Earth, Life & Social Sciences
Utrechtseweg 48
3704 HE Zeist
P.O. Box 360
3700 AJ Zeist
The Netherlands

www.tno.nl

T +31 88 866 60 00
F +31 88 866 87 28

TNO report

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Applicability of provisional NRVs to synthetic nanomaterials. A study within the frame of the project Update of the NRVs

Date	30 March 2017
Author(s)	H.E. Buist M.T.T. Oosterwijk
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This study was carried out on behalf of the Netherlands Ministries of Infrastructure and the Environment (I&M); Health, Welfare and Sport (VWS) and Social Affairs and Employment (SZW).
The study was guided by the Risks of Nanotechnology Knowledge and Information Centre (KIR nano) of the RIVM.
The study was carried out within the frame of the project '*Update of the NRVs'*

Preface

This report relates the results of an assignment by the RIVM to investigate if the Dutch provisional nano reference values, as proposed by the SER in 2012 (SER, 2012) and recommended as a tool for risk management by the Minister of Social Affairs in 2012, are still in line with the current scientific knowledge. The purpose of this assignment is put into perspective and the questions to be addressed are specified below.
As common practice and based on European chemical legislation, the risk of occupational exposure to chemicals is assessed based on accepted limit values. This is also desirable for nanomaterials, but for most nanomaterials the current scientific knowledge is insufficient to derive nanospecific health-based occupational exposure limit values. Because of the lack of specific health-based limit values combined with limited information on exposure to nanomaterials, the Minister of Social Affairs proposed to manage the possible risks by applying the precautionary principle[1]. In 2011 provisional nano reference values (NRVs) were developed (van Broekhuizen et al., 2011). These NRVs provide a pragmatic limit value for four classes of engineered nanomaterials, which are based on the precautionary principle (SER, 2012).These values, however, are not health-based and therefore do not guarantee that an exposure lower than the NRVs is safe.
Since the drafting of the NRVs in 2011 more (international) scientific knowledge has become available on the possible occupational risks and the dose-effect relationship of nanomaterials. For a number of nanomaterials health-based occupational limit values are proposed, such as the limit values on carbon nanotubes (0.1 µg/m^3) and titanium dioxide nanoparticles (0.3 mg/m^3) proposed by the US NIOSH (NIOSH, 2013, 2011). The NRV for nanotubes is based on the limit value for asbestos, and it must be noted that as per 01-01-2017 the Dutch OEL for asbestos fibres has been lowered to 0.002 fibres/cm^3 (Staatsblad, 2016).
Following the progress made in scientific knowledge and the lowered limit value for asbestos, the Ministry of Social Affairs and employment (SZW) has asked KIR-nano to determine whether adjustment of the current NRVs is needed.

[1] According to the European Commission, the precautionary principle covers those specific circumstances where scientific evidence is insufficient, inconclusive or uncertain and where there are indications through preliminary objective scientific evaluation that there are reasonable grounds for concern that the potentially dangerous effects on the environment, human, animal or plant health may be inconsistent with the chosen level of protection (EC, 2000). Application of the precautionary principle is part of risk management, but principle can under no circumstances be used to justify the adoption of arbitrary decisions. The appropriate response in a given situation is thus the result of an eminently political decision, a function of the risk level that is "acceptable" to the society on which the risk is imposed.

Table 1 Dutch nano reference values (NRVs) for four classes of synthetic nanomaterials (SER, 2012)

