing in the pulmonary (gas exchange) region of the lungs, which in humans are particles with an aerodynamic diameter of less than approximately 10 μm. Particles with an aerodynamic diameter of 4 μm have a 50% probability of depositing in the pulmonary region of human lungs (ACGIH, 2015; ICRP, 1994; ISO, 1995).

For particles larger than approximately 300–500 nm in diameter, deposition in the human respiratory tract depends on aerodynamic diameter, and for smaller particles including nanoparticles, deposition depends on the diffusion diameter and density (Kuempel et al., 2015; Vincent, 1998; Volkwein et al., 2011). Shape and orientation are additional factors influencing deposition of fibers and other non-spherical particles.

If the diameter of an agglomerate of HARNs is <3 μm and the length of the agglomerate is >5 μm, these agglomerates or aligned bundles are considered by the panel as WHO-fiber-like HARNs based on the WHO fiber criteria. Although there is evidence that at least some of the agglomerated HARNs fall apart into single fibers with a length < 5 μm in the lung (Knudsen et al., 2019; Mercer et al., 2013), the expert panel agreed that this may not be the case for all agglomerated HARNs and the practical cut-off value for the diameter of <3 μm and the length of >5 μm, should also apply to the agglomerated or aligned bundles of HARNs.

**3.2.4. How should the HNRV for WHO-fiber-like HARNs (category A) be derived?—**The expert panel considered asbestos a suitable benchmark material on which to base an HNRV for WHO-fiber-like HARNs. This is based on the length, diameter and biopersistence of characteristics of certain types of CNTs that are comparable to asbestos fibers. When using asbestos as a worst-case benchmark material, it should be noted that different national OELs have been established, ranging from 2000 to 300,000 fibers/$m^3$ according to the IFA GESTIS database of international limit values (IFA, 2021). The differences between national OELs for asbestos reflect differences in the interpretation of scientific studies and alternative ways of dealing with technical and economic constraints when setting OELs. Without taking technical and economic feasibility issues into account, an OEL may have been set at a lower value. For example, the Health Council of the Netherlands recommends an exposure limit of 400 fibers/$m^3$ for amphibole asbestos (Gezondheidsraad, 2010).

There is emerging evidence to support the induction of mesothelioma for some types of multi-walled carbon nanotubes (MWCNTs) (Chernova et al., 2017; Dong and Ma, 2015; Dong and Ma, 2019; Nagai et al., 2011). There are no data in humans on the development of mesothelioma or lung cancer from exposure to WHO-fiber-like nanomaterial, such as a specific type of long and rigid CNTs, Mitsui-7. Hence, classification by the International Agency for Research on Cancer (IARC) on Mitsui-7 as class 2B (“possibly carcinogenic to humans”) is on the basis of available data from in vivo studies in rodents. The other fibrous CNTs are classified as class 3 (“not classifiable as to its carcinogenicity to humans”) due to a lack of (in vivo) data (Grosse et al., 2014; IARC., 2017). Within the European chemical legislation REACH, a proposal for classification of certain types of MWCNTs as carcinogenic was recently received by the European Chemicals Agency (ECHA) (see: ECHA Registry of Classification and labelling (CLH) intentions until outcome).

In estimating the occupationally attributable risk, the incidence of lung cancer in exposed workers is compared to the background incidence in a similar but unexposed population. Worldwide, the lung cancer incidence in the general population is approximately 11% (Sung et al., 2021). Currently, the relatively low number of workers exposed to these

types of CNTs, as well as the long latency times do not allow to determine such an effect. Some animal toxicity data on the carcinogenicity of nanofibers are available. Rats exposed to Mitsui-7 (MWCNT-7) by inhalation for two years developed lung cancer, but not mesothelioma. This could be due to the expected small amount of fibers reaching the pleura ($1 \times 10^3$ fibers) compared to the amount of fibers reaching the lung (between $1.38 \times 10^9$ and $10.4 \times 10^9$ fibers). However, since the generated aerosol was deemed to be relevant for workplace exposures, this study provides an indication of the carcinogenic potency of Mitsui-7 (Kasai et al., 2016). Those authors estimated that for a similar dose that led to tumor formation in 16/50 = 32% of male rats, humans need to be exposed to 8.5 μg/m$^3$ ($7.7 \times 10^7$ fibers/m$^3$) for 8 h per day, 5 days per week, 52 weeks per year during 45 working years. For a risk level of 1:1000, and based on a linear exposure-response model, this would correspond to (8.5/320 μg/m$^3$) = 0.027 μg/m$^3$, corresponding to $9.03 \times 10^6$ MWCNT/μg x 0.027 μg/m$^3$ = $0.24 \times 10^6$ (240,000) fibers/m$^3$ (one μg of MWNT-7 was determined to equal $9.03 \times 10^6$ MWNT-7 fibers by SEM examination) (Kasai et al., 2016). Based on the rat data for this particular type of CNT, the health based exposure limit for amphibole asbestos of 400 fibers/m$^3$ recommended by the Health Council of the Netherlands (Gezondheidsraad, 2010) would be sufficiently worst-case to use as HNRV for the category of WHO-fiber-like HARNs as it corresponds to an excess risk level of approximately 1:500,000. The expert panel agreed that using a health based risk estimate for asbestos would be reasonable in the absence of specific data.

**3.2.5. Definition of non-WHO-fiber-like HARNs and other non-spheroidal ENMs (category B)—**Some non-spheroidal ENMs do not meet the criteria of WHO-fiber-like HARNs as described in 3.2.2. Examples are HARNs with a length < 5 μm, that are not biopersistent, or non-rigid (flexible or entangled HARNs), and platelets. These non-spheroidal ENMs may also cause lung cancer (Saleh et al., 2020), but the mechanisms of toxicity and the potency may be different from that of WHO-fiber-like HARNs.

It was discussed whether entangled flexible fibers and platelets could be grouped together with biopersistent spheroidal ENMs. The experts' opinion is that this is not possible, because evidence suggests a difference in potency between them. This difference may be due to greater surface area per unit mass of the entangled fibers or platelet and/or release of individual fibers in the lungs as shown in rats (Mercer et al., 2013).

Therefore, the panel placed both non-WHO-fiber-like HARNs as well as other non-spheroidal ENMs in category B.

**3.2.6. How should the HNRV for non -WHO-fiber-like HARNs and other non-spheroidal ENMs (category B) be determined?—**For carbon-based non-WHO-fiber-like HARNs, HNRVs may be derived using existing recommended exposure limit values for CNTs as a benchmark, for example those proposed by the U.S. NIOSH (NIOSH, 2013) or the Danish NFA (Poulsen et al., 2018).

For other non-spheroidal ENMs, it was considered not possible to derive an HNRV at the moment, because the toxicological data are currently insufficient. For now, it is practical to group the non-WHO-fiber-like HARNs and platelets that have insufficient data, but future

data may indicate that further separation is justified. For example, more information may emerge from discoveries of 2D materials beyond graphene (He et al., 2017).

### 3.3. Readily soluble spheroidal ENMs (category C)

Differences in dissolution have been suggested as a promising approach for grouping ENMs, although dissolution is not comprehensive of all biological mechanisms that influence ENM degradation in the lungs or the regulation of the innate response to particle thereof (Arts et al., 2015; Braakhuis et al., 2016). The toxicity of ENMs that are readily soluble could be considered similar to the toxicity of their soluble ionic or molecular counterparts. For these ENMs using the OEL of their ionic or molecular counterpart would be sufficient. In theory, the constituents of such a readily soluble ENM could still stay within certain compartments of the body for a prolonged time, but that would be similar to the ionic or molecular counterparts.

A question is how to determine whether an ENM is readily soluble? Pragmatic thresholds have been suggested, since there are currently no scientifically sound cut-off thresholds to define groups according to dissolution rate as the transition from very slow to quick dissolution rate is a continuous scale. In the EU project GRACIOUS, the following proposal is given:

1. Instantaneously dissolving nanoforms (NFs): threshold of $t_{½}$ < 10 min in lung lining fluid.
2. Quickly dissolving NFs: threshold of $t_{½}$ < 48 h in lung lining or lysosomal fluid.
3. Gradually dissolving NFs: threshold of $t_{½}$ > 48 h and < 60 days in lung lining or lysosomal fluid.
4. Very slowly dissolving NFs: threshold of $t_{½}$ > 60 days in lysosomal fluid (Braakhuis et al., 2021).

