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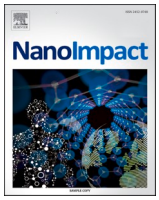

Research Paper

Health-based nanomaterial guidance value (HNGV) for occupational exposure to spheroidal biodurable engineered nanomaterials of relatively low substance-specific toxicity

Petra C.E. van Kesteren [a,1], Damiën van Berlo [a,1], Delphine Bard [b], Owen Butler [b], Susan Dekkers [c], Niels Hadrup [d], Karin Lovén [e,f], Michael Riediker [g], Roel P.F. Schins [h,i], Christoph van Thriel [j], Ulla Vogel [d], Ilse Gosens [a,*]

[a] National Institute for Public Health and the Environment, Bilthoven, the Netherlands
[b] Health and Safety Executive (HSE) Science and Research Centre, Buxton, United Kingdom
[c] TNO, Risk Analysis for Prevention, Innovation & Development, Utrecht, the Netherlands
[d] National Research Centre for the Working Environment, Copenhagen, Denmark
[e] Division of Occupational and Environmental Medicine, Lund University, Lund, Sweden
[f] Department of Occupational and Environmental Medicine, Skåne University Hospital, Lund, Sweden
[g] Swiss Centre for Occupational and Environmental Health, Winterthur, Switzerland
[h] IUF– Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany
[i] Department of Pharmacology and Toxicology, Research Institute for Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University, Maastricht, the Netherlands
[j] Leibniz Research Centre for Working Environment and Human Factors (IfADo), Dortmund, Germany

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ABSTRACT

Exposure to engineered nanomaterials (ENMs) at the workplace can adversely affect human health via inhalation. Occupational exposure limits (OELs) for specific ENMs remain scarce due to a lack of nano-specific data and consensus on the most appropriate dose metric for exposure assessment. In 2022, recommendations were provided on how to derive Health-based Nano Reference Values (HNRVs) for different categories of ENMs. Here, we have updated these recommendations based on new insights and information and changed the name into Health-based Nanomaterial Guidance Values (HNGVs) to distinguish from existing pragmatic Nano Reference Values. Using expert consultation, we derived a general HNGV for spheroidal biodurable ENMs with relatively low substance-specific toxicity. Benchmark ENMs were selected based on criteria such as low dissolution rate in physiologically relevant media and absence of substance-specific toxicity. For these ENMs, several human health endpoints were evaluated and pulmonary inflammation was selected as the critical effect. Persistent inflammation is considered an important driver for chronic adverse effects and keeping exposures below levels causing neutrophil influx is expected to protect against effects such as ENM-induced lung fibrosis and lung cancer. Subsequently, no-observed-adverse-effect-concentrations (NOAECs) or lowest-observed-adverse-effect-concentrations (LOAECs) were derived from high quality in vivo studies to provide a range of Derived No Effect Levels (DNELs). Based on these DNELs, we recommend an HNGV value of 4 μg/m^3 averaged over an 8-h workshift. This HNGV can be practically assessed at the workplace for ENMs that have a clear chemical signature such as metal-based ENMs.

Abbreviations: BET, Brunauer-Emmett-Teller; CNF, Carbon nanofibers; CNT, Carbon nanotubes; DNEL, Derived No Effect Level; ECHA, European Chemicals Agency; ENM, Engineered Nanomaterial; FCNPs, Nanoparticles' fractions in conventional components; GSD, Geometric Standard Deviation; HARNs, High-aspect ratio nanomaterials; HNGV, Health-based Nano Reference Value; LLF, Lung lining fluid; LOAEC, Lowest Observed Adverse Effect Concentration; LDSA, Lung Deposited Surface Area; MMAD, Mass Median Aerodynamic Diameter; NOAEC, No Observed Adverse Effect Concentration; OEL, Occupational Exposure Limit; PGNPs, Process generated nanoparticles; PoD, Point of Departure; PSF, Phagolysosomal fluid; WHO-fiber, Fiber that follows criteria for counting according to World Health Organization definition.

* Corresponding author.
E-mail address: ilse.gosens@rivm.nl (I. Gosens).
[1] Both authors contributed equally.

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1. Introduction

Establishing substance-specific occupational exposure limits (OELs) for engineered nanomaterials (ENMs) remains a complex challenge. To date, no legally binding OELs have been set (Mihalache et al., 2017; Riediker et al., 2012). This is partly due to the complexity in characterization, interpretation of toxicity and worker-exposure data. Furthermore, there is no clear consensus on the most appropriate exposure assessment dose metric for such a limit. Several metrics have been proposed such as particle number, mass concentration or surface area per volume of air or the lung deposited surface area (OECD, 2025a). In a pragmatic approach providing some much-needed guidance for workplace exposure assessment, the Netherlands adopted provisional 'Nano Reference Values (NRVs)' in 2011 (SER, 2012) that are based on the Institute for Occupational Safety and Health of the German Social Accident Insurance approach and that provide particle number concentrations limit values (IFA, 2008). Companies can show due diligence by using these guidance values to protect their workers' health.

The NRVs cover four different classes of ENMs and are accepted as pragmatic benchmark values in risk assessment in the Netherlands. NRVs are not health-based as they are not based on data from health hazard studies. There are concerns that the NRVs may not be protective enough for workers based on reports on the 'Applicability of provisional NRVs to synthetic nanomaterials' (Buist and Oosterwijk, 2017), and the 'Applicability of provisional NRVs to process-generated nanoparticles (PGNPs) and nanoparticles' fractions in conventional components (FCNPs)' (van Broekhuizen, 2017). As part of those evaluations, the few proposed OELs (WHO, 2017), that are health-based but not legally binding, for ENMs were compared to the NRVs. Results showed that for some ENMs the available proposed OELs were lower than the corresponding NRVs, suggesting that the NRVs may offer insufficient protection in some cases despite the uncertainties (Buist and Oosterwijk, 2017). It should be noted that a direct comparison of available proposed OELs and NRVs is challenging. This is because of the difference in metrics (mass-based OELs versus number-based NRVs). Particle number counts can be converted into mass concentrations but the critical information such as particle size distribution, density and shape of ENMs is often lacking or incomplete. Thus, several assumptions on these parameters had to be used in calculations of mass to particle number concentrations and vice versa, leading to uncertainties in the quantitative comparison (Visser et al., 2022).

Based on the evaluations of the NRVs and considering recent scientific advances in the risk assessment of nanomaterials, Visser et al. previously explored the possibilities to derive health-based values for different categories of ENMs using a panel approach (Visser et al., 2022). This resulted in a framework comprising of different categories of ENMs based on nanoform (non-spheroidal versus spheroidal; referring to the constituent particle shape/form, not the agglomerates and aggregates that are formed which can deviate significantly in shape) and biodurability (for the spheroidal ones) which are further discerned by low dissolution in physiologically relevant fluids and absence or presence of substance-specific toxicity (Visser et al., 2022).

By performing a similar Expert Panel consultation, the previous recommendations on how to derive health-based reference values by Visser et al. are now revisited and updated if deemed beneficial, using progression in scientific knowledge and insights. Subsequently, the panel members derived a health-based nanomaterial guidance value (HNGV) for the specific category of spheroidal biodurable ENMs with relatively low substance-specific toxicity.

2. Methods

2.1. Composition of the expert panel

A core panel of eleven members participated in on-line discussion meetings. The panel members did not represent organizations or interest groups, but all contributed as recognized scientific experts in their respective fields of epidemiology (2 experts), toxicology (5 experts), risk assessment (2 experts), occupational and environmental hygiene (2 experts) and occupational exposure sciences (2 experts). Some experts have more than 1 area of expertise. Three writers prepared the draft project proposal, the discussion questions, documents and meeting notes and the draft publication. The core panel provided written comments on the discussion questions, the meeting notes and the draft version(s) of the publication. An additional writer was involved in preparing the section on technical measurements at the workplace (paragraph 3.3.).

2.2. Discussion meetings

A total of five meetings were held in 2024–2025. Before every panel discussion meeting, a discussion document with relevant literature together with questions to be discussed were distributed as background information to the expert panel. The first meeting was focused on discussing new insights from recent scientific advancements with respect to the previously formulated recommendations per ENM category. The questions were:

1. Does the Panel support the suggested framework on grouping of ENM for the purpose of deriving HNGVs developed by Visser et al. (Visser et al., 2022)?
2. Given recent international scientific advances, which data gaps described by Visser et al. have been filled or which advancements have taken place?
3. Does the Panel support the goal to derive a health-based guidance value for the group of spheroidal biodurable ENMs with relatively low substance-specific toxicity?

The answer by the panel to question 1 and 3 was 'yes' and the answers to question 2 are described in paragraph 3.1 Updated recommendations for derivation of health-based guidance values. The subsequent four meetings were centered on finding a strategy to derive an HNGV for spheroidal biodurable ENMs with relatively low substance-specific toxicity and the derivation of the HNGV itself and addressed the following questions:

1. What strategy is deemed appropriate to successfully derive an HNGV for spheroidal biodurable ENMs with relatively low substance-specific toxicity?
2. What selection criteria shall be applied to identify ENMs as spheroidal biodurable ENMs with relatively low substance-specific toxicity?
3. Which ENMs are selected as benchmark materials for the assessment and which ones are disregarded, and why?
4. What are the toxicological effects caused by these ENMs and what is the critical effect?
5. What selection criteria shall be applied to select the key studies, NOAECs/LOAECs and assessment factors?
6. What is the dose metric in which the HNGV should be expressed?
7. How should the HNGV be determined from the range of obtained values?
8. Can the derived HNGV practically be assessed in the workplace?
9. What is the applicability domain for the HNGV for spheroidal biodurable ENMs with relatively low substance-specific toxicity?