Class	**Description**	**NRV (8-hr TWA)**
1	Rigid, biopersistent, insoluble, fibre form nanomaterials for which effects similar to those of asbestos are not excluded • *SWCNT or MWCNT or metal oxide fibres for which asbestos-like effects are not excluded by the manufacturer*	**0.01 fibres/cm^3**
2a	Non-biodegradable granular nanomaterials in the range of 1–100nm and density > 6 kg/litre • *Ag, Au, CeO_2, CoO, CuO, Fe, Fe_xO_y, La, Pb, Sb_2O_5, SnO_2,*	**20,000 particles/ cm^3**
2b	Non-biodegradable granular nanomaterials in the range of 1–100nm and density < 6 kg/litre • *Al_2O_3, SiO_2, TiN, TiO_2, ZnO, nanoclay Carbon Black, C_{60}, dendrimers, polystyrene Nanotubes, nanofibres and nanowires for which asbestos-like effects are excluded by the manufacturer*	**40,000 particles/ cm^3**
3	Biodegradable/soluble granular nanomaterials in the range of 1–100nm • *e.g. NaCl-, fats, flower, siloxane particles*	**Appli-cable OEL**

The purpose of this assignment is to determine whether the provisional NRVs developed in 2011 are still in line with currently available scientific knowledge or whether adjustment of the NRVs is needed. The following issues are addressed:

Q1. Which specific air limit values for nanomaterials are currently being proposed (worldwide) in scientific publications. Focus is on the nanomaterials for which previously provisional NRVs have been derived or other commonly used materials.

Q2. An overview of proposed (by authorities) substance-specific and non-specific air limit values for occupational exposure to nanomaterials.

Q3. How do the provisional NRVs from 2011 compare to current scientific knowledge and the recommended air limit values for occupational exposure in terms of height, dose metric and scientific basis?

Q4. a. Based on the current state of knowledge and understanding (questions 1-3), would adjustment of the provisional NRVs be recommended for synthetic nanomaterials?

b. In case adjustment of the current NRVs is recommended, what is needed to achieve this adjustment?.

Q5. Is the current classification of provisional NRV values suitable for process generated nanoparticles (PGNPs) and for the fraction of nanoparticles in conventional products (FCNPs)?

Q6. a. Based on the current state of knowledge and understanding (questions 1-4), would adjustment of the provisional NRVs from 2011 be recommended for PGNPs and FCNPs?

b. In case adjustment of the current NRVs is recommended for PGNPs and FCNPs, what is needed to achieve this adjustment?

This report, titled “Applicability of provisional NRVs to synthetic nanomaterials”, addresses Q1-Q4 and is authored by Harrie Buist and Thies Oosterwijk (TNO). Q5-Q6 are addressed in a separate report, titled “Applicability of provisional NRVs to PGNPs and FCNPs”, authored by Pieter van Broekhuizen.

Summary

The purpose of this research is to assess whether the provisional NRVs developed in 2011 are still in line with currently available scientific knowledge and whether adjustment of the NRVs is needed for manufactured nanomaterials (MNMs). The following questions are addressed:

Q1. Which specific air limit values for nanomaterials are currently being proposed (worldwide) in scientific publications. Focus is on the nanomaterials for which previously provisional NRVs have been derived or other commonly used materials.
Q2. An overview of proposed (by authorities) substance-specific and non-specific air limit values for occupational exposure to nanomaterials.
Q3. How do the provisional NRVs from 2011 compare to current scientific knowledge and the recommended air limit values for occupational exposure in terms of height, dose metric and scientific basis?
Q4. a. Based on the current state of knowledge and understanding (questions 1-3), would adjustment of the provisional NRVs be recommended for synthetic nanomaterials?
b. In case adjustment of the current NRVs is recommended, what is needed to achieve this adjustment?

Q1, Q2 and Q3:
Research was carried out on the basis of a paper by Mihalache et al. (2016), in which the results of an inventory of public literature on occupational exposure limits (OELs) for manufactured nanomaterials (MNMs) are reported and a search of public literature and websites. The OELS were collected in two inventories:

- Inventory of published generic exposure limits using a grouping approach
- Inventory of published exposure limits proposed for specific manufactured nanomaterials

The exposure limit values that were retrieved from literature all consider MNMs that are classified in NRV classes 1 to 3. No exposure limit values were encountered for MNMs classified in NRV class 4.