Here, we would advise to base the classification of a nanomaterial in category C on the rate of dissolution in lung lining fluid. As a minimum requirement, this could be similar to the category suggested in GRACIOUS of “instantaneously dissolving NFs” with a threshold of a half-life of less than 10 min in lung lining fluid”. However, we expect that only very few NMs will dissolve this quickly.

There is need for further discussion whether the second category suggested by the GRACIOUS project (quickly dissolving NFs with a half-life of less than 48 h in lung lining fluid or lysosomal fluid) should be included as well to group ENMs in category C of the HNRVs. In this case, ENMs could have been taken up by phagocytosing cells, but then quickly dissolve to constituent ions in the acidic environment of the lysosome. The GRACIOUS approach suggests to gather data on reactivity and inflammatory potential (in vitro) after the dissolution rate in lung lining and lysosomal fluid has been determined. To then find a source material, such as an ionic form or microscale particles to read-across to, sufficient data on these hazard endpoints are needed. Grouping and read-across based on molecular structural similarity alone is deemed not sufficient for nanomaterials as ENMs

with the same chemical composition can deviated in e.g. particle size or shape that lead to different behavior and effects (OECD, 2020).

With respect to read-across to microscale particles, nanoscale particles would likely result in a faster dissolution rate due to the greater surface area per unit mass than microscale particles. For some ENMs, dissolution would tend to decrease the toxicity if this is due to the reactivity at the surface. Indeed, the surface area of a particle is becoming smaller upon dissolution. For other ENMs, dissolution could result in increased toxicity if the toxic effects are due to release of toxic ions. Categorization of ENMs in C should include these considerations. Some hazard banding approaches recommend assigning the next more stringent band to address this uncertainty in using data from the microscale particles to categorize the nanoscale forms in hazard bands (NIOSH, 2019).

### 3.4. Biopersistent spheroidal ENMs (category D, E and F)

**3.4.1. Rational behind these categories—**Biopersistent spheroidal ENMs (defined as ENMs that are poorly soluble or are poorly cleared from the lung) may be less efficiently cleared from the body compared to more soluble ENMs. This may lead to increased retention at the site of deposition and local tissue containment reactions. In the alveoli, this retention may cause persistent inflammation, sometimes leading to fibrosis or lung cancer (Bevan et al., 2018; Braakhuis et al., 2020). Poorly soluble ENMs can also translocate from the original site of deposition, can become systemically available and can accumulate in secondary organs (ISO, 2019; Landsiedel et al., 2012). For example, effects of ENMs have been found in the brain (Heusinkveld et al., 2016), liver (Modrzynska et al., 2018) and cardiovascular system (Stone et al., 2017). Also, translocation of very small engineered particles is possible via the olfactory epithelium in the nose or via uptake into the circulation in rodent studies (Heusinkveld et al., 2016).

Within this category of biopersistent or poorly soluble spheroidal ENMs, different approaches for further categorization are possible. Distinguishing subgroups was considered preferable by the expert panel to better describe the toxicity of materials within this category, including materials with relatively low toxicity versus those with material-specific toxicity.

Previously, the Dutch NRVs have been based on the IFA approach (IFA, 2008) discerning two categories based on differences in density (below or above 6000 $kg/m^3$). The BSI approach (BSI, 2007) discerns 2 categories: with or without intrinsic toxicity. The DF4Nano-grouping (Arts et al., 2015) defined passive or non-passive ENMs with specific cut-off points for toxic components, surface reactivity, dispersibility and No Observed Adverse Effect Concentrations (NOAECs) in short-term inhalation study (STIS) >10 $mg/m^3$ (see Table 1).

Some experts have applied the term ‘Respirable Granular Biopersistent Particles’ (GBP) (BAuA, 2015) for particles without known significant specific toxicity that show a very low solubility in physiological lung fluids (extracellular lung lining fluid, intracellular lysosomal fluid) and do not exhibit a specific chemically related toxicity at volumetric non-overload conditions (Creutzenberg et al., 2017). In other words, their toxicity is not mediated by their specific chemical composition or surface characteristics, but because these particles

are poorly soluble and persist in the lung, they may cause inflammation and secondary mutagenicity that may finally lead to lung cancer, also referred to here as “particle toxicity” (Gebel et al., 2014).

Others use the term Poorly Soluble Low Toxicity particles (PSLT). These have been defined as inhaled particles that are considered poorly soluble. Their dissolution half-life, measured in artificial lung fluids i.e. artificial interstitial fluid (pH 7.4), artificial lysosomal fluid (pH 4.5), and artificial alveolar fluid (pH 7.4) is longer than macrophage mediated clearance times (ECETOC, 2013).

Although there are no generally acceptable objective criteria for ‘low toxicity,’ some criteria have been published. Guest has categorized substances as very toxic if the OEL is less than 0.1 $mg/m^3$ and toxic if the OEL is 0.1 to 1 $mg/m^3$ (Guest, 1998). This toxicity definition was used in the NanoSafer framework as described by Brouwer (2012) (Brouwer, 2012). Also, as mentioned above, a STIS NOAEC of 10 $mg/m^3$ in a rodent study has been used as an indicator of toxicity (Arts et al., 2015).

For the purpose of derivation of HNRVs, the expert panel preferred to consider the chemical toxicity estimated by toxicity data of microscale particles, the ionic or molecular counterpart of the substance. When no toxicity data are available on the chemical components of the biopersistent nanomaterial, is not possible to apply HNRVs and these ENMs should be tested on a case-by-case basis (category D in Fig. 3). The panel recommends, from a precautionary point of view, that exposure to these ENMs should be minimized. If the substance-specific toxicity is expected to exceed the particle toxicity effect, the ENM would be placed in category E (ENMs with substance-specific toxicity). When the toxicity is driven by the particle effect, rather than by chemical toxicity these ENMs would be placed in category F (ENMs with relatively low specific toxicity).

**3.4.2. Definition of biopersistence for categories D, E, and F—**The availability of cut off values for biopersistence has been discussed among the experts. Solubility of ENMs in water is considered not predictive enough, as the ENMs interact with the biological environment where they deposit or subsequently end up e.g. in macrophages. After inhalation, once deposited in the alveoli, the initial contact would be with the lung lining fluid. ENMs that slowly dissolve in lung lining fluid are grouped in category D, E or F.

Once particles are phagocytosed by cells of the immune system, the dissolution behavior in lysosomal fluid becomes relevant for their toxicity. As the pH and the composition of the fluid (e.g. presence of salts and proteins) will influence dissolution behavior, information on dissolution rates (the amount of dissolved substance versus time) in both fluids (lung lining fluid and lysosomal fluids) is needed. If there is slow dissolution in lung lining fluid, but there is quick dissolution in lysosomal fluid, particles can still be relatively easily cleared by the body but could still cause lung cell damage and inflammation. This can be the case for certain metal oxide nanoparticles such as copper oxide nanoparticles (Gosens et al., 2016). Please note in case there is quick dissolution in lung lining fluid, the dissolution rate in

lysosomal fluid is less important since particles will already dissolve in the lung lining fluid and ENMS are grouped under Category C.

As mentioned in 3.3, dissolution rates from ENMs are particularly important in determining adverse effects. Dissolution could decrease toxicity if this is due to the reactivity at the surface or increase it if toxic effects are due to released ions or direct cellular interactions.

The expert panel agreed that the dissolution rate in lung lining fluid can be used to group ENMs that are expected to cause particle-related effects even if the particles dissolve eventually, for example in lysosomal fluid. At the time of the panel discussions, no literature was available on cut-off values for biopersistence. Recently, a proposal for such cut-off values was published within the GRACIOUS project(Braa-khuis et al., 2021). Ongoing work should evaluate this and other literature for dissolution data to make recommendations on an appropriate definition for biopersistence.