Although screening of the literature was performed in preparation of the meetings, extensive systematic literature reviews were not performed. Rather, this paper presents the results of the expert panel discussions, supplemented by relevant literature on the discussed topics. A quality screening of the literature studies to be considered in the preparation of the meetings was done based on e.g. sufficient number of animals per group to allow statistical analysis, inclusion of an appropriate concurrent control group, background variation and the absence

of an effect in the control group, the test material needed to be sufficiently characterized to be able to identify ENMs for inclusion in the category of spheroidal biodurable ENMs with relatively low substance-specific toxicity, exposure (with a minimum duration of 28 days) should have occurred via inhalation to allow for DNEL derivation and information on the key event (inflammation assessed at least 24 h after the last exposure) should be suitable related to the specific endpoint measured and the exposure duration. GLP compliance or adherence to OECD guidance documents and test guidelines were not strict requirements but were considered as clear indications of sufficient quality.

3. Results and discussion

3.1. Updated recommendations for derivation of health-based guidance values

The previously developed framework by Visser et al. identified six different groups of nanomaterials (category A-F) to derive a health-based guidance value for and was called 'Health-based Nano Reference Values (HNRVs)' (Fig. 1). Upon reflection, the experts decided to change the name of the approach first described in Visser et al. into Health-based Nanomaterial Guidance Values (HNGVs) in order to make

Fig. 1. Framework on the proposed grouping strategy to derive an HNGV (previously named HNRV) for six categories of ENMs as proposed by Visser et al. (2022) with updates on the recommendation for category D and the considerations for category A, B, C and E in bold. Note that the word biopersistence has been changed to biodurability as this more accurately refers to dissolution rate testing in vitro in relevant physiological media (after 'check nanoform'). For category F, slow dissolution in lysosomal fluid has been added to the definition, next to slow dissolution in lung lining fluid.

it more distinctive from existing pragmatic NRVs (Visser et al., 2022). The word nano was also replaced by the word nanomaterials' to reflect a broader applicability of the framework then only to nanoparticles. Continued discussion on accurate terminology has led to changes of some of the wording used to define the categories (adaptations are indicated in Fig. 1 in **bold**). For example, the term biopersistence has generally been used to indicate long-term presence of a chemical in a biological system due to resistance to degradation or elimination. In the previous framework, we used the term ambiguously to indicate materials that were poorly soluble or poorly cleared from the lung. Since the term biopersistence is not synonymous with poor solubility, this could create confusion. Also, since we proposed to base the grouping of the spheroidal ENMs, category D-F, on the dissolution rate in physiologically relevant media such as lung lining fluid and did not involve in vivo lung clearance as a criterium, in hindsight, we believe that the term biodurable would be a more accurate term (Maxim et al., 2006; Muhle and Bellmann, 1995). In the updated framework, the term biopersistent is replaced with the term 'biodurability' when referring to dissolution in relevant physiological media assessed in vitro or *in chemico* (Fig. 1 in **bold**).

Also, based on new scientific insights, some of the recommendations or research needs were amended (in **bold** in Fig. 1) which are all explained in more detail per category below.

3.1.1. Category A: WHO-fiber-like high aspect ratio ENMs (HARNs)

The WHO defines fibers as greater than 5 μm in length, less than 3 μm in diameter and having an aspect ratio of 3:1 or greater. This means that the length of the fiber is at least three times the diameter. The WHO-fiber-like HARNs for the grouping approach was 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)' (Visser et al., 2022). Asbestos was deemed a suitable benchmark material to base the derivation of a health-based value on. The previous recommendation was to count fiber-like HARNs as defined by the WHO and use the health-based risk estimates for asbestos. Based on the recent developments, no change in the recommendations for this category is required. Note that since October 2023, the European Parliament agreed to lower the OEL for asbestos fibers to 0.01 fibers/cm^3, without a transition period (Directive (EU), 2023). After a maximum transition period of six years, member states will have to switch to new techniques such as electron microscopy (EM) instead of phase-contrast microscopy, to allow the counting of thin fibers. Member states will then have the option to a) use new techniques and keep the level at 0.01 fibers/cm^3 including thin fibers, or b) decrease the level to 0.002 fibers/cm^3 excluding thin fibers.

3.1.2. Category B: Non-WHO-fiber-like HARNs and other non-spheroidal ENMs

For this category, it was deemed not possible to set one HNGV for the whole category due to the great diversity of materials. For carbon-based non-WHO-fiber-like HARNs, HNGVs may be derived using existing recommended exposure limit values for carbon nanotubes (CNTs) and carbon nanofibers (CNFs) as a benchmark, for example those proposed by the U.S. National Institute for Occupational Health and Safety (NIOSH) (NIOSH, 2013) or the Danish National Research Centre for the Working Environment (NFA) (Poulsen et al., 2018), while for nanomaterials with other shapes more data is needed. Although new publications on the adverse effects of mainly graphene and graphene-like materials are available (Andrews et al., 2024; Lin et al., 2024; Vogel, 2024; Lee et al., 2019; European, Chemicals, Agency, 2022), the expert panel concluded it was premature to start a full evaluation of new evidence. Hence, no changes in the previous recommendations were made and this category was not selected for derivation of a HNGV at this moment (Fig. 1, in **bold**).

3.1.3. Category C: spheroidal ENMs readily soluble in lung lining fluid

Solubility refers to the quantity of solute that dissolves in each quantity of solvent to form a saturated solution. Solubility and dissolution rate (the time needed for a certain amount of substance dissolved (solute) into a solvent) are both important aspects to understand their biological behavior and predict bioavailability, toxicity and the distribution through the body. Dissolution rates are deemed more important for ENM hazard assessment and grouping, because the speed of releasing of ions or molecules before they react or form complexes with biological ligands may be more important than equilibrium concentrations (OECD, 2025b). Effects of readily soluble spheroidal ENMs are expected to be like that of their chemical or ionic constituents. Therefore, our previous recommendation included taking an existing OEL for the bulk material or ionic counterpart of the nanomaterial as a starting point. The panel highlighted that care should be taken to use good quality (not outdated) OELs and case-by-case toxicity testing is still needed when a good quality OEL is not available for the non-nanoform.

3.1.4. Category D: spheroidal biodurable ENMs with unknown toxicity

This category of biodurable ENMs comprises mainly new materials for which toxicity data is lacking or for which it is unknown whether toxicity is driven mainly by particle effects or by substance-specific toxicity. It was previously suggested to test and evaluate these ENMs on a case-by-case basis. Discussions in the expert group led to the suggestion of selecting a worst-case ENM for derivation of a Category D HNGV. Although case-by-case testing will provide more accurate data, a worst-case approach is a more practical solution in the short term. Hence, the panel decided the new recommendation is to select a worst-case ENM (i.e. the ENM for which adverse health effects have been observed at the lowest concentrations) with substance specific toxicity for this category. The method on how to select these worst-case materials has not been further explored here.

3.1.5. Category E: spheroidal biodurable ENMs with known substance-specific toxicity

For ENMs in this category, toxicity is expected to be driven by substance-specific (chemical) effects, rather than by a 'particle effect'. Previously, it was suggested to derive an assessment factor that can be applied to (proposed) OELs set on bulk materials to calculate an HNGV for that specific nanomaterial. As was already mentioned for category C ENMs, the expert group noted that some existing OELs for larger scale materials may be outdated and not sufficiently protective. For example, the NFA health-based OEL published by the National Research Centre for the Working Environment in Denmark derived for ZnO is 0.04 mg/m^3 as compared to the current OEL of 4 mg/m^3 ZnO measured as Zn content (Hadrup et al., 2021a). Further, it was the expert's opinion that the type of toxicity of nanomaterials may not necessarily be the same as shown for bulk material. Therefore, it was suggested to approach risk assessment of materials in category E by using OELs for the non-nanoform (if available) with a precautionary extra assessment factor to account for the possible higher toxicity of the nanoform as compared to the non-nanoform. Both aspects need to be considered when deriving an HNGV for this category. Based on the new insights, the recommendation to derive an assessment factor is still supported, however, care should be taken in the selection of OELs to be used for this assessment factor derivation.

3.1.6. Category F: spheroidal biodurable ENMs with relatively low substance-specific toxicity

For spheroidal ENMs with relatively low substance-specific toxicity and high biodurability in lung lining fluid, the adverse effects are expected to be driven by physical properties (size, solubility, shape), also known as the 'particle effect', rather than by chemical toxicity. It should be noted that for such substances, dissolution rate in lysosomes is considered as a driver of substance-specific toxicity, as this is commonly associated with ion-related toxicity (Hadrup et al., 2023). Thus, high

dissolution rate in lysosomal fluid is considered as an indicator of substance-specific toxicity and would place the material outside category F. Derivation of a generic HNGV value for category F was considered feasible by the Panel in terms of data availability. New data that seemed particularly useful included an overview of inhalation toxicity data on 13 ENMs, including a database of available study details of these 13 materials (Hadrup et al., 2023), and published documentation for setting health-based OELs for carbon black and titanium dioxide (TiO_2) nanomaterials (Saber et al., 2018a; Jacobsen et al., 2018).

3.2. HNGV derivation for spheroidal biodurable ENMs with relatively low substance-specific toxicity

Previously, it was stated that human exposure and adverse health effect data provide useful information on the mode of action of ENMs, but are not sufficient for a quantitative estimation of an HNGV for spheroidal biodurable ENMs with relatively low substance-specific toxicity (category F) (Visser et al., 2022). The literature has been screened by the panel to identify new information generated since Visser et al. (2022), but human data were still found to be scarce. For substances in category F, these new human data are not sufficiently relevant to describe or predict adverse effects and thus cannot be used quantitively as a point-of-departure for HNGV derivation (see chapter 3.2.2). Thus, for pragmatic reasons, the expert panel agreed to use rodent inhalation toxicity data as the quantitative starting point.

A strategy was discussed among the panel members to derive an HNGV for these category F ENMs (Fig. 2).