To compare the existing proposed mass based OELs to number based NRVs, different methods were used. For NRV class 2 and 3, two different conversion methods were compared. In both methods the conversion is done based on the density and calculated volume of the particles. One method is based on the assumption that all particles are spherical and 100 nm in diameter, which can be considered to be a worst case approach. The second method is based on the actual size distribution of the nanoparticles on which the OELs were based. For this an algorithm derived from Hinds (1999) was used.

For NRV class 1, two different approaches were used to calculate fibre weight for carbon nanotubes, depending on the available data. In both cases the fibres were assumed to be cylindrical in shape. When data on the specific surface area (SSA) were available in combination with data on fibre dimensions, a formula from Aschberger et al. (2005) using the SSA was applied. When data on SSA were not available, the density of the fibre was calculated using a formula adapted from Laurent et al. (2010). The calculated weights were subsequently used to convert mass concentrations to fibre concentrations.

The general limit values

For biopersistent nanofibres, the proposed OELs are all identical to the NRV value for class 1 (0.01 fibre/cm^3) except for the one legal value published by the Belgian government: 2 fibres/cm^3. For classes 2 and 3, all exposure limit values found were higher than those of the corresponding provisional NRV values, with two exceptions: as proposed by the British Standards Institution (BSI), low density globular[2] biopersistent particles will have a limit value of 20,000/cm^3 while they fall into NRV class 3 with a value of 40,000/cm^3 and, as proposed by the Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (BAuA), high density globular nanoparticles with specific toxicity like nanogold will have a limit value of 9,900/cm^3 while they belong to NRV class 2 (20,000/cm^3).

Specific limit value

The specific exposure limit values are health-based, and therefore of special interest for the evaluation of the existing NRVs.

Among the nanomaterials classified into NRV class 2, a specific health-based OEL was found only for nanosilver. In whatever way the particle number concentration of this OEL has been derived, it is lower than the corresponding NRV value of 20,000 particles/cm^3. However, when the OEL in question would have been derived from a better founded, less conservative point of departure, the OEL for nanosilver would be above the NRV class 2 value, indicating this NRV is conservative in relation to the nanosilver OEL.

Based on particle number concentrations calculated from the size distributions of the actual nanoparticles dispersed into the air, which is the most relevant method to calculate these concentrations, eight of nine OELs are lower than the corresponding NRV of 40,000 particles/cm^3, implying NRV class 3 may not be conservative.

The comparison of OELs with corresponding NRVs proved to be complicated by variations in the methodology to derive OELs and the conversion of number concentrations into mass concentrations (and vice versa). This is discussed in more detail in chapter 3, when addressing which dose metric(s) should be preferred for setting NRVs for MNMs.

Q4a:

At the moment, it is not clear whether MWCNTs cause carcinogenicity via the same mechanism as asbestos nor whether they have a comparable potency. Furthermore, it is debatable whether the mechanism of carcinogenicity of asbestos justifies the linear extrapolation of observed dose-response relationships to low doses, and if so, whether this also would be valid for MWCNT-7. Therefore, it is not yet clear whether a lowering of the class 1 NRV is scientifically justifiable. Also it is not clear whether the present NRV class 1 is conservative or not. Therefore, it is advised not to decide on the possible modification of the present class 1 NRV until this publication is available for inclusion in the considerations.

Overall, too few OELs have been retrieved from public sources to draw a firm conclusions on the conservativeness of the present class 2 and 3 NRVs. However, there are strong indications that the class 3 NRV is too high. Based on this, and the uncertainty surrounding the distinction between class 2 and class 3 NRVs, it is recommended to reconsider the values attributed to class 2 and 3 NRVs.

[2] The SER (2012) in its advice on provisions NRVs uses the term “granular” instead of “globular”. In this document both used as synonyms. As a rule the designation of the cited institution or author is followed.

Class 4 NRVs were not evaluated due to lack of data.