**3.4.3. Definition of biopersistent spheroidal ENMs with specific toxicity (category E)—**Within the category of biopersistent ENMs, the expert panel considered it useful to distinguish between ENMs for which the toxicity is driven by a general particle effect, and ENMs for which the toxicity is driven by specific material properties. Besides particle size, also shape, crystal phase and coatings can be determinants of the toxic effect. The latter two characteristics can directly influence surface reactivity that correlates well to pulmonary inflammation (Braakhuis et al., 2014).

Some ENMs contain chemical components that have carcinogenic, mutagenic, teratogenic, reproduction toxic (CMTR) or sensitizing properties. It was suggested to use available hazard banding methods to help distinguishing expected specific toxicity versus non-specific or particle-driven toxicity based on the chemical identity of the ENM (NIOSH, 2019). Hazard bands can be based on OELs of the chemical components, No Observed Adverse Effect Level (NOAEL), Benchmark dose lower confidence limit (BMDL) data or other toxicity data on the non-nanoscale form, or more qualitatively based on harmonized classification and labelling of the chemical components, e.g. using the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

Some ENMs, especially metal oxide ENMs, can initiate oxidative stress via reactive oxygen species (ROS). It was suggested to use surface reactivity or the ability to produce ROS to identify ENMs with surface reactive specific toxicity. Several acellular assays have been used to measure the reactive oxygen species (ROS), including the Ferric Reducing Ability of Serum (FRAS) assay (Arts et al., 2015; Braakhuis et al., 2021), the Electron Paramagnetic Resonance (EPR) assay and the Dichlorodihydrofluorescein diacetate assay ($DCFH_2$-DA) (Braakhuis et al., 2021). The most suitable assay to assess the surface reactivity of an ENM depends on the type of mechanism with which ROS are generated. Furthermore, the experimental conditions (media, pH, temperature, proteins, salts, light, etc.) should be carefully selected based on the expected environmental conditions. To analyze the biological consequences of the surface reactivity, several cellular assays such as cellular $DCFH_2$-DA assay, protein carbonylation, Nrf2 antioxidant response pathway, Endoplasmic Reticulum stress, Heat Shock Protein activation, glutathione depletion and lipid peroxidation have been

suggested. However, more work is needed to enable the selection of the most appropriate cellular assay or battery of assays (Braakhuis et al., 2021).

The expert panel agreed that information on toxicity of the larger scale material and chemical components, and acellular and/or in vitro reactivity and inflammatory potential of the ENM could be used to distinguish ENMs with and without nanomaterial-specific toxicity as suggested by Braakhuis et al. (Braakhuis et al., 2021).

**3.4.4. How should the HNRV be determined for biopersistent spheroidal ENMs with specific toxicity (category E)?—**xA starting point for a worst-case approach could be to use of the OEL for larger scale materials (if available) and then apply an assessment factor for the nanoforms. This is only possible for materials for which a safe exposure threshold can be derived (below which no adverse effects are expected). Ideally, to be able to do this, the size particle distribution of the material used in the studies that were used to derive the OEL would need to be known. However, in practice this information is not always available. A (worst-case) approximation would be to assume that the OEL for micron-sized material refers to the upper limit of the respirable range. It was recommended that a (worst-case) assessment factor could be determined by accounting for the difference in toxicity between larger scale material and the nanoform(s). For example, toxicity data on both nanoparticles and microscale particles are available for several ENMs, e.g. titanium dioxide ($TiO_2$) and silver (NIOSH, 2011, 2021). To determine a worst-case assessment factor, datasets of several different types of ENMs with different toxicological mechanisms should be compared to datasets of their larger scale counterparts, taking into account differences in (expected or measured) lung deposition.

Subsequently, the resulting assessment factor can be applied to calculate separate HNRVs for specific ENMs, based on the OEL of their corresponding larger scale material. The calculated HNRV for the nanomaterial should be more restrictive than the general HNRV estimated for biopersistent ENMs with relatively low substance-specific toxicity (Category F) (see 3.4.5), indicating that the substance-specific toxicity indeed exceeds the generic particle toxicity.

**3.4.5. Definition of biopersistent spheroidal ENMs with relatively low specific toxicity (category F)—**For biopersistent ENMs with relatively low substance-specific toxicity, it is assumed that the particle effect is greater than the substance-specific toxicity. In toxicity tests, rats developed cancer only after exposure to high concentrations of poorly soluble particles of relatively low toxicity (Borm et al., 2004; ECETOC, 2013; IARC, 2010; Olin, 2000).

A high ‘overload’ inhalation dose of poorly soluble particles of relatively low toxicity in rats can lead to impaired particle clearance. This alters the distribution of these particles in the rat lung towards the interstitium. In this region of the lungs, adverse effects such as inflammation and fibrosis are also found in humans (Bos et al., 2019). Coal miners, who have historically been exposed to high levels of coal dust, can develop lung inflammation and fibrosis, and as some evidence suggests, lung cancer (Graber et al., 2014).

At doses below overload conditions, there may already be prolonged lung clearance, but the clearance is not completely impaired, as is shown in studies on the clearance rate of carbon black (Elder et al., 2005). Rats exposed to carbon black at doses that prolong the clearance (but not completely impair clearance) can develop lung cancer (IARC, 2010). Since not only completely impaired clearance, but also prolonged clearance may lead to lung cancer, no specific recommendation can be made on a threshold or cut-off point for a dissolution rate in lysosomal or lung lining fluid to categorize ENMs.

**3.4.6. How should the HNRV for biopersistent spheroidal ENMs with relatively low specific toxicity (category F) be determined?—**The expert panel agreed that potentially useful data are available to derive an HNRV for biopersistent ENMs with relatively low substance-specific toxicity, as described in this section. They suggested to evaluate data on chronic endpoints such as (lung) cancer and determine the NOAEL or BMDL if possible. In addition, it was also recommended to evaluate data on other effects also of relevance to humans, such as inflammation or fibrosis, and determine the NOAEL or BMDL if possible and then select the most sensitive endpoint to derive an HNRV.

**3.4.6.1. Human data.:** Human epidemiological data are available for carbon black and $TiO_2$. However, those studies often have limitations such as lack of exposure-response information and lack of information on particle size distributions. These limitations affect the usability of such studies in the derivation of occupational exposure limits (Jacobsen et al., 2018).

The panel agreed that data from exposure to mixtures that contain a nanosized particle fraction, such as diesel engine emissions (DEE), respirable dust and ultrafine dust particles (UFP) could also be used in a weight of evidence approach. For example, OELs are available for respirable dust, such as the UK action level for general dust under The Control of Substances Hazardous to Health Regulations (CoSHH) of 4 $mg/m^3$ for the respirable fraction, and the German OEL for respirable dust of 0.3 $mg/m^3$. The German OEL specifically applies for granular biopersistent dusts without substance-specific toxicity (GBS) with a density of 1 $g/cm^3$. In the USA, regulatory limits for occupational exposure to “inert or nuisance” dust are covered under the Particles Not Otherwise Regulated (PNOR) permissible exposure limit (PEL) of 5 $mg/m^3$ (respirable fraction) and 15 $mg/m^3$ (total dust) (OSHA, 2022).

UFP is an example of a complex mixture, for which epidemiological data has provided insight about the effect of exposure to particles. Exposure to UFPs can result in adverse short-term associations with inflammatory and cardiovascular changes (Ohlwein et al., 2019). In addition, some exposure studies compared UFP dust with dust exposure in the workplace (Stone et al., 2017; Viitanen et al., 2017). Thus, UFP data could be used in a weight of evidence approach or sensitivity analysis.

Epidemiological studies on DEE are available that have information about exposure characterization such as particle size distribution including the nanosized fraction. The Health Council of the Netherlands published cancer risk estimates for DEE exposure based on epidemiological data (Gezondheidsraad., 2019). However, DEE are complex

mixtures of chemicals in gas and particulate form. The particulate fraction consists of a core of elemental carbon and adsorbed organic compounds including polycyclic aromatic hydrocarbons (PAHs), metals, and other trace elements. The possibility that some of the DEE carcinogenic effects may be related to particle-transported chemicals rather than the inert particle core should be considered before using these data to derive an HNRV for biopersistent spheroidal ENMs.