In short, a stepwise approach was suggested by first selecting potential benchmark ENMs based on several criteria, e.g. particle shape and size, slow dissolution rate in physiologically relevant media (mimicking lung lining fluid), absence of substance-specific toxicity (chapter 3.2.1). An overview of the toxicological effects caused by ENMs in category F is given. For some potential benchmark materials, this resulted in the identification of substance-specific effects and these ENMs were excluded from the set of benchmark materials (Table 1).

In addition, arguments for selecting the critical effect are listed (chapter 3.2.2). For each of the selected benchmark materials, point of departures (PoDs), such as NOAEC(s) or a LOAEC(s), were identified from published toxicological studies (chapter 3.2.3). These were then extrapolated by the panel to a Derived No Effect Level (DNEL), taking into account exposure duration, intraspecies variability and interspecies differences and applying assessment factors according to the approach suggested by the REACH guidance R8 (ECHA, 2012). This is a similar approach that has been used to derive health-based OELs in Denmark (Saber et al., 2018a; Jacobsen et al., 2018; Saber et al., 2018b) and it provides a transparent approach for others to re-use, including the DNELs itself, the identified NOAECs/LOAECs or the key studies. From the range of DNELs derived in this work, a DNEL value of 4 $\mu g/m^3$ was selected to be used as the proposed HNGV for biodurable ENMs with no substance-specific toxicity, which is further explained in chapter 3.2.4.

3.2.1. Step 1 – selection of benchmark materials

Benchmark materials representative for the category of spheroidal biodurable ENMs with relatively low substance-specific toxicity (category F) were selected based on the following criteria:

3.2.1.1. Origin, surface and shape. Only manufactured, pristine, non-surface-treated nanomaterials were included as benchmark materials; natural particles (e.g. volcanic ash or wood-smoke particles) were excluded since these are mostly mixtures that can cause effects by the presence of the particle and by the additional chemicals adsorbed to the surface. This also excludes data on process-generated nanoparticles (e.g. from welding, combustion of diesel fuel), as the chemical composition of these is often not known or are mixtures making it hard to determine if there is only a particle effect. Eligible ENMs are spheroidal as defined by the European Chemical Agency (ECHA) (ECHA, 2022), and this definition includes particles with an aspect ratio up to 3:1. Examples of shapes included in this category are spherical, pyramidal, cubic or other approximately equiaxial shapes. Fibers and platelets were excluded as they fall under other categories (ECHA, 2022).

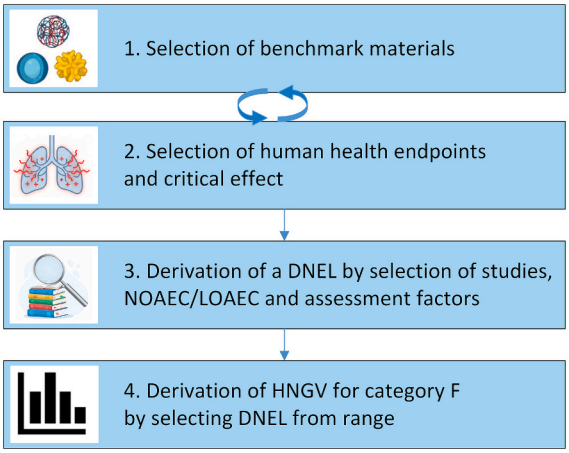

Fig. 2. Strategy for the derivation of an HNGV for spheroidal biodurable ENMs with relatively low substance-specific toxicity (category F ENMs) in four steps. Step 1 is the selection of potential benchmark materials that are representative for category F ENMs. Step 2 is the selection of relevant human health endpoints. Based on the resulting human health endpoints, exclusion of certain ENMs was indicated based on substance specific toxicity, leading to adaptations in step 1 and 2. From the final list of human health endpoints, the critical toxicological effect was identified. In step 3, assessment factors are applied to the concentration that does not lead to the identified critical effect (No Observed Adverse Effect Concentration, NOAEC) to derive DNELs for every benchmark material. In one case, only a lowest observed adverse effect concentration (LOAEC) was available. In step 4, the range of DNEL values for the selected benchmark materials is evaluated. Source illustrations in step 1 (Sheikh and Jirvankar, 2024), step 2 and 3: Google Gemini version 2.5 pro, step 4: Microsoft Visio.

3.2.1.2. Size range. The authors adhered to EU definition of nanomaterials as solid particles of 1–100 nm, either on their own or as identifiable constituent particles in aggregates or agglomerates (EU, 2022). A deviation from the 1–100 nm size range was discussed, mainly in relation to the option to increase the lower boundary on particle size for the applicability domain of this HNGV value. This was based on indications that extremely small nanoparticles (<10 nm) may be less toxic after inhalation. Such extremely small particles do not easily deposit in the airways by the mechanisms known for larger nanoparticles (e.g., impaction, gravitational sedimentation) and behave like a gas rather than a solid particle (Braakhuis et al., 2014a). In addition, a study with 2 nm gold nanoparticles demonstrated relatively fast clearance once inside the body after intravenous injection, likely by renal clearance (Sadauskas et al., 2007). When particles are cleared rapidly from the body, less toxicity is expected. It is plausible that with decreasing size, the same mass concentration may have an increased toxic potential due to the higher surface to volume ratio, or even due to the fact that the total number of particles rapidly increases. But the nanoform is not always more toxic compared to micron-sized particles, since this also depends on the chemical composition. Also, very small sized nanoparticles can migrate to the brain and have high olfactory deposition, leading to potential neurotoxic effects (Garcia et al., 2015). Overall, some characteristics of smaller sized nanoparticles will result in less toxicity, others in higher toxicity. Hence, from a precautionary point of view, the authors decided to adhere to the lower size limit of 1 nm recommended in the EU definition for selecting benchmark materials. The 100 nm upper limit was maintained for consistency, acknowledging that toxicity does not change abruptly at this cut-off.

Table 1
Complete list of potential benchmark ENMs for category F, sorted according to the conclusion on their suitability as a benchmark material based on their solubility behavior in water or dissolution rate in lung lining fluid (LLF) in combination with phagolysosomal simulant fluid (PSF) and information on substance specific toxicity and availability of data. The reason for not being selected as a benchmark material for category F is marked by a grey box (Avramescu et al., 2022; Braun et al., 2016; Gosens et al., 2016; Gouin et al., 2024; Hadrup et al., 2022; Keller et al., 2021; Lim et al., 2021; McCarrick et al., 2021; Merck Index, 1976; Nishi et al., 2020; NTP, 2004; Sharoyko et al., 2021; Shinohara et al., 2017; Sørli et al., 2023; Stefaniak, 2010; Turci et al., 2016; Zanoni et al., 2022).

Nanomaterial	Molecular formula	CAS	Solubility in water	Dissolution rate in LLF (t1/2)	Dissolution rate in PSF (t1/2)	Substance-specific toxicity	Availability of relevant toxicological data	Benchmark material for category F
Included as benchmark material								
Carbon black	C	1333-86-4	**Not soluble** 1 mg/L[a] insoluble[b]	-	-	No	Yes	Yes
Iron (III) oxide	Fe_2O_3	1309-37-1; 1317-60-8	**Poor solubility** pH = 8; 20°C: <1 µg/L[a] Insoluble[b] pH ca. 8; 20 °Ca: > 0.03 < 0.07 g/L[a]	3859- 5776 days (26)	2352-3610 days (26)	No	Yes	Yes
Cerium oxide	CeO_2	1306-38-3	**Poor solubility** < 0.123 - 1.76 µg/L[a] Insoluble[b]	6209-7220 days (26)	2001-2888 days (26, 27)	No	Yes	Yes
Fullerene	C_{60}	99685-96-8	**Not soluble** <10–11 g/l (28)	Cannot be tested due to high hydrophobicity(29)	Cannot be tested due to high hydrophobicity (29)	No	Yes	Yes
Aluminum oxide	Al_2O_3	1344-28-1	**Poor solubility** 0.00002 g/L bulk, 82.0 µg/L nanoform[a]	Losses due to sedimentation in DMEM, therefore not further tested (30)	Losses due to sedimentation in DMEM, therefore not further tested (30)	No	Yes	Yes
Aluminum oxyhydroxide	AlO(OH) (Boehmite)	1318-23-6; 24623-77-6	**Poor solubility** Insoluble. Instability in solution; precipitation[a]	-	-	No	Yes	Yes
Titanium dioxide	TiO_2	1317-70-0; 1317-80-2	**Poor solubility** Rutile: insoluble Anatase: - [b]	320-413 Days (26)	>700 days (26)	No	Yes	Yes
Excluded as benchmark material (reason in grey box)								
Nano silver	Ag	?	**Moderate solubility** 0.03 µg/L – 1 mg/L[a] >100 mg/L (22)	52-72 days (26)	284-289 days (26)	Toxicity due to ion release	Yes	No
Copper (II) oxide	CuO	1317-38-0	**Moderate solubility** >100 mg/L (22)	1% dissolved after 24 hrs in Gamble's solution (pH7.4) (31)	Quickly dissolving: 60% of CuO NPs dissolved within 1 hour (31)	Toxicity due to ion release		No
Nickel (II) oxide	NiO	1313-99-1	**Poor solubility** 0 g/L[a]		7 days in vitro compared to 1 month in vivo (32) 11-33% dissolved in 9 days (33)	Toxicity due to ion release		No
Cobalt(II) sulfate	$CoSO_4$	10124-43-3; 13455-34-0 (monohydrate)	**Moderate solubility**	-	-	Toxicity due to ion release and carcinogenicity of both particle and ion (34)		No
Zinc oxide	ZnO	1314-13-2	**Moderate solubility** 1-2.9 mg/L[a]	3552-4126 days (26)	0.7-0.9 days (26)	Toxicity (partly) due to ion release	Yes	No
Mesoporous silica	SiO_2	7631-86-9	**Quick/ Moderate solubility**	Quickly/moderately soluble in lung lining fluid, 3 hours (35)			No	No
Silicon dioxide, amorphous (Nano)	SiO_2	7631-86-9	**Moderate solubility** > 100 mg/L[a]	3.6-4.5 days (26)	29-35 days (26)	Toxicity partly related to silanol groups at the surface	Yes	No