Q4b:

The following step-wise approach for updating of the NRVs for MNMs is proposed:

1. Collect a database of *in vivo* inhalation toxicity studies with well-characterised nanoparticles for which the dose can be expressed in terms of mass, particle number and surface area.
2. For specific nanoparticles, select a combination of studies that allow analysis of dose-response relationships in term of mass and particle number and surface area.
3. Perform dose-response analyses for each suitable set of specific nanoparticles using a software package that provides statistical measures of 'goodness of fit', which may be used to rank the different dose metrics used.
4. Based on the results thus obtained, draw conclusions on the most appropriate dose metric to express nanoparticle toxicity. If no unequivocal choice can be made, investigate whether the most appropriate dose metric for a given nanoparticle can be linked to a specific physicochemical property.
5. Extend the database collected under point 1 with other inhalation studies with well-characterised nanoparticles that did not meet all the criteria for characterization mentioned there (mass, particle number and surface area), but do provide toxicity data that can be expressed in the dose metric(s) selected under point 4.
6. Based on the collected database, derive limit values for as many specific nanoparticles as possible using the methods described by ECHA to derive long-term occupational DNELs or DMELs, expressed in the metric(s) selected under point 4.
7. Based on the results obtained under points 4 and 6, identify classes of MNMs and propose NRVs for these classes.
8. Develop a practical method to translate the proposed NRVs to practical measures for use in an occupational hygiene setting.

Contents

1 Comparison of current provisional nano reference values with nano exposure limit values from public sources (Q1-3)

1.1 Introduction

In this chapter, questions 1-3 of the assignment are answered. In section 1.3, a description of conversion methods from mass concentration to particle concentration is provided, used to convert mass-based units of the OELs proposed in literature to the number-based unit of the NRVs.

1.2 Retrieval of public nano exposure limit values (Q1 and 2)

Basis for the collection of public values was a recent paper of Mihalache et al. (2016), in which the results of an inventory of public literature on occupational exposure limits (OELs) for manufactured nanomaterials (MNMs) is described. This inventory was supplemented with the results of searches in Scopus and Toxline/Medline for OELs on specific MNMs, more in particular those that are part of the OECD nanotoxicology research programme and those that were mentioned as examples in the publication on the NRVs by the SER (SER, 2012). The search terms and results, the OECD MNMs and the exemplary MNMs are listed in in sections 5.1 to 5.4. Furthermore the GESTIS database of OELs and the sites of NIOSH, BAuA, SER and SCOEL were scrutinized for additional OELs. The result of these searches were one paper by Katsnelson et al. (2015), who proposed four additional OELs on metal (oxide) nanoparticles, and one legal OEL, from Belgium (ELSD, 2014) .
Besides long-term inhalatory OELs, the Mihalache paper also lists dermal and oral OELs as well as short-term inhalatory OELs. Since the NRVs as published by the SER only address long-term inhalatory occupational exposure (SER, 2012), these dermal, oral and short-term inhalatory OELs were not considered here.

1.3 Conversion of mass-based OELs to number-based OELs (Q3)

1.3.1 Mass to number conversion assuming spherical particles with a diameter of 100 nm

Some studies used by the SER for verification of NRVs were based on mass concentrations (SER, 2012). For these studies, conversion from mass concentration to particles' number concentrations was applied by the Dutch Social and Economic Council, assuming primary particles or fibres to be present with a spherical, respectively, a cylindrical form (see table 6 of appendix 1 of SER (2012)). It should be noted that the conversion applied by the SER was not specified in detail. Therefore the conversion from mass to particle concentration as applied in the current report is provided below.
By definition, primary nanoparticles have a diameter between 1 and 100 nm and NRVs should cover this entire size range. For the purpose of comparison of those OELs retrieved from literature which were solely expressed in mass per m^3, these units were converted into particles' number concentrations assuming a primary particle size of 100 nm, as this would lead to the lowest limit value ("worst case" NRV method). For that purpose the following formula, based on the basic relation weight = volume times density and on adjustments in connection with the mixture of units used, was applied to obtain particle weight expressed in µg:

$$W = \frac{{}^4/_3\pi\,({}^1/_2 d)^3\,\rho_p}{10^{15}} = \frac{\pi\,d^3\,\rho_p}{6\,10^{15}} \quad \ldots. (1)$$

in which W = particle weight (µg/particle), d = particle diameter in nm and ρ_p = particle density in g/cm^3. Table 2 lists the densities used in the calculations. The mass concentration expressed in µg/m^3 was then divided by W and multiplied with 10^{-6} (m^3/cm^3) to obtain the number of (nano)particles/cm^3.