It is furthermore possible that endotoxin contamination may have influenced inflammatory effects of nanomaterials in older studies (Dobrovolskaia et al., 2010; Oostingh et al., 2011; Shi et al., 2010; Smulders et al., 2012; Vallhov et al., 2006). However, recent studies have reproduced a strong surface-area dependence of nanomaterial- induced inflammation up to 26 weeks post-exposure, (Cosnier et al., 2021) and thus not expected to be confounded by endotoxin, since endotoxin-induced inflammation peaks within 24 h and ceases within days.

**3.4.6.2. Animal data.:** Chronic inhalation exposure studies to $TiO_2$ NPs, carbon black and DEE in rats performed in the same study showed that the lung cancer incidence from the three types of exposure was very similar (Heinrich et al., 1995). This supported the hypothesis that the carbon core of the diesel soot is the main causative agent for DEE-related carcinogenicity. In a recent study in mice (Bendtsen et al., 2020), ROS was a good predictor of DNA damage, while PAHs did not predict DNA damage. In addition, animal studies have shown that DEE without particles (only gas phase) is not carcinogenic (Brightwell et al., 1989; Heinrich et al., 1986), whereas there is evidence that extractable contaminants on DEE as well as the carbon core may contribute to carcinogenicity (Bendtsen et al., 2020; Siegel et al., 2004; Xia et al., 2004). Chronic inhalation exposure studies to $TiO_2$ ENMs, carbon black and DEE in rats performed in the same study showed that the lung cancer incidence from the three types of exposure was very similar (Heinrich et al., 1995) and induced lung cancer at air concentrations below the air concentrations that inhibit particle clearance in rats (Saber et al., 2019). Logistic regression modeling did not demonstrate significant differences between the carcinogenic potencies of carbon black and DEE in male or female rats. This result supports the hypothesis that the carbon core of the diesel soot contributes to DEE-related carcinogenicity (Nikula et al., 1995). Both inhalation of diesel exhaust and pulmonary instillation of DEE and DEE extracts increased the mutant frequency in lung tissue in genetically more susceptible mice (Hashimoto et al., 2007). DEE extracts constituted 50% of DEE mass, and the authors concluded that the mutagenic potential of DEE could be explained in that study by the extractable mutagens including PAHs and nitrated PAHs (Hashimoto et al., 2007).

Inhalation studies of carbon black in rats reported adverse effects based on a threshold mechanism for inflammation and a non-threshold mechanism for cancer at doses below the current Danish OEL level for carbon black of 3.5 $mg/m^3$ (Jacobsen et al., 2018). Therefore, the Danish working group of the National Research Centre for the Working Environment re-evaluated the scientific basis for setting health-based occupational exposure limits for carbon black. For the estimation of a derived no effect level (DNEL) they selected the following information: a 12-month chronic inhalation study in rats (mass concentrations: 0, 2.5, and 6.5 $mg/m^3$); a 13-week sub-chronic inhalation study in mice, rats, and hamsters (0, 1, 7, and 50 $mg/m^3$); and a 13-week sub-chronic inhalation study in rats (0, 1, 7, and 53

mg/m$^3$). The DNEL was set at 0.02 mg/m$^3$ for CB ENMs (aggregates or agglomerates of primary particles within a size range of 10–100 nm) based on the threshold approach for the inflammatory potential. Excess cancer risk was derived from a 2-year chronic inhalation study in rats (0 and 12 mg/m$^3$) and a 2- year chronic inhalation study in rats (0, 2.5 and, 6.5 mg/m$^3$) resulting in exposure levels of 0.00003 mg/m$^3$, 0.0003 mg/m$^3$ and 0.003 mg/m$^3$ corresponding to excess cancer risk of 1:100000, 1:10000 and 1:1000, respectively (Jacobsen et al., 2018).

**3.4.6.3. Comparison of human and animal data.:** The rat inhalation assay is an area of scientific debate that relates to the consequences of the prolonged clearance rates observed in rats following inhalation and lung deposition of particles and the relevance of those events in humans (Bevan et al., 2018; Borm and Driscoll, 2019; Driscoll and Borm, 2020; Saber et al., 2019; Warheit et al., 2016). Rats are more sensitive than other rodents (mice or hamsters) to developing adverse lung effects from inhaled nanoparticles such as carbon black and titanium dioxide. These lung effects include prolonged particle clearance, persistent pulmonary inflammation, and lung cancer (Elder et al., 2005; Heinrich et al., 1995).

This greater sensitivity of the rat has been cited as overpredicting the particle-associated lung effects in humans. However, in an evaluation of inhaled particles classified by IARC as human carcinogens, rats were shown to better predict the human cancer hazard than were mice or hamsters, which gave false negatives (Mauderly, 1997).

In addition, quantitative comparisons of rat- and human-based excess risk estimates for lung cancer have been derived for working lifetime exposures to some inhaled poorly-soluble particles (Kuempel et al., 2009). Risk estimates for lung cancer were not statistically significantly different based on human or rat data for either carbon black, titanium dioxide, coal mine dust, or crystalline silica, although the human risk estimates included high variability. For diesel exhaust particulates, the rat-based excess risk estimates generally under-predicted the estimates based on the human studies, as shown in summary estimates across studies (Kuempel et al., 2009; Stayner et al., 1998).

Saber et al. (2019) also highlighted the higher lung cancer rates in human populations exposed to diesel engine exhaust compared to those in rats. A systematic meta-analysis of three epidemiological studies reported an estimated 170 excess cases of lung cancer per 100,000 persons exposed (Vermeulen et al., 2014), which is more than two orders of magnitude higher than the estimated 1.3 excess cases per 100,000 based on the rat chronic inhalation studies (Saber et al., 2019). These studies suggest that the rat studies do not overpredict the risk in humans.

Currently, for quantitative data on particle effects it is necessary to rely on animal studies. In the future, it would be preferable to utilize information from alternative models (Hartung, 2009; Rovida et al., 2015) once predictive assays and computational models for EMNs become more widely available and validated. The currently available human data provide useful information on mode of action in humans, but are not sufficient for a quantitative estimation of an HNRV for biopersistent spheroidal ENMs. The experts agreed that by

combining dose-response data from animal studies and evidence from human data, a weight-of-evidence approach could be used to derive an HNRV for the category of biopersistent spheroidal ENMs with relatively low substance-specific toxicity.

### 3.5. Discussion on dose metric

The metric for measuring ENMs is a well-known point of debate and not one dose metric is preferred for all categories of ENMs. Rather, the most suitable dose metric depends on the endpoint measured and on the expected exposure circumstances.

For WHO fiber-like HARNs (category A), the panel agreed that fiber number is the preferred dose metric. However, a concern is that the WHO (1997) method to count asbestos fibers is not applicable to thin fibers (diameter below 200 nm), because it uses optical microscopy. Individual nanoscale fibers of diameter < 100 nm will not be visible for counting using phase contrast microscopy either, even if these fibers are longer than 5 μm (Baron, 2016). Thus, nanofibers will be in many cases too thin to be observed in the monitoring methods for asbestos. In addition, nanofibers and nanotubes occur at the workplace in many shapes and forms: as fibers or bundles, as agglomerates or aggregates, as entangled fibers or composite particles. Detection of individual nanoscale structures would likely require other methods such as transmission or scanning electron microscopy (TEM or SEM). Electron microscopy has the resolution to count fibers down to few nanometers but there is a need for a harmonized counting method. This counting method by EM could be time consuming and may not be a method affordable for all countries or all companies. Also, when using conventional manual counting techniques, it is difficult to recognize WHO-fiber-like HARNs in agglomerates and composite particles and thereby increases the possibility of underestimating their numbers (Tromp et al., 2017). Research is currently underway to develop and harmonize counting methods by EM, including ways for semi-automatization of the method. There are several projects, in which alternative methods have been proposed (NIOSH, 2013; Ogura, 2013; Tromp et al., 2017). Recently, BAuA published a methodology to count nanoscale fibers using SEM (Meyer-Plath et al., 2020; Plitzko et al., 2018b; Plitzko et al., 2018a). The method is based on the WHO methodology for counting asbestos fibers with specific adaptations for nanoscale fibers. It also includes specific recommendations on sampling, compliance checking and documentation. NIOSH recommends airborne sampling of carbon nanotubes in workplaces and analyzing the filters for Elemental Carbon mass concentration. In addition, qualitative analysis of the airborne carbon nanotubes collected on the filters can be carried out by EM (Dement et al., 2015; NIOSH, 2003; NIOSH, 2013).