Gold	Au		**Poor solubility** Generally very insoluble in water [36]	-	Macrophage assisted solubility [37]		No	No
Barium sulphate	$BaSO_4$	7727-43-7	**Poor solubility** (bulk) 3.1 mg/L[a]	9898-14441 days [26]	6.3-7.2 days [26]			No
Quartz	SiO_2	14808-60-7	**Poor solubility**	-	-	Toxicity is very specific for this material due to crystal fragmentation [38]	Yes	No
Aluminum hydroxide	$Al(OH)_3$	21645-51-2	**Poor solubility** 0.00009 g/L[a]	-	-		No, lack of toxicological data	No
Aluminum	Al	7429-90-5	**Poor solubility**	-	- (Soluble in acid solutions)		No, lack of toxicological data	No
Zirconium (IV) oxide	ZrO_2	1314-23-4	**Poor solubility** < 55 µg/L[a]	-	-		No, lack of toxicological data	No
Tungsten (IV) oxide	WO_2	12036-22-5	**Poor solubility** 4.2 mg/L [a]	-	-		No, absence of persistent inflammatory response	No
Iron (II,III) oxide	Fe_3O_4	1317-61-9	**Poor solubility** 1.52 µg/L[a]	-	-		No, lack of toxicological data	No
Tungsten (VI) oxide	WO_3	1314-35-8	**Poor solubility** 84 mg/L[a]	4-11 days (not nano) [39]	9893-21541 days [39]		No, absence of persistent lung inflammatory response [40, 41]	No
Polystyrene	$(C_8H_8)_n$	9003-53-6	**Not soluble** Not soluble in water[a]	-	-	-	Equivocal data, not always persistent lung inflammation [42, 43]	No

a REACH registration dossier – lead dossier.
b CRC Handbook of Chemistry and Physics.

3.2.1.3. Biodurability. Particles are called biopersistent if they remain in the lung for a prolonged period of time despite various clearance mechanisms such as clearance via the mucociliary escalator, macrophage assisted clearance, breakdown, dissolution or translocation. Biopersistence is an in vivo phenomenon that can be assessed in laboratory animals or humans. This is influenced by ENM interaction with macrophages and neutrophils as well as the shape and constituent particle size. For example, a well-known effect of fibrous, poorly soluble materials is a phenomenon called frustrated phagocytosis, in which immune cells that function to remove foreign/toxic materials and organisms from the lung, are not capable of internalizing (due to its size) and digesting these fibers. Spherical nanomaterials, however, can also lead to impaired phagocytosis when they, for instance, are toxic to macrophages. Less efficient phagocytosis by macrophages can also occur when they are very small (e.g. < 10 nm) compared to larger particles (Geiser et al., 2008; Geiser, 2010).

ENMs can reside in the lung as particles for a prolonged period exerting adverse effects if they are poorly soluble in water and dissolve slowly in the lung lining fluid or within the phagosomes of resident macrophages and lung-recruited neutrophilic leukocytes. This behavior can be assessed in vitro or *in chemico* and has been identified as an important property for nanomaterial grouping (Visser et al., 2022). As indicated in section 3.1, we use the term biodurability here, since the grouping of category D-F is based on the dissolution rate in physiologically relevant media and did not involve assessing the in vivo lung clearance as a criterium.

To qualify as a benchmark material, ENMs were considered by the dissolution rate in lung lining fluid and lysosomal fluid (Table 1). Gamble's solution is often used as a representative of the extracellular lung lining fluid (LLF). Resident alveolar macrophages, containing many lysosomes filled with digestive enzymes (e.g. proteases, lipases, phosphatases) in an acidic environment (pH around 4.5–5), are mainly responsible for clearance of the particles. A simulant fluid for this lysosomal environment that has been developed is phagolysosomal simulant fluid (PSF).

Currently, no generally accepted definitions for biodurable ENMs and their dissolution behavior in relevant physiological media exists. Also, there is not yet scientific consensus on how this behavior *in chemico* or in vitro relates to the ENMs behavior in vivo. With respect to measuring the solubility and dissolution rate of nanomaterials, an OECD Guidance Document on determination of solubility and dissolution rate of nanomaterials in water and relevant synthetic biological media (WNT Project 1.5) is being developed (Heunisch et al., 2022). For ENMs, OECD Test Guideline 105 is inadequate for testing water solubility and is also not developed for dissolution rate testing. In this work, accessible databases and scientific literature are searched for measured data on solubility and dissolution in physiologically relevant media for LLF (Gamble's solution) and PSF (see Table 1). In addition, fibroblast lysosome and macrophage assisted degradation in an in vitro assay as described for gold nanoparticles was found and used for the evaluation of their biodurability (Carlander et al., 2019; Balfourier et al., 2020). No distinction was made between methods used to obtain the data although uncertainties in the value may arise depending on the laboratory conditions and method used. Therefore, the data can only be used as a rough estimate.

To determine which ENMs are slowly dissolving in LLF and PSF, the pragmatic cutoffs previously proposed by the GRACIOUS project (Braakhuis et al., 2021a) and previously described by Visser et al. (Visser et al., 2022) were used for the selection. Based on the available data (Table 1), these ENMs turned out to have a half-time (time when equal to or more than 50% is dissolved, $t_{1/2}$) > 320 days in LLF (pH 7.4) and $t_{1/2}$ > 700 days in PSF and are considered relevant for category F, since a greater $t_{1/2}$ than the pragmatic threshold of $t_{1/2}$ > 60 days in lysosomal fluid proposed by GRACIOUS would opt for inclusion. This resulted in the selection of TiO_2, aluminum oxide (Al_2O_3), iron (III) oxide (Fe_2O_3), and cerium dioxide (CeO_2) as potential benchmark materials.

Where dissolution data were lacking, water solubility served as a proxy. This was the case for several iron, tungsten and aluminum oxides,

but also gold (Au), barium sulphate ($BaSO_4$), CeO_2, TiO_2, nickel(*II*)oxide (NiO), cobalt(*II*)sulfate, zirconium oxide (ZrO_2) ENMs and quartz particles. Highly hydrophobic materials such as polystyrene, carbon black (CB) and fullerene (C60) that have a poor wettability and cannot be tested for water solubility without adding solvents or surfactants are listed as not soluble in water and were evaluated further (Vance et al., 2016; Iveson et al., 2001). ENMs with water solubility greater than 100 mg/L are categorized as 'moderately soluble' in Table 1. This categorization, often combined with the release of toxic ion from ENMs with specific toxicity, was a reason to not further evaluate the ENM as a benchmark material.

3.2.1.4. Substance-specific toxicity. ENMs with known toxicity due to ion release (e.g., silver, copper, nickel, cobalt) (Hadrup et al., 2023) or specific hazards (e.g. quartz causing silicosis) were excluded ((Pavan et al., 2024).

3.2.1.5. Toxicological data availability. For all potential benchmark ENMs, toxicity data were identified and further discussed in paragraph 3.2.2. For some materials such as aluminum (Al), aluminum hydroxide ($Al(OH)_3$) and zirconium oxide (ZrO_2), insufficient toxicological data was available for inclusion as a benchmark material. For tungsten oxide and polystyrene, no (consistent) data on a persistent inflammatory response was found. Therefore, these were also excluded from being considered as a benchmark material.

3.2.1.6. Final selection. ENMs prioritized as benchmarks included those with poor solubility in water and/or physiological fluids, low substance-specific toxicity, and robust toxicological datasets: carbon black, fullerene (C60), aluminum oxyhydroxide (AlOOH), iron oxide (Fe_2O_3), aluminum oxide (Al_2O_3), cerium dioxide (CeO_2), and titanium dioxide (TiO_2). These are detailed in Table 1; excluded ENMs are also listed for reference.

3.2.2. Step 2 – selection of human health endpoints and the critical effect

An overview of toxicological effects caused by ENMs was prepared for two reasons. First, this overview was used to identify toxicological effects caused by spheroidal biopersistent ENMs to determine if any of these effects would be related to substance-specific toxicity. ENMs inducing such effects were not selected as benchmark material. Second, the overview was used to select a specific health endpoint as point-of-departure for HNGV derivation. The panel decided on the following four requirements to select appropriate adverse health effect(s): The adverse health effect should have.

1. Relevance for humans and/or animals
2. Been observed in a reliable and relevant animal or human study (i.e. exposure via inhalation, ENMs sufficiently characterized, study does not have obvious shortcomings/deficiencies as described in 2. Method section)
3. A relation to chronic pathology associated with ENM exposure (e.g., chronic inflammation, fibrosis, granuloma, lung cancer)
4. High sensitivity: i.e., the effect must occur at a relatively low exposure level and preferably be the critical effect (worst-case approach).

A selection of health effects was made based on recommendations from the expert panel that met the requirements listed above. These health effects are discussed below to identify the critical effect.