Table 2 Particle densities used in calculations

Particle	density		Source
	(g/cm³)[a]	Type	
Carbon black: CB ultrafine	1.7 -1.9[b]	particle	Cabot (2016)
Multiwalled carbon nanotubes: MWCNT 140 nm Baytubes®	0.31	particle	Pauluhn (2010)
Carbon: Fullerenes, C_{60}	1.729	particle	Shinohara (2011)
General dust ("A-Staub")	2.5	particle	BAuA (2016)
Nanoclay (BYK Cloisite® Na+ Nanoclay)	2.86	particle	MatWeb - Material properties data (http://www.matweb.com)
Nanosilver	10.5	chemical product	Handbook of Chem. & Physics (CRC, 2005), Table Physical constants of inorganic compounds
Silicon dioxide (amorphous)	2.2	particle	Evonik (2015)
Titanium dioxide	4.23	chemical product	Handbook of Chem. & Physics (CRC, 2005), Table Physical constants of inorganic compounds
Zinc oxide	5.6	chemical product	Handbook of Chem. & Physics (CRC, 2005), Table Physical constants of inorganic compounds

[a] If no value was available for the air-borne particle, the density of the chemical product was used as a worst case estimate due to assuming the highest possible density.
[b] A value of 1.8 g/cm³ was used in the calculations

It should be noted that in these calculations the number of particles is inversely proportional to their density. When in these calculations, the density of the chemical compound is used instead of the density of the particles dispersed into the air, the particle density may be overestimated and consequently the particle number may be underestimated. In Table 7 ,calculated particle numbers based on chemical compound density instead of particle density are marked with an asterisk to indicate potential underestimation of the particle number.

1.3.2 Mass to number conversion based on size distribution assuming spherical particles

Often, the concentration of nanoparticles in the air is composed of agglomerates or even aggregates of primary particles, instead of solely primary particles. The measured mass concentration will reflect the entire distribution of these primary particles, agglomerates and aggregates within the measured particle size range. Therefore, it could be argued that the entire size distribution should be taken into account when calculating number concentrations. Many respiratory studies provide descriptors of the size distributions of the particles dispersed into air, mostly Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD). Therefore, in the current report number concentrations were also calculated based on the size distribution of the nanoparticles concerned.
When MMAD and GSD were used to describe the particle size distribution, the following formula was used to derive the number concentration:

$$C_N = 10^9 C_m \frac{6}{\rho\, \pi\, MMAD^3 \left(\sqrt{\frac{\rho_0}{\rho}}\right)^3 e^{-4.5\, ln^2\, \sigma_g}} \quad \text{....(2)}$$

When MMAD and GSD were used to describe the particle size distribution, the following formula was used to derive the number concentration:

$$C_N = 10^9 C_m \frac{6}{\rho\, \pi\, CMD^3 e^{4.5\, ln^2\, \sigma_g}} \quad \text{....(3)}$$

The derivation of formulas (2) and (3) is described in appendix 5.5.

1.3.3 Mass to number conversion for fibres

Two different approaches were used to calculate fibre weight for carbon nanotubes, depending on the available data. In both cases the fibres were assumed to be

cylindrical in shape. When data on the specific surface are were available in combination with data on fibre dimensions, the following formula was applied:

$C_N = 10^6 C_m \frac{SSA}{\pi d L}$ ….(4)

in which C_N = the number of (nano)fibres/cm^3, C_m = the mass concentration in µg/m^3, SSA = specific surface area in m^2/g, d = fibre diameter (nm), L = fibre length (nm).