In relation to exposure to primary airborne spheroidal ENMs, particle number or surface area may be the appropriate dose metric, while volume or mass-based methods may be more appropriate when a high level of agglomeration is expected. In addition, few real-time instruments can measure the surface area concentration of agglomerates larger than 500 nm up to several micrometers in size (e.g., during powder handling). This may favor the derivation of volume or mass-based limit values. Kuuluvainen et al. suggested to combine mass and surface area (e.g. Lung Deposited Surface Area (LDSA) concentration) for most health- based endpoints (Kuuluvainen et al., 2016).

The panel discussed whether aerosol surface area concentration should be recommended as the most relevant dose metric for the category of biopersistent nanoparticles without substance-specific toxicity. The experts did not agree on making such a recommendation. Different specific surface properties may be good predictors for certain surface- related endpoints but not for all endpoints. The link between specific surface endpoints and toxicity is not always clear and for some material groups, mass may be better at predicting endpoints such as ROS- generation (Sauvain et al., 2013). Although the importance of surface reactions (e.g., ROS generation) is acknowledged for ENMs, the measurement of relevant surface properties is challenging.

The ideal approach would be to measure functional group reactivity, but this is complicated (Setyan et al., 2010). Measuring Fuchs or active surface area gives a good approximation for the specific surface area (OECD, 2009). However, this does not work for complicated structures or porous ENMs as it cannot take inner surface into account. For the workplace, the use of a diffusion charger has been discussed. This technique has an upper measurement limit of <500–700 nm, so large agglomerates would be missed and results could be biased when mixtures of nanoparticles and larger particles are present (Ku and Kulkarni, 2012). As workplace measurements have shown that exposure to ENMs often involves agglomerates and aggregates (Guseva Canu et al., 2020; Kaluza et al., 2009; Kuhlbusch et al., 2011; Oberbek et al., 2019) this method is less suitable for measuring surface area in the workplace.

Number-based limits are highly dependent on the degree of agglomeration, and would not be suitable in cases where (large) agglomerates are present, as is illustrated in Table 2 and by calculations from the Institute for Occupational Safety and Health (IFA), which clearly show the variance in particle number in relation to particle size and density for different nanomaterials (IFA, 2008). In addition, using a number-based limit requires stable background measurements to differentiate between ENMs and background particles such as process-generated nanoparticles.

Most but not all experts agreed that using mass as a dose metric in workplace exposure measurements may be sufficient for spheroidal ENMs. The OELs for many particles are in the units of airborne mass concentration. Mass measurements of aggregates and agglomerates of ENMs are easier and more stable to perform. For ENMs that are not highly agglomerated, surface area as dose metric might be more appropriate to predict adverse effects. However, surface area measurements are more difficult to perform in workplaces. In many cases, a combination of different dose metrics would be the most suitable option, for example a combination of mass-based measurements along with estimates of primary particle size and agglomerated particle size. Further discussion is needed on how to handle the measurement of ENMs in workplaces and to identify suitable proxy measures.

### 3.6. How to handle surface modified ENMs

Surfaces of nanoparticles can be modified to give them specific functionalities such as a water-repellent function, a change in conductivity or to withstand corrosion. Also these modifications could prevent unwanted interactions that lead to a ENM decay, agglomeration or surface-mediated catalytic reactions (Zhao et al., 2019). For example, an ENM surface

that is capable to produce toxic substances such as the formation of ROS, can be shielded with a surface modification. As long as the surface is covered, hazardous effects may be prevented. Alternatively, if the modification is causing toxic effects, the negative effects may disappear or get reduced once the modification has disappeared.

It was suggested that surface modified and non-modified ENMs should be dealt with separately (meaning that a separate risk assessment for both forms should be undertaken) and that the approach of the Swiss Precautionary Matrix (Höck et al., 2018) could be applied to coated ENMs. The Swiss Precautionary Matrix states that depending on the stability of the surface modification, separate HNRVs may apply to the modified and the non-modified nanoforms. The most stringent HNRV value should then be used.

### 3.7. Process-generated nanoparticles (PGNPs)

In many occupational settings, exposure to nanoparticles may not exclusively be caused by the handling of ENMs. Exposure may also occur to nanoparticles that are formed and released unintentionally, for example by heating and combustion processes, electrical motors, or high-energy mechanical processes like sawing, grinding and drilling. In addition, handling of nanocomposites may release unbound nanoparticles as well as nanoparticles embedded in the composite matrix (Ding et al., 2017b). Unintentionally formed and released particles are referred to as process-generated nanoparticles (PGNPs). Current knowledge on toxicity of ENMs justifies greater attention on the risks of exposure to PGNPs in workplaces (Bessa et al., 2021; Oberbek et al., 2019; van Broekhuizen et al., 2012).

The experts agreed that if the composition of the PGNPs can be estimated, HNRVs can be applied in the same manner as for ENMs. Based on the starting material and the process, a reliable indication of the composition of PGNPs can be anticipated for many materials and processes (van Broekhuizen, 2017). If the composition and chemical toxicity of the PGNPs is estimated, they can be assigned into one of the categories based on their composition, e.g., to category C or D. If information on size, composition and expected chemical toxicity is insufficient, PGNPs cannot be assigned to one of the categories of HNRVs. However, they should still be considered in overall risk management measures or risk assessments, e.g., using ambient air quality standards.

Furthermore, for some specific types of PGNPs, e.g. welding fumes and diesel exhaust, legally binding OELs are available in several countries (IFA, 2021). The use of these OELs is preferred over assigning these types of PGNPs to one of the HNRV categories.

### 3.8. Fraction of nanoparticles in conventional products (FCNPs)

Some materials have a particle size distribution in both the micrometer-range and the nanometer-range. Materials with a relatively small fraction of particles in the nanometer-range are not necessarily considered to be ENMs. For example, in the EU recommendation of the definition of nanomaterial, a particle number size distribution threshold of 50% is given (EU, 2011), below which materials are not considered ENMs. However, when handling these materials, exposure to nanoparticles may occur. This has been shown in workplace exposure measurements, for example with calcium carbonate (van Broekhuizen et al., 2012). Whether FCNPs should be included with HNRVs depends on the relative

difference in biodistribution and toxicity between the nanosized fraction and non-nanosized fraction. Are data available to assess this, and can uncertainty factors be applied when these data are lacking? Do we expect toxic effects that are specific for the nanosized fraction or that are greater per unit mass dose? If yes, the risk assessment would need to give separate consideration of the nanoscale fraction. If not, the available OEL for the non-nanoform should be sufficient. However, quantitatively discriminating which toxic effects were caused by nanosized particles versus agglomerates/aggregates or larger sized particles in toxicity studies and workplace air monitoring studies is very challenging.

In the derivation of the REL for $TiO_2$, NIOSH determined the fraction of $TiO_2$ in the nanoscale size range and applied separate RELs for the nanoscale and the microscale fractions. By analogy, HNRVs could be applied to materials in which a relatively small fraction (< 50%) consists of particles in the nanoscale range, but with a disclaimer that the HNRVs should only apply for the released nanosized fraction. The panel agreed on the need for more epidemiological studies in which exposure to different size fractions of released ENMs airborne dust can be measured and related health effects monitored. Most early epidemiological studies on nanoscale particles (e.g., $TiO_2$, carbon black) published to date did not report particle size-dependent exposure data (IARC, 2010; NIOSH, 2011). Fortunately, more extensive exposure characterization is emerging in more recent studies (Dahm et al., 2018; Fonseca et al., 2016).

### 3.9. Perspectives on implementation of HNRVs

The framework proposed in this paper may assist national implementation of advisory exposure limits for groups of ENMs. How national regulators set these exposure limits is outside the scope of this project. Regulations differ quite substantially per country and so, concrete recommendations for actions, or policy options, have not been considered by the expert panel.