3.2.2.1. Identifying key effects from animal data. Several adverse health effects and diseases are known to occur in animals upon inhalation of ENMs with low substance-specific toxicity. The main pathologies are listed below:

- **Pulmonary inflammation**. This is determined typically by measuring markers in bronchoalveolar lavage fluid (BALF) such as influx of neutrophils or polymorphonuclear leukocytes (PMN), increased numbers of other immune cells, increased concentration of protein (indicating vascular permeability), and oxidative stress or immune markers. Histopathological assessment can be used to identify alveolar wall thickening, edema, inflammatory cell infiltration and general injury to epithelium or endothelium. Measuring the wet-to-dry lung weight ratio provides information on pulmonary edema (Salem and Katz, 2014).
- **Lung fibrosis**. This is assessed visually by a pathologist or through investigation of collagen deposition, the hallmark of lung fibrosis (e.g. by measuring hydroxyproline or specific staining for collagen, such as Masson trichrome or Sirius Red that are quantifiable using image analysis tools). In relation to particle exposure, fibrosis mainly occurs in humans after long-term exposure to specific particle types, such as crystalline silica and asbestos (Churg and Muller, 2024; Jamrozik et al., 2011) or coal dust (Graber et al., 2014). Chronic lung inflammation is considered a key driver for lung fibrosis induced by particle exposure. Recently, a two-year inhalation study of anatase TiO_2 NPs in rats has shown that TiO_2 NPs cause a specific type of interstitial lung disease related to fibrosis, i.e. pneumoconiosis (Yamano and Umeda, 2025). The fibrotic dust foci were already observed in a 13-week inhalation exposure study (Yamano et al., 2022). The lesions required longer to develop, and histopathological analysis revealed that these fibrotic lesions were similar to those seen in humans.
- **Carcinogenicity**. Exposure to ambient particulate matter has been associated with lung cancer induction (Hamra et al., 2014), leading to a classification by the International Agency for Research on Cancer (IARC) of particulate matter (PM2.5 and, PM10, which both include an ultrafine fraction in the nanosized range) in outdoor air pollution as a Group 1 carcinogen (Loomis et al., 2013). There are two mechanisms of particle-associated carcinogenesis, that have implications for HNGV setting: there is a genotoxic mechanism, via the direct induction of DNA damage and/or mutations by the particles themselves, and a non-genotoxic mechanism whereby the particles elicit chronic inflammation, leading to a persistent presence of inflammatory cells that secrete reactive species capable of damaging the DNA. It is assumed that there is no threshold dose for genotoxic carcinogenicity (a single hit event can result in a malignant tumor), where for non-genotoxic carcinogenicity, a threshold dose is assumed to be applicable (Hartwig et al., 2020; Nohmi, 2018; Bolt and Huici-Montagud, 2008). Genotoxicity can for instance be determined by measuring DNA strand breaks, induction of mutations or structural (clastogenicity) or numerical (aneugenicity) chromosomal alterations. There is abundant evidence for the genotoxic potential of several types of nanomaterials (e.g. for ZnO, CuO, MnO, NiO, Al_2O_3, CeO_2, and Fe_3O_4 (Solorio-Rodriguez et al., 2024)). For other ENMs, such as TiO_2, SiO_2 and CB, the picture is not as clear, with some conflicting evidence available pointing towards genotoxicity while other studies have not found indications for genotoxicity (Braakhuis et al., 2021b; Zheng et al., 2024; Di Ianni et al., 2022).
- **Cardiovascular effects**. These include changes in heart rate, cardiac output, or ventricular pressure; cardiac arrhythmias; blood pressure; vascular dysfunction; and increased release of acute phase proteins (Hadrup et al., 2020). Histopathological assessment of the heart allows for identification of structural changes to the heart or vasculature, including myocardial degeneration, cardiac fibrosis, or inflammation of the myocard, endocard or pericard. Associations between PM2.5 or ultrafine particle exposure and cardiovascular toxicity have been shown in numerous epidemiological studies (Stapleton et al., 2012; Franck et al., 2011). Exposure to diesel engine exhaust has been shown to be associated with myocardial infarction (Wils et al., 2024; Wils et al., 2025). Occupational exposure to carbon

black nanoparticles has been associated with an increasing risk of developing cardiovascular diseases via endothelial cell activation (Tang et al., 2020). In addition, controlled exposure studies on human volunteers have shown dose-dependent induction of acute phase response in humans following exposure to (nano)particles of zinc oxide, copper oxide, paper dust, wood smoke, particulate matter PM (summarized in Hadrup et al. 2020, (Hadrup et al., 2020)) while exposure to 0.2 mg/m^3 graphene oxide did not induce toxic effects (Andrews et al., 2024).

- **Neurotoxicity**. Nanoparticles have the potential to reach the brain via two routes: (a) via the olfactory system (after deposition in the nose) and, (b) after deposition in the lower airways, via systemic translocation, after which they can also cross the blood-brain barrier. Epidemiological studies have reported an association between exposure to ambient air pollution (in particular PM2.5) and neurodegenerative diseases such as dementia, Alzheimer's disease and Parkinson's disease (Huang et al., 2025; Cristaldi et al., 2022; Xie et al., 2025). A role for the smallest inhalable PM fractions, (ultrafine particles (UFP) or nano-sized particles), in inducing effects is plausible via a direct and indirect mechanism (Heusinkveld et al., 2016). A third indirect pathway for toxic effects occurs via systemic inflammation, where inflammatory mediators released by lung cells migrate to other tissues and organs via the circulation and are capable of eliciting inflammatory effects on the central brain (Millán Solano et al., 2023). It should be noted that neuro-inflammation can also be caused by translocated particles directly, with microglia shown to be activated directly via translocated UFPs (Jayaraj et al., 2017).
- **Reproductive toxicity**. Because nanoparticles can translocate systemically, they can reach organs distal to the portal of entry. These include the ovaries (Hou and Zhu, 2017) and the testicles (Saeki et al., 2024), while effects in the offspring after exposure of the mother have also been reported (Jackson et al., 2011).

For each relevant benchmark ENMs, critical effects were identified based on the requirements previously listed (in the first paragraph of Section 3.2.2). This selection was informed by scientific literature, existing reviews and proposed exposure limit derivations. For example, for carbon black, inflammation, genotoxicity and cancer, cardiovascular toxicity and reproductive toxicity have been reported (Jacobsen et al., 2018). When applying the four requirements, lung inflammation was considered as the primary critical effect. While genotoxicity is an important indicator for and driver of carcinogenicity, findings for carbon black are not conclusive which is reflected by the absence of a harmonized classification for mutagenicity for this substance. Also, genotoxicity is not causally associated with chronic pathologies other than cancer, e.g. chronic inflammation and fibrosis. Conclusive evidence for genotoxicity would warrant assigning a substance to 'spheroidal biodurable ENMs with substance specific toxicity' (Category E) (Visser et al., 2022), emphasizing the need to investigate the state-of-the-art experimental evidence on a case-by-case basis.

3.2.2.2. Identifying key effects from human exposure and health effects data. Of the above list of adverse health effects, cardiovascular and functional respiratory effects have also been described in human controlled exposure studies involving exposures to nanosized materials in a qualitative manner. For diesel engine exhaust (predominantly composed of nano-sized particles of 5 to 50 nm,) for instance, studies are available where healthy human volunteers were exposed for a short duration at concentrations of a few hundred μg/m^3 (Hesterberg et al., 2010). In another study, the kinetic fate (as measured in blood and urine) of inhaled gold nanoparticles was investigated (Miller et al., 2017). Although information on effects in humans is the 'gold standard', in these studies, the exposure dose levels were relatively low (compared to animal studies) and effects observed were either not useful as a Point of Departure (PoD) in the current approach because of their nature (i.e., translocation and clearance) or were mild and transient (e.g., lung and systemic inflammation, thrombogenesis, vascular function, and brain activity). In addition, insoluble nanoparticles accumulate in the lung, and thus, repeated-dose exposure studies are needed to be able to assess the toxicity/point-of-departure/no-effect levels. Thus, it is not clear if the effects found in controlled single exposure studies should be considered as adverse (Hesterberg et al., 2010).

Another source of human evidence can be obtained from epidemiological studies. Here, large numbers of individuals are followed up with the aim of finding associations between health effects and exposure. Using such approaches, associations have for instance been found between exposure to ultrafine particles and lung function (Turner et al., 2022), cumulative exposure to nanomaterials in an occupational setting and worse pulmonary function parameters that could be mediated via lung inflammation (Squillacioti et al., 2024), cardiovascular disease and effects on the central nervous system (Werder et al., 2021). However, it should be noted that exposure in these epidemiology studies involves a complex mixture of substances that is highly variable in time. As a result, it is very difficult (if possible, at all) to attribute possible occurring health effects to specific nanomaterials. In the context of the current approach, where the aim is to use benchmark materials to derive a quantitative HNGV for occupational settings and specific groups of materials, such data are of limited value.

3.2.2.3. Supporting information from identifying key effects from adverse outcome pathways. As an additional source of mechanistic information for identifying early key events, relevant Adverse Outcome Pathways (AOPs) were reviewed. AOP 451 describes how interaction with lung resident cell membrane components leads to lung cancer (Nymark et al., 2021). This AOP describes that, for biodurable particles, prolonged retention in the alveolar region induces the release of inflammatory mediators leading to an increased recruitment of inflammatory cells. These cells then release reactive species that can cause damage to the DNA in addition to potential damage caused by the particles themselves. Together, this primary and secondary DNA damage results in an increased risk of lung cancer. In AOP 237, the mechanism by which nanomaterial exposure of lung cells can lead to atherosclerosis is described (Gutierrez et al., 2025). This pathway is also driven by inflammatory processes triggered in the lung that can lead to a systemic acute phase response and finally atherosclerosis. The OECD-endorsed AOP 173, describes how an interaction of a substance, such as a nanomaterial, with lung cells can lead to lung fibrosis (AOPwiki173, 2023). This process is, once more, heavily driven by the recruitment of inflammatory cells, leading to cell death, fibroblast proliferation and finally enhanced collagen deposition, which is the hallmark of lung fibrosis. In AOP 481, the mechanism by which amorphous silica nanoparticles can induce respiratory disease is outlined (AOPwiki481, 2025). For such nanoparticles, exposure elicits the recruitment of inflammatory cells and generation of ROS, leading to toxicity, fibroblast activation/proliferation and persistent inflammation and lung fibrosis. The information from the AOPs developed using in vitro data supports the pathway of lung inflammation.