In addition to defining categories of ENMs for which HNRVs can be derived, guidance and possibly research is needed for the practical implementation of these limits in workplaces. Behavioral sciences should explore the question how the division in 6 HNRV categories would fit into daily occupational safety and health practice. The details of this practical implementation will probably differ between countries, depending on the national system for regulation of occupational exposure. Also, the derivation of the HNRVs should take into account possible limitations in what can practically be measured at the workplace. Implementation of HNRVs in conjunction with other hazard and control banding approaches is another practical consideration. The expert panel did not discuss these considerations in depth.

Some general recommendations can be made about how and when the HNRVs can be used in relation to existing occupational health and safety regulations. It is important to note that the HNRVs are not meant to replace any existing OELs for ENMs. They are intended to supplement existing regulations. In the assessment of occupational exposure to ENMs, the preferred hierarchy would be:

1. check if an OEL or other health-based exposure limit for the specific ENM is available from an international or national body, for example: NIOSH REL, German occupational exposure limits: Arbeitsplatzgrenz-werte (AGW) or Maximale Arbeitsplatz-Konzentrationen (MAK value), DNELs or OEL recommendations derived by the Risk Assessment Committee (RAC) of the European Chemicals Agency (ECHA), former Scientific Committee on Occupational Exposure Limits (SCOEL) recommendations, etc.
2. if no binding or non-binding health-based OEL or other reference value is available for the specific nanomaterial, but specific toxicity data for the nanomaterial is available, use this data to derive an advisory OEL.
3. if insufficient toxicity data on the specific nanomaterial are available to derive an advisory OEL, use the HNRV. A precaution-based approach is advised: if insufficient information is available to place a nanomaterial in one of the HNRV categories, exposure to these ENMs should be minimized.

A broad dialogue with authorities is needed to ensure this approach will be acknowledged and accepted as a complementary approach to existing OELs.

## 4. Conclusion

Our expert panel discussed the possibilities of using state-of-the-art scientific knowledge about the health effects caused by ENMs to advise on provisional advisory health-based occupational limit values (HNRVs) to support the protection of workers. The panel addressed the following questions: 1) What ENM-categories should be distinguished to derive HNRVs? 2) What evidence would be needed to define values for these categories and 3) How much effort would it take to achieve this? The expert panel proposed the following categories of ENMs:

**A.** *WHO-fiber-like HARNs*. Within the group of non-spheroidal ENMs, the scientific evidence was considered sufficient to define a category of ‘WHO-fiber-like HARNs’ and estimate an HNRV for this specific category. Agreement was reached on the definition of the category and the approach of using health-based exposure limits for asbestos to define the HNRV. However, effort is needed to develop harmonized counting methods for nanofibers.

**B.** *Non-WHO-fiber-like HARNs*. For the non-WHO-fiber-like HARNs, it is not possible to set one HNRV for the whole category, because of the great diversity of materials within this category. For carbon nanotubes and nanofibers, existing recommended exposure limits for specific carbon nanotubes and nanofibers could be used. For some graphene platelets, notably the recent Graphene Flagship initiative has generated toxicity data (Fadeel et al., 2018). However, more toxicity data are needed to estimate appropriate HNRV values for entangled fibers, platelets and other non-spheroidal ENMs.

**C.** *Readily soluble spheroidal ENMs*. Toxicity of ENMs in this category is expected to be similar to acute toxicity of dissolved ions or molecules. If an OEL of the larger scale or ionic counterpart of the nanoform is available, this may be used as

a HNRV. In absence of such data, case-by-case assessment of the nanomaterial is needed and the highest level of protective measures to prevent exposure should be applied on a precautionary basis.

**D.** *Biopersistent spheroidal ENMs with unknown toxicity*. The experts agreed that when toxicity of a nanomaterial is unknown and cannot be estimated by using toxicity data on its chemical composition or larger scale counterpart, an HNRV cannot be applied. These materials should be assessed separately on a case-by-case basis. The expert panel stressed that when the toxicity of nanomaterial is unknown, the highest level of protective measures to prevent exposure should be applied on a precautionary basis.

**E.** *Biopersistent spheroidal ENMs with substance-specific toxicity*. An assumption of substance-specific toxicity of the nanomaterial is warranted based on the toxicity of the larger scale counterpart material and the chemical components. The expert panel recommended that HNRVs for this category may be derived by applying an assessment factor on the OEL of the larger scale counterpart. A general precautionary assessment factor should be derived that accounts for the increase in toxicity of nanosized particles as compared to larger sized particles of the same substance. This could be done by comparing toxicity data of nanoforms and their larger scale counterpart. As most OELs for larger scale materials are mass-based, the resulting HNRVs for ENMs in category E should probably be mass-based as well. However, doses can be converted between mass and particle numbers or volumes, given sufficient information. A check should be made if the substance-specific HNRV is indeed lower than the HNRV of category F, indicating that the substance-specific toxicity of the nanomaterial exceeds the particle toxicity effect.

**F.** *Biopersistent spheroidal ENMs with relatively low substance-specific toxicity*. In this category, toxicity is considered to be driven by particle effects. A substantial body of data on biopersistent particles with relatively low substance-specific toxicity is available for example, data on carbon black, ultrafine particles and diesel exhaust particles. The experts were of the opinion that these data can be combined in a weight-of-evidence approach, to arrive at a general HNRV for this category. Depending on the available data, the expert panel suggests flexibility in the dose metrics.

Regarding question 2, the panel concluded that more work is needed to fill remaining data gaps to recommend appropriate HNRVs:

1. Developing a harmonized methodology to measure and count nanofibers for category A.
2. Generate more hazard data for the materials in category B (non-WHO-fiber-like HARNs and other non-spheroidal ENMs) and connect hazard to specific physicochemical properties.

3. Evaluate all proposals for cut-off values for dissolution rates in relevant physiological conditions as well as their application as an estimate of biopersistence to distinguish between categories C and D-F.

4. Evaluate data on nanoforms and larger scale materials of the same substance to derive a precautionary assessment factor which can be applied to OELs of larger scale counterparts in category E (biopersistent spheroidal ENMs with substance-specific toxicity).

5. Combining human and animal data in a weight-of-evidence approach to derive a general value for HNRV of category F (biopersistent spheroidal ENMs with relatively low substance-specific toxicity).

Work on this has already started with members of the panel presenting these findings to relevant stakeholders and projects so that these gaps and needs can be addressed in ongoing and future projects and initiatives. We think recommendations 1 and 3 could be achieved with reasonable efforts, as work is already in progress in several initiatives. Recommendation 4 and 5 could be possible in the near future considering the work being done in the collection of ENM hazard data in international databases. More attention is needed to ensure that reproducible high quality data are generated for nanomaterials (Halappanavar et al., 2021; Krug, 2014, 2018), including through the development of guidance on standardized testing methods such as those of the OECD (Rasmussen et al., 2019) and full reporting of test results for quantitative analyses. Recommendation 2 may need more time and effort as to our knowledge there are currently no concerted efforts to solve the data needs.

Further work is also needed on the most suitable dose metric for the HNRVs, except for category A in which fiber counts are recommended. In contrast to the current Dutch provisional NRVs, where particle number is the applied dose metric, we suggest flexibility in the dose metric, depending on the toxicity mode-of-action information, the type of workplace activities and the feasible measurement techniques.

Also, the practical feasibility of applying these 6 categories of HNRVs in the workplace should be explored, including available methods to measure and characterize ENM exposure at the workplace, and preferably involving social and behavioral sciences to investigate compliance with these HNRVs in daily practice. Working through some examples within the proposed HNRV categories and performing pilot studies in facilities that work with nanomaterials may help to evaluate the feasibility of the HNRVs. Eventually practical guidance for implementation of HNRV in workplace settings will have to be developed, but this is outside the scope of this study.

In the meantime, until HNRVs are available, the provisional NRVs may still be used as benchmark values for workplace risk assessment in the absence of nanomaterial-specific data, following the hierarchy as described. The proposed categories for HNRVs and recommendations as outlined by this expert panel can serve as a reference point for upcoming derivation of HNRVs.

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## Abbreviations:

| | |
|---|---|
| **BAuA** | the German Federal Institute for Occupational Safety and Health |
| **BMDL** | Benchmark dose lower confidence limit |
| **CLP** | harmonized classification and labelling |
| **DCFH$_2$-DA** | Dichlorodihydrofluorescein diacetate assay |
| **DNEL** | derived no effect level |
| **ECHA** | European Chemicals Agency |
| **ENMs** | engineered nanomaterials |
| **EPR** | Electron Paramagnetic Resonance |
| **FCNPs** | Fraction of nanoparticles in conventional products |
| **FRAS** | Ferric Reducing Ability of Serum |
| **GHS** | Globally Harmonized System of Classification and Labelling of Chemicals |
| **HARNs** | high aspect ratio ENMs |
| **HNRVs** | health-based nano reference values |
| **IFA** | German Social Accident Insurance |
| **MAK** | maximale arbeitsplatz konzentration (German occupational exposure limit) |
| **MWCNTs** | multi-walled carbon nanotubes |
| **NFA** | National Research Center for the Working Environment |
| **NFs** | nanoforms |
| **NIOSH** | National Institute of Occupational Safety and Health |
| **NOAEL** | No Observed Adverse Effect Level |
| **OEL** | Occupational exposure limit |

| | |
|---|---|
| **PAHs** | including polycyclic aromatic hydrocarbons |
| **PGNPs** | Process generated nanoparticles |
| **PNOR** | Particles Not Otherwise Regulated |
| **REACH** | Registration, Evaluation, Authorisation and Restriction of Chemicals |
| **RAC** | Risk Assessment Committee of the European Chemicals Agency |
| **ROS** | reactive oxygen species |
| **SCOEL** | scientific committee on occupational exposure limits |
| **SDS** | safety data sheet |
| **$TiO_2$** | titanium dioxide |
| **UFP** | ultrafine particles |
| **WHO** | World Health Organisation |

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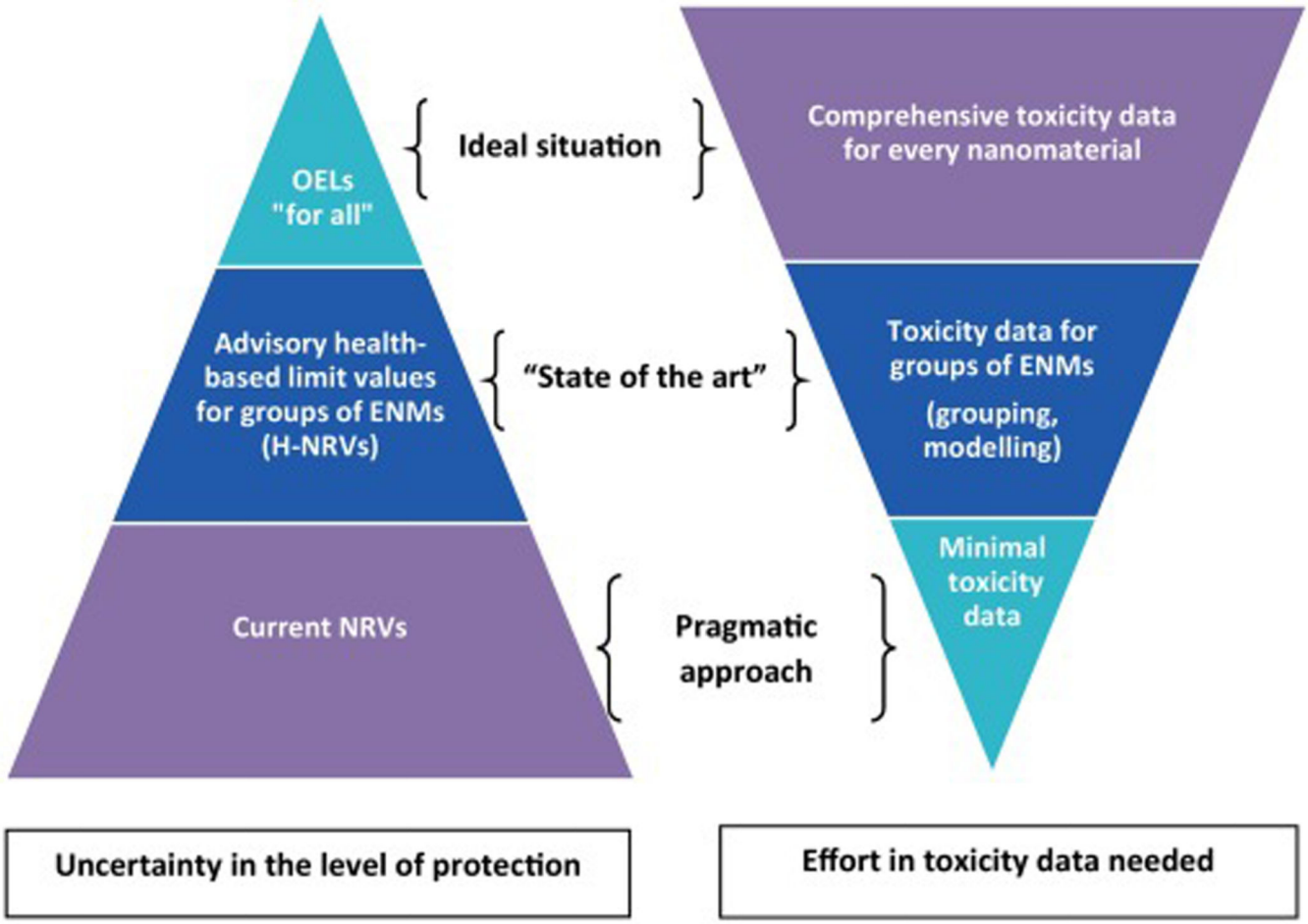


**Fig. 1.**

Uncertainty in the level of protection (left) and effort needed to decrease the uncertainty in the level of protection (right).


**Fig. 2.**

Schematic view of the different steps in the Expert Panel Approach used in this project. CP = Core Panel, RP = Review Panel.