3.2.2.4. Indicators of a pro-inflammatory response. The common denominator in the AOPs is the induction of a pro-inflammatory response. There are several key events in the inflammatory cascade that could support the selection of a PoD. In mice, strong correlations have been observed between neutrophil influx in bronchoalveolar lavage fluid, acute phase protein levels in blood and gene expression of acute phase proteins in lung tissue following pulmonary exposure to e.g. metal oxides (Gutierrez et al., 2023). Data on the acute phase response protein CRP is available and may be a useful biomarker of particle-induced inflammation in controlled exposure studies or in large cross-sectional studies. However, since there are many sources of CRP biosynthesis it

is an unspecific biomarker.

Also, the release of pro-inflammatory cytokines and chemokines is an early characteristic of acute lung inflammation. However, these very early endpoints do not always result in inflammation. For the choice of the PoD, a key event with stronger relation to lung inflammation would be preferable. After discussion in the expert panel, the influx of inflammatory cells, notably polymorphonuclear (PMN) leukocytes or neutrophils (that form the largest part of PMNs) which is considered a hallmark of lung inflammation, was selected.

For many nanomaterials, rodent data on their ability to induce lung inflammation including assessment of PMN influx (number of cells are usually counted in bronchoalveolar lavage fluid) is available. In humans, a value of approximately 4% PMNs in BALF was associated with respiratory impairment in workers in dusty jobs; and 10% PMNs in BALF in humans is considered to be clinically abnormal (NIOSH, 2021).

The indicator PMN influx meets most of the pre-defined criteria for the selection of a PoD. It is relevant to humans and there are many relevant and sufficiently reliable inhalation studies in animals, where the response is persistent. There is also a relation to nearly all chronic lung pathologies i.e., chronic inflammation, lung fibrosis and non-genotoxic carcinogenicity. Furthermore, PMN influx is an early event in the inflammatory cascade, indicated by a statistically significant increase in neutrophils or PMNs compared to controls (determined by cell count in BALF or by histopathological assessment) at the end of exposure. It was considered especially relevant when detected in toxicity studies with an exposure duration of 28 days or more as the exposure should model the potential continuous 40-year work-life exposure of workers to ENMs. For example, exposure to 2 mg/m^3 TiO_2 for 13 weeks resulted in 6.5% PMNs (relative amount of PMNs in differential cell count in BALF), while in controls this is 0.4%. Exposure to 0.5 mg/m^3 TiO_2 for 13 weeks did not lead to a significant increase in PMNs (relative cell number in BALF was 0.5%) (Bermudez et al., 2004). Moreover, the persistence of the inflammatory response is an important predictor of particle-induced lung pathology (Poland et al., 2024). In most of the studies on the benchmark materials, an increase in PMNs was even observed weeks after stopping the exposure. For example, in rats exposed to 1 mg/m^3 CB for 13 weeks, 6.5 and 13 weeks after stopping the exposure (Driscoll et al., 1996) or 3 and 11 months after stopping the exposure (Elder et al., 2005), the percentage BALF PMNs were similar as in controls, whereas in rats exposed to 7 mg/m^3 CB, the percentage PMNs were still elevated compared to controls. After a 28-day recovery period, the percentage BALF PMNs were still elevated after exposure to 5 mg/m^3 Al_2O_3 for 28 days, but not after exposure to 1 mg/m^3 (Kim et al., 2018). After a 12, 33 and 91-day recovery period, absolute PMN numbers were still elevated after exposure to 28 mg/m^3 AlOOH for 28 days, but not after exposure to 3 mg/m^3 or lower (Pauluhn, 2009). After a 28 and 90-day recovery period, the percentage BALF PMNs were still elevated after exposure to 1 mg/m^3 CeO_2 for 90 days, but not after exposure to 0.3 mg/m^3 or lower mass concentrations (Schwotzer et al., 2017). The exposure concentration that does not lead to an increase in (the percentage) neutrophils or PMNs at the end of a 28 day or 90 day exposure is considered the NOAEC (Supplementary Table S1).

Interestingly, it has been described that particle surface reactivity correlates well to the ability of some ENMs to elicit lung inflammation (Braakhuis et al., 2014b). Although this is not useful as a criterion for the selection of a PoD, it can be used to determine whether ENMs should be included in the category of spheroidal biopersistent ENMs with or without substance specific toxicity. It might be worthwhile (but not within the scope of the current work) to explore whether measurement of reactivity of particles sampled at the workplace could provide additional information useful to protect workers, e.g. as an exposure metric.

3.2.2.5. Final choice of adverse health effect as point-of-departure for HNGV derivation. Based on the overview above, we identified three mechanism-based scenarios to derive the HNGV:

1. Adverse health effects based on inflammation via a threshold mechanism. This inflammatory response is characterized by the influx of neutrophils.
2. Adverse health effects based on non-genotoxic carcinogenicity via a threshold. The precursor for this effect is persistent inflammation. It could be argued that the exposure level that prevents inflammation (observed in animal studies with exposure durations of 28 days or more), also protects against subsequent downstream effects such as tumor formation and fibrosis.
3. Adverse health effects based on genotoxic carcinogenicity via a non-threshold mechanism. The non-threshold mechanism approach results in lower proposed OELs, see e.g. the excess lung cancer risk in relation to workplace exposure to carbon black CB and TiO_2 of 1:1000 at 3–4 μg/m^3 (Saber et al., 2018a; Jacobsen et al., 2018). The OEL is highly dependent on the choice of acceptance level of the excess lung cancer risk and this differs per country.

Option 1 and 2 are both based on inflammation, and it was argued by the panel that when choosing option 1, the HNGV would also be protective against health effects mentioned in option 2. Therefore, by choosing option 1, this value would also likely protect against effects observed under option 2. When basing an HRNV for category F on option 3 (based on genotoxic carcinogenicity), it was argued, that a genotoxic substance should not be included in Category F because it shows substance specific toxicity, leading to inclusion in Category E (see paragraph 3.2.4).

3.2.3. Step 3 – derivation of a DNEL by selection of studies, NOAEC/LOAEC and assessment factors

All key studies were considered relevant with respect to deposition of benchmark materials in the pulmonary region of the lung. All ENMs administered to rodents are respirable, given the fact that the Mass Median Aerodynamic Diameters (MMAD) ranged between 0.38 and 2.4 with a Geometric Standard Deviation (GSD) of 1.7 to 4.23 μm (Supplementary Table S1). The OECD guidance for sub-acute and sub-chronic inhalation studies in rodents designed to accommodate the testing of airborne nanoparticles specifies an MMAD of ≤2 μm with a GSD of 1–3 μm to enhance deposition in the pulmonary region (OECD, 2017; OECD, 2009). Only for benchmark material Fe_2O_3, with a constituent particle size of 14 nm, no MMAD was reported. Since the particles are spark-generated from an iron rod, the agglomerated particle size is likely smaller than 2 μm and would still lead to sufficient deposition in the pulmonary region of the rodents.

An exposure concentration that leads to a statistically significant increase in PMNs (number of neutrophils relative to the total number of cells (%); or the absolute number of neutrophils) compared to controls at the end of exposure was considered as the LOAEC. In the five out of six key studies, the effect size was at least an added 4% increase in PMNs compared to controls (Supplementary Table S1). In the sixth study, PMNs were reported in absolute numbers without the total cell counts given. This increase of at least 4% is considered biologically relevant in rodents and has been associated with respiratory impairment in workers in dusty jobs (NIOSH, 2021). The exposure concentration that does not lead to a statistically significant increase in neutrophils compared to controls was considered the NOAEC. In five out of six benchmark material key studies, recovery periods were included and showed that the PMN response, detected at the end of exposure, was not completely

resolved after the subsequent recovery periods (varying from 4 weeks up to 1 year). This supports the selection of this response as the critical effect (Supplementary Table S1).

For each benchmark material, a suggested DNEL was derived by the expert panel based on ECHA guidance R.8 (Table 2 and see Supplementary Table S1 for more details on the selection of NOAEC or LOAEC and assessment factors used in the derivation of the DNELs) using the following formula (ECHA, 2012):

$$\text{Suggested DNEL} = \frac{\textbf{corrected NOAEC}}{\textbf{interspecies AF*intraspecies AF*subchronic to chronic AF}} \text{ or}$$

$$\text{Suggested DNEL} = \frac{\textbf{corrected LOAEC}}{\textbf{interspecies AF*intraspecies AF*subchronic to chronic AF*LOAEC to NOAEC AF}}$$

The NOAEC or LOAEC was corrected for the exposure duration per day (duration of animal exposure, typically 6 h per day to a human 8-h working day) and for the difference in breathing rate between rats and humans. Assessment factors (AF) were applied for interspecies differences, intraspecies differences, study duration and (if applicable) to extrapolate from LOAEC to NOAEC. In addition, two DNELs based on Benchmark Doses (BMDs) calculated from human lung burden were also included. These are 4 μg/m^3 for TiO_2 as derived by NIOSH (NIOSH, 2011) and 24.8 μg/m^3 for CeO_2 as derived by Boots et al. (Boots et al., 2021).

3.2.4. Step 4 – derivation of an HNGV by selecting DNEL from range

The DNELs for the selected benchmark materials are between 0.4 and 25 μg/m^3. For the overall assessment of these data, some studies were given less weight and were considered as supporting data (Table 2). For example, Kim et al. (2018) studied Al_2O_3, demonstrating a small but statistically significant increase in BALF PMNs at the lowest concentration tested (Kim et al., 2018). However, it was noticed that the percentage PMN was 2.5% in control animals at the end of the exposure and 4% after a recovery period of 28 days, which is relatively high for unexposed animals. The key studies have reported percentage PMNs in controls of around 0.5% or lower. This results in uncertainty in the interpretation of the result. Further, at the lowest exposure concentration, no other parameters indicative of a lung inflammatory response were observed. Due to the high background in PMNs in control animals and the lack of an increase in other inflammatory markers in the lowest dose group, this study is given less weight and considered as supporting data. A study on Fe_2O_3 by Suntunkova et al. (2016) has a similar issue, where control animals had high inflammation, and not all data were reported, making it hard to judge the results. (Sutunkova et al., 2016). This study was also considered as supporting data.

For nano-sized fullerene, the highest concentration tested was the NOAEC, leaving the possibility of the true NOAEC to be even higher. Inhalation of 1 μm fullerene resulted in pulmonary inflammation (Sayers et al., 2016). Instillation studies have shown mild PMN effects for fullerenes (Morimoto et al., 2010), but nano-sized fullerenes are also reported to inhibit activated inflammatory responses which may explain the absence of pulmonary inflammation (Zhen et al., 2024). These indications suggest that nano-sized fullerene could cause pulmonary

Table 2
Suggested DNELs for the benchmark materials for category F.

Nanomaterial	Critical effect	Point of departure	Suggested DNEL	Reference
Key studies				
TiO_2	Pulmonary influx of neutrophils [a]	BMD = 0.11 mg/m^3 [b]	4 μg/m^3 [b]	Bermudez et al., 2004, NIOSH, 2011
CeO_2	Pulmonary influx of neutrophils	NOAEC = 0.3 mg/m^3	6 μg/m^3 [c]	Schwotzer et al., 2017
TiO_2	Pulmonary influx of neutrophils	NOAEC = 0.5 mg/m^3	10 μg/m^3 [c]	Bermudez et al., 2004
AlOOH	Pulmonary influx of neutrophils	NOAEC = 3 mg/m^3	20 μg/m^3 [c]	(Pauluhn, 2009
Carbon black	Pulmonary influx of neutrophils	NOAEC = 1 mg/m^3	20 μg/m^3 [c]	Elder et al., 2005
	Pulmonary influx of neutrophils	NOAEC = 1.1 mg/m^3	22 μg/m^3 [c]	Driscoll et al., 1996
CeO_2	added 4% PMN in BALF	BMD: 0.17 μg/g lung [d]	24.8 μg/m^3 [d]	Boots et al., 2021
Supporting studies				
Al_2O_3	Pulmonary influx of neutrophils	LOAEC = 0.2 mg/m^3	0.4 μg/m^3 [c]	Kim et al., 2018
Fe_2O_3	Pulmonary influx of neutrophils [e]	LOAEC = 1 mg/m^3	4 μg/m^3 [c]	Sutunkova et al., 2016
Fullerene	Inflammation markers	NOAEC 2 mg/m^3	20 μg/m^3 [c]	Sayers et al., 2016

[a] Bermudez et al. 2004 describes a sub-chronic inhalation study in mouse, rat and hamster. The rat was found to be the most sensitive species.
[b] BMD and the effect level derived by (NIOSH, 2011).
[c] DNEL as derived by the expert panel. The DNEL was derived by 1) correcting the NOAEC/LOAEC for exposure duration per day, 2) correcting for difference in breathing rate between animals and humans, and 3) by applying assessment factors to account for interspecies differences, intraspecies differences, study duration, and (if applicable) for extrapolating from LOAEC to NOAEC. The corrections and assessment factors were based on ECHA guidance R.8. See Supplementary Table S1 for more details on the derivation of the DNELs.
[d] BMD and effect level derived by (Boots et al., 2021).
[e] For Fe_2O_3, the key study is identified by Moen et al. (Moen et al., 2024) and describes pulmonary inflammation characterized by PMN influx as the critical effect (Sutunkova et al., 2016) which has also been reported in a human instillation study of 5 mg into a subsegment of the lingula lobe (LOAEC 35 mg/m^3) (Lay et al., 1999).

inflammation if higher concentrations were tested. However, due to a lack of response at the highest concentration tested, this study was given less weight.

Based on the data from the key studies, the DNEL range is 4 to 25 μg/m^3 (Table 2). Upon assessment of the key study data and in order to provide optimal worker protection, we recommend the lowest value of the range of 4 μg/m^3 as HNGV for spheroidal biodurable ENMs with no substance-specific toxicity. This HNGV would protect against pulmonary inflammatory effects, as well as subsequent persistent lung effects such as lung fibrosis. Two of the three supporting studies, on fullerene and Fe_2O_3, have values that fit within this range. A single supporting study on Al_2O_3 resulted in a DNEL of a factor 10 lower. The panel concluded that a single supporting study with high uncertainty should not drive the derivation of the value. It is emphasized that Al_2O_3 may not be sufficiently covered by this HNGV. More data are needed on this material to conclude on the NOAEC and a specific OEL may need to be considered for Al_2O_3.

3.3. Practical feasibility of workplace measurements

Practical and reliable quantitative measurements are essential to assess a worker's exposure against the suggested 4 μg/m^3 HNGV for spheroidal biodurable ENMs with low substance specific toxicity in the workplace. Therefore, the practical feasibility of measuring this concentration at the workplace was reviewed. In summary, for assessing respiratory exposure to airborne particulates, this involves collecting airborne particles from workplace air onto a filter that is placed within a personal air sampler subsequently mounted on the torso within the breathing zone of a worker (CEN, 2018). Air sampling is conducted over a period that is representative of the work process undertaken and represents the potential worker's inhalation exposure.

The European standard EN 481 defines three particle size fractions that are commonly determined in workplace air namely: the inhalable fraction (this approximates to the fraction of airborne particulate matter of a size that enters the nose and mouth during breathing, and is therefore available for deposition anywhere in the respiratory tract); the thoracic fraction (the inhaled airborne particulate fraction of a size that can penetrate beyond the larynx) and the respirable fraction (the inhaled airborne particulate fraction of a size that penetrates to the lower gas exchange region of the lung) (CEN, 1993). A variety of size selective particle samplers mapping these three defined particle fractions are available for personal monitoring although most workplace exposure limits e.g. metallic workplace particles, are based upon either the inhalable or respirable size fractions. When handled in powder form, ENMs tend to form airborne aggregates and agglomerates, sometimes reaching tens of micrometers in size. In several countries, there are also legal requirements to measure the inhalable fraction for metal particles. Therefore, we recommend measuring both the respirable and inhalable fraction (ISO, 1995; Brown et al., 2013).

Filters from air sampling are analyzed in the laboratory using different methods. For metallic-based ENMs, elemental analysis via the commonly used inductively coupled plasma-mass spectrometry enables low (analytical) mass quantification limits per filter of <100 ng/filter to be achieved (ISO, 2010). For carbon-based ENMs, such as carbon black or fullerene, a combustion-based analysis measuring an elemental carbon moiety that is representative of such materials is employed (NIOSH, 1993). Here an analytical quantification limit of ~500 ng/filter is achievable (for typical 25-mm diameter air sampling filter employed). For such low quantification limits to be reliably achieved, the purity of filter media and reagents used needs to be assured, and effective plus rigorous cleaning protocols of both sampling and analytical equipment followed. This minimizes the potential for sample (cross) contamination and hence degradation in achievable analytical quantification limits. For carbon-based ENMs, background sources of black carbon (elemental carbon, EC), such as diesel exhaust particles or other combustion derived particles, can interfere with the analysis, making the necessary background measurements challenging to distinguish specific carbonaceous ENMs (NIOSH, 2022).

Ideally, the anticipated air sampling duration, hence the volume of air sampled in the workplace, should not only be representative of the work process in question under examination but should be sufficient so as to ideally ensure that the achievable method (exposure) quantification is ≤ one-tenth of the exposure limit benchmark to be applied thus reducing measurement uncertainty as far is practical. Here a priori conversations with laboratory analysts to ascertain what analytical quantification limits are readily achievable to then assist in estimating what suitable air sampling volumes should and can be taken is therefore recommended. Furthermore, given that workplace airborne pollutant concentrations can vary both in space and in time, resulting in exposure datasets from similarly exposed worker cohorts that demonstrate a log-normal data distributions profile, taking and measuring multiple personal samples is required to get a representative picture of exposure that is statistically robust.

There are ongoing developments in air sampling and measuring devices. New personal air samplers include: single use disposable samplers (to eliminate the potential for cross-contamination issues); dual-fraction samplers (to sample both inhalable and respirable particle size fractions simultaneously); samplers that can operate at higher flow rates (so as to collect more sample mass per unit time and hence improve method limit of quantification) and smaller, lighter and easier to use air sampler devices that can be deployed potentially at scale. Samplers working on alternative principles to penetrative samplers mentioned above are also emerging. One example is the personal Nanoparticle Respiratory Deposition (NRD) sampler designed to efficiently mimic the deposition of nanoparticles of <300 nm in the human respiratory system (McCollom et al., 2019).

Many experts consider (chemical) mass a suitable metric for measuring spheroidal airborne ENMs, with real-time monitors providing valuable complementary data, especially those measuring surface area or particle number concentrations (Eastlake et al., 2016; ISO, 2018). However, obtaining reliable real-time measurements, such as those mentioned in the NIOSH NEAT strategy (Eastlake et al., 2016) at the proposed low HNGV value could be difficult as these instruments cannot distinguish particles by chemical composition and are susceptible to confounding backgrounds from non-ENM particles. In addition, electron microscopy can be a valuable tool for distinguishing substance-specific ENMs smaller than 100 nm from those larger than 100 nm (NIOSH, 2011; Mudunkotuwa et al., 2016).

In summary, practical and reliable measurements for category F HNGV ENMs are possible using personal air samplers combined with off-line chemical mass analysis. Emerging technical developments could however further enhance workers' exposure assessments in the future.

3.4. Dose metric of the HNGV for spheroidal biodurable ENMs with low substance specific toxicity

The reason for selecting mass concentration as the metric to represent the HNGV has been a practical one. All key animal studies used here have reported the ENM exposure concentration in milligram per cubic meter air. Also, as discussed above, mass concentration measurements are performed at the workplace.