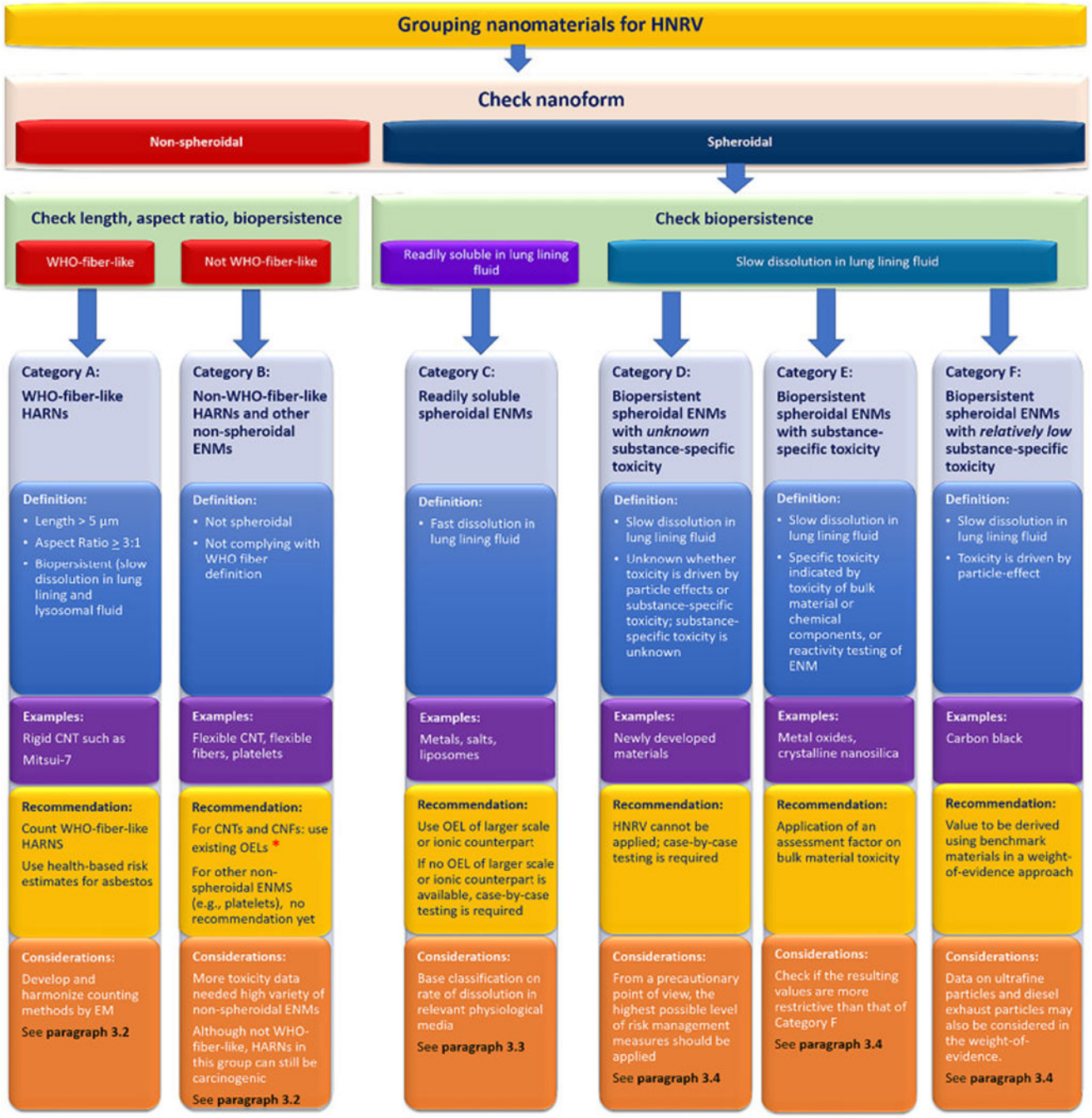


**Fig. 3.**

Graphical representation of proposed HNRV categories, characteristics of the categories and recommendations and considerations on HNRV values described in this article.

* For example, workplace exposure limits recommended by the U.S. NIOSH (NIOSH, 2013) or the Danish NFA (Poulsen et al., 2018).

**Table 1**

Overview of different approaches for grouping ENMs and setting benchmark occupational exposure values.

| **Nanomaterial group ↓** | **Previous initiatives to set benchmark levels and their criteria for grouping →** | | | | | |
|---|---|---|---|---|---|---|
| | **British standards institution (BSI, 2007)** | **German social accident insurance (IFA, 2008)** | **Nano reference values (SER, 2012)** | **Ausschuss fur Gefahrsstoffe (AGS, 2016) *1*** | **US NIOSH (Kuempel et al., 2012)** | **DF4NanoGrouping (Arts et al., 2015)*2*** |
| High aspect ratio nanomaterials (HARNs) with asbestos-like effects | Non-soluble fibers; Aspect ratio > 3:1, length > 5 um. **Value: 0.01 fiber/ml (10,000 fibers/$m^3$)** | Carbon nanotubes for which asbestos-like effects cannot be excluded. **Value: 10,000 fibers/ $m^3$(based on OEL for asbestos)** | Rigid, biopersistent nanofibers for which effects similar to those of asbestos cannot be excluded. **Value: 0.01 fibers/$cm^3$(10,000 fibers/$m^3$)** | Aspect ratio: >3:1, length: >5 um; diameter: <3 um; (WHO, 1997); asbestos-like effects cannot be excluded. **Value: 10,000 fibers/$m^3$** | Not specified. Benchmark materials: carbon nanotubes, carbon nanofibers | Aspect ratio: >3:1, Length: >5 um; Diameter: <3 um;(WHO, 1997) Biopersistence: dissolution rate > 100 mg/L ( BAuA, 2014) or pulmonary half-life of 40 days upon intratracheal instillation (BAuA., 2016). Fiber diameter as a proxy for rigidity. |
| Biopersistent or poorly soluble ENMs, regardless of intrinsic toxicity | | Density > 6000 $kg/m^3$: Metals, metal oxides and other granular biopersistent nanoparticles. **Value: 20,000 particles/$m^3$above background, 8-h TWA;** Density < 6000 kg/m3: Granular biopersistent nanoparticles. **Value: 40,000 particles/$m^3$above background, 8-h TWA** | Biopersistent granular nanomaterial in the range of 1 and 100 nm and a density of >6000 $kg/m^3$**Value: 20,000 particles/$cm^3$, 8-h TWA;** Biopersistent granular and fiber form ENMs in the range of 1 and 100 nm and a density of <6000 $kg/m^3$**Value: 40,000 particles/$cm^3$, 8-h TWA** | | | |
| Biopersistent or poorly soluble ENMs with intrinsic toxicity | Classification of non-nanoform as carcinogenic, mutagenic, asthma genic or reprotoxic. **Value: 0.1 * OEL for non-nanoform** | | | | Surface area, volume, mass or number by particle size fraction, and surface reactivity. Benchmark materials: crystalline silica, nickel (III) oxide, chromium (III) oxide. | Not HARN, not “passive”, not soluble |
| Biopersistent or poorly soluble ENMs without intrinsic toxicity | With OEL for non-nanoform: **Value: 0.06 * OEL for non-nanoform ($mg/m^3$)** Without OEL for non-nanoform: Value based on urban background UFP | | | With OEL for non-nanoform: Based on toxicity of the non-nanoform. **Specific toxicity assessment needed for the nanoform.** Without OEL for non-nanoform: No substance-specific toxicity; | Surface area, volume, mass or number by particle size fraction. Benchmark materials: Titanium dioxide, carbon black. | “Passive” nanomaterials: Toxic component <0.1% (EU, E. A. C. O. T, 2008); surface reactivity: <10% of $Mn_2O_3$ reactivity in FRAS or cytochrome *c* assays; Dispersibility: average agglomeration number ≥ 3; no cellular effects at 610 lg/$cm^2$ |

| Nanomaterial group ↓ | Previous initiatives to set benchmark levels and their criteria for grouping → | | | | | |
|---|---|---|---|---|---|---|
| | **British standards institution (BSI, 2007)** | **German social accident insurance (IFA, 2008)** | **Nano reference values (SER, 2012)** | **Ausschuss fur Gefahrsstoffe (AGS, 2016)** *1* | **US NIOSH (Kuempel et al., 2012)** | **DF4NanoGrouping (Arts et al., 2015)** *2* |
| | concentration. **Value: 20,000 particles/mL above background** | | | no OEL available. **Value: 0.5 mg/m$^3$, respirable fraction** (for particles with a median agglomerate density of 1.5 mg/cm$^3$ and a mass fraction of 20% granular biopersistent particles | | (Kroll et al., 2011). Confirmatory threshold value for low toxic potency: NOAEC in short-term inhalation study >10 mg/m$^3$. |
| Soluble or non-biopersistent ENMs | Cut-off point for solubility not defined. **Value: 0.5 * OEL for non-nanoform (mg/m$^3$)** | | Non-biopersistent granular ENMs in the range of 1 and 100 nm **Value: use OEL for the non-nano form** | **Solubility in water >100 mg/L. Value: use OEL for non-nanoform** | Higher soluble particles (no cut-off). Benchmark materials; zinc oxide, copper(I) oxide | Water solubility >100 mg/L (BAuA, 2014) |

OEL: Occupational Exposure Limit; TWA: Time-weighted Average (concentration); NOAEC: No Observed Adverse Effect Concentration.

*1* If data on solubility in relevant biological media are available, these prevail over solubility in water. Unless asbestos-like effects can be excluded based on data for the specific nanomaterial, all fiber-like materials that fulfil the WHO criteria are assigned to category HARNs with asbestos-like effects.

*2* Exposure-based waiving: if no release/exposure is expected, e.g., because the nanomaterial is trapped in a matrix, then Group 3 (NM without intrinsic toxicity) is assigned.

**Table 2**

Variation of particle number concentrations with agglomeration size for a hypothetical material with an arbitrary density of 2 kg/m$^3$ at an arbitrary mass concentration of 0.1 mg/m$^3$.

| Mass concentration (mg/m$^3$) | Particle/agglomerate size (nm) | Number concentration (#/cm$^3$) |
|---|---|---|
| 0.1 | Primary particles, 10 nm | ~ 95,500,000 |
| 0.1 | Agglomerates, 100 nm | ~ 76,000 |
| 0.1 | Agglomerates, 500 nm | ~ 610 |
| 0.1 | Agglomerates, 1000 nm | ~ 76 |