The range of proposed DNEL values expressed in μg/m^3 could not easily be transformed into particle number concentrations to compare the outcome with the current NRVs. Particle number concentrations have not directly been determined in the key animal studies (see Supplementary Table S1 for the particle characteristics listed per benchmark material). To convert a mass concentration to a particle number concentration, among information on particle size distribution and shape (spherical), information on the density is needed which is lacking for most of the benchmark materials (Supplementary Table S1). Particle number concentrations are in principle easy to measure in the workplace as such, but it requires stable background measurements to differentiate

between ENMs and background particles such as process-generated nanoparticles to apply a number-based limit, which is more complicated (Visser et al., 2022). There is little information available to support the idea that the sheer number of particles are driving adverse effects compared to deposited mass. Compared to a mass-based limit, number-based limits have the additional disadvantage that they are highly dependent on the degree of agglomeration and would not be suitable when ENMs are present in (large) agglomerates which has been described in the workplace.

The range of DNEL values could be expressed as a range of 0.00005–0.0048 m^2/m^3 by converting the particle mass concentration into the particle surface area concentration using the Brunauer-Emmett-Teller (BET) particle surface area (Supplementary Table 1). Specific surface area data were provided in the key animal studies for all materials except for the freshly spark generated Fe_2O_3 for which BET surface area was not reported. In the workplace, surface area measurements by the BET method are difficult to perform. Previously, we noted that 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) (Visser et al., 2022).

The retained particle surface area has been suggested as a driver of adverse effects of certain ENMs, especially for lung inflammation in rats which is selected here as the mechanism-based scenario for the derivation of the HNGV (Cosnier et al., 2021; Oberdorster, 1996). Differences in porosity of ENMs could influence the level of lung inflammation. It has been shown that more porous silica particles by introducing internal surface area induced more inflammation compared to solid particles (Hadrup et al., 2021b). Converting the NOAEC or LOAEC values to a retained particle surface area would require information on retained lung burden measurements and particle surface area. This information is lacking for some of the benchmark materials or is only presented in graphs and would result some uncertainty in estimating the value (Supplementary Table S1). Therefore, we did not attempt to express the HNGV as a surface area-based metric. A surface area related metric for *airborne* particles is the Lung Deposited Surface Area (LDSA) concentration. This predicts the portion of the airborne surface area concentration that settles in the alveolar area of the human respiratory system, assessed by electrically charging aerosol particles using a unipolar diffusion charger, and then measuring the current generated by the charged particles. The measured current is directly proportional to the LDSA concentration. There were no LDSA measurements performed in the key animal studies. A more in depth discussion on different methodology and instruments to determine surface area related metrics is beyond the scope here, but is provided in the OECD guidance on 'The identification of exposure metrics for use in evaluation of inhalation exposure to nano-objects and their aggregates and agglomerates (NOAA) in the workplace' when made public for details (OECD, 2025a).

3.5. Restrictions to the application of the HNGV

If there is evidence indicating substance-specific toxicity of an ENM, including direct genotoxicity, the HNGV for spheroidal biodurable ENMs with low substance specific toxicity derived here may not be sufficiently protective. In such cases, the ENM shall be considered under the category of spheroidal biodurable ENMs with substance specific toxicity and further evaluation is needed to derive a safe level below which no adverse effects are expected. For some ENMs such as carbon black and titanium dioxide, positive as well as negative data for genotoxicity are available. At present, these data are not considered to be conclusive and thus these ENMs were included as benchmark materials and used for the derivation of the DNELs. When conclusive genotoxicity data become available, their inclusion as a benchmark material may need to be re-evaluated.

Identification of genotoxic effects by ENMs can also be done by looking for their classifications for carcinogenicity or, especially, mutagenicity. Several different classifications exist. The IARC classifies substances for carcinogenicity, which is considered as a scientific opinion that holds authority but has no direct legal implications. Also, the mechanism by which carcinogenicity is induced, is not reflected in the IARC classification system. In the European Union, The European Classification, Labelling and Packaging (CLP; EU regulation No. 1272/2008) regulation implements the Globally Harmonized System of Classification and Labelling of Chemicals (GHS); a harmonized classification according to CLP has legal implications, e.g. for all producers and importers of the substance. In addition, individual registrants self-classify substances, which is only valid for their own purpose. Consequently, the registrant then needs to take appropriate protective measures for exposed workers. The main CLP classification categories of relevance are carcinogenicity and mutagenicity, with subclasses 1 A, 1B and 2 to indicate the strength of evidence. For Germ Cell Mutagenicity (Muta Cat. 1 or 2), the situation is different, with Muta 1 indicative of mutagenicity in germ cells, and Muta 2 indicative of mutagenicity in somatic cells. Thus, harmonized classification for both Muta 1 and Muta 2 would indicate that a (nano)material is genotoxic, which implies that the substance classified as such should not be allocated to Category F. For self-classification (instead of harmonized classification) for Muta 1 or 2, the same could apply, but this should be considered on case-by-case basis. A CLP classification for carcinogenicity is not in itself proof for the genotoxicity of a nanomaterial. However, it might serve as an indicator triggering further scrutiny related to the assessment of an ENM for genotoxicity. This is also the case for IARC classification on carcinogenicity. In any case, when there are indications that an ENM is genotoxic/mutagenic, the evidence should be carefully evaluated for evidence regarding a potential non-threshold mechanism.

Another method would be to screen the scientific literature in a case-by-case approach based on weight-of-evidence. However, care should be taken in assessment of data quality and relevance of methods used. It should be noted here that genotoxicity observed in vivo upon ENM exposure can also be mediated by inflammatory cells. It should be carefully assessed whether the genotoxic findings occur at doses causing marked inflammation.

4. Summary and conclusion

An expert panel previously distinguished six possible categories of ENMs and now provided updated recommendations on how to derive health-based guidance values for some of the categories (Visser et al., 2022). Moreover, an HNGV for spheroidal biodurable ENMs with relatively low substance-specific toxicity has been derived with a value of 4 $\mu g/m^3$, based on pulmonary inflammation as the critical adverse health effect.

Several aspects for this derived HNGV need to be considered: i) this value is health -based and does not take into account the technical feasibility and socio-economic factors of implementing this value at the workplace, ii) this value is based on animal data, iii) this value assumes that the selected benchmark materials are representative for all spheroidal biodurable ENMs with no substance-specific toxicity. However, in case new data is available for a specific material that suggests a more stringent value, it is recommended to use that substance-specific data for derivation of an occupational exposure limit. This may be the case for Al_2O_3, for which there are indications of a neurological effect at lower concentrations than 4 $\mu g/m^3$ (Zhang et al., 2015), iv) this value is derived to protect against persistent pulmonary inflammation induced by particles based on a discussion on the current information. In the future, other more critical effects may be identified that this HNGV should protect workers for. In that case, this HNGV value should be re-evaluated. v) other derivations are possible using more complex inhalation dosimetry methods or models that use the internal lung dose or account for toxicokinetic differences in e.g. particle clearance in rats versus humans (Kuempel et al., 2015). The approach chosen in this paper was selected for its transparency, ease of implementation, and reproducibility, in line with regulatory guidance. It is important to note

that, in case a reliable proposal for an OEL for a specific ENM is already available, this proposed OEL should be prioritized over this proposed HNGV for workplace risk assessments. This also applies for legally binding exposure limits for processes that release nanoparticles (PGNPs) such as welding fumes or diesel engine emissions. In case there is no proposed or legally binding exposure limit and there is no specific information available, we propose to use the HNGV derived here, instead of using non-health-based NRVs, for all ENMs that fall into the group of spheroidal biodurable ENMs with relatively low substance-specific toxicity (category F).

We foresee that this HNGV may also be informative for a worker risk assessment on advanced nanomaterials or non-engineered nanomaterials such as PGNPs (for which no OELs exist), provided that their dimensions are in the nanometer size range, they have a spheroidal shape and their chemical identity can be determined to assess the biodurability and non-substance specific toxicity. Also, the source and the process through which they are generated is important to identify. For example, for PGNPs, reactivity can differ between aged versus freshly generated PGNPs. Only if the process by which PGNPs are produced is known and it does not lead to reactive particles that (often) have substance specific toxicity, the HNGV presented here can be used. Similarly, for ENMs that would meet category F requirements, but are functionalized or otherwise modified, the effects of this modification on their toxicological potential should be investigated. Thus, the applicability to other particles, like PGNPs requires careful consideration of their specific properties and generation processes. Newly obtained information on dissolution in physiological relevant media and reactivity, or existing information related to the chemical identity could serve to estimate whether the HNGV for spheroidal biodurable ENMs with relatively low substance-specific toxicity is sufficiently protective for these materials.

Based on considerations on sampling and measuring ENMs at the workplace, the proposed HNGV should be feasible to apply in practice, although a practical guidance for implementation of the HNGV in workplace settings is beyond the scope of this work. The results of this project may be used as input for (inter)national implementation of exposure limits (such as a European indicative OEL) for spheroidal biodurable ENMs with low substance-specific toxicity.

CRediT authorship contribution statement

Petra C.E. van Kesteren: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Damiën van Berlo:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Delphine Bard:** Writing – review & editing, Writing – original draft, Investigation. **Owen Butler:** Writing – original draft. **Susan Dekkers:** Writing – review & editing, Writing – original draft, Investigation. **Niels Hadrup:** Writing – review & editing, Writing – original draft, Investigation. **Karin Lovén:** Writing – review & editing, Writing – original draft, Investigation. **Michael Riediker:** Writing – review & editing, Writing – original draft, Investigation. **Roel P.F. Schins:** Writing – review & editing, Writing – original draft, Investigation. **Christoph van Thriel:** Writing – review & editing, Writing – original draft, Investigation. **Ulla Vogel:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Ilse Gosens:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization.

Disclaimer

The paper's contents, including any opinions and/or conclusions expressed, are those of the authors alone and do not necessarily reflect HSE policy.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Data availability

No data was used for the research described in the article.

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