When no data on SSA were available, the following formula was applied:

$C_N = 1.315\ 10^9 C_m / \{[n\ d_{out} - (0.34\ n\ (n-1)]\ \pi\ L\}$ ….(5)

in which n = number walls of the CNT and d_{out} = outer diameter (nm).

Since Baytubes are flexible carbon nanotubes, the mass to number conversion was executed assuming these fibres have a globular shape (convoluted like a ball of wool), applying the methods described in sections 1.3.1and 1.3.2.

The derivation of formulas (4) and (5) is described in appendix 5.6.

1.4 Results

1.4.1 Introduction

The OELs retrieved from public literature were divided into two categories:

1. values for groups of MNMs or based on read-across to a group of MNMs, and
2. values for specific MNMs based on quantitative risk assessment.

The exposure limit values that were retrieved from literature all consider MNMs that are classified in NRV classes 1 to 3. No exposure limit values were encountered for MNMs classified in NRV class 4.

Since the generic limit values lack a clear scientific basis, these are only summarily compared with the Dutch provisional NRVs in sections 1.4.2.1and 1.4.3.1. The nanomaterial specific limit values do have a scientific basis, which is evaluated in sections 1.4.2.2and 1.4.3.2. Based on this evaluation, the OELs that were proven to have a clear and sound scientific basis were taken forward to the comparison of the provisional NRVs with current scientific knowledge made in section 1.4.4.

1.4.2 OELs from scientific publications (Q1)

1.4.2.1 Generic limit values

NRV class 1 (fibres)

The general limit values listed in Table 3 for biopersistent nanofibres are all identical to the NRV value for class 1 (0.01 fibre/cm^3).

NRV class 2 (biopersistent high-density granular particles)

When applicable, for this class all exposure limit values found were higher than those of the corresponding provisional NRV values.

NRV class 3 (biopersistent low-density granular particles)

Also for this class all exposure limit values found were higher than those of the corresponding provisional NRV values.

Table 3 Inventory of generic exposure limits from scientific publications using a grouping approach

Category	Nanomaterials and specifications	Reference	Concentration		NRV equivalence		OEL/NRV
			Mass (µg/m³)	Particles/fibres (#/cm³)	Class	Part. Conc. (#/cm³)	
MNM	Fine particulate matter ≤ 2500nm	Guidotti et al. (2010)	30	n/a	1-4	undetermined	n/a
MNM	Airborne particles from nanotechnology processes	McGarry et al. (2013)		3 times LBPC for over 30 minutes	1-4	undetermined	n/a
Fibres	Carbon nanofibres, CNFs	Stockmann-Juvala et al. (2014)		0.01	1	0.01	1
Fibres	Nanocellulose[e]	Stockmann-Juvala et al. (2014)		0.01	1	0.01	1
GBP	Inhaled poorly soluble particles	Pauluhn (2011)	0.5 µl $PM_{respirable}/m^3$ x agglomerate density	n/a	2-3	20,000 - 40,000	n/a
Low-toxicity dust		Stockmann-Juvala et al. (2014)	300 (respirable) 4,000 (inhalable)	230,000[a]	2-3	20,000 - 40,000	5.7 - 11
Low-toxicity dust	Nanoclays[c]	Stockmann-Juvala et al. (2014)	300 (respirable) 4,000 (inhalable)	200,000[d]	3	40,000	5

[a] calculated from the respirable mass concentration, using the particle density of 2.5 g/cm³ specified by BAuA for general dust
[b] calculated from mass concentration using the particle density of 2.5 g/cm³ specified by BAuA as typical for this class of MNMs
[c] included under substance specific approaches by Mihalache et al. (2016), but since the general low-toxicity dust value was attributed to nanoclays by Stockmann-Juvala (2014) because of lack of substance-specific toxicity data, it is more appropriate to regard it as a group approach.
[d] calculated from the respirable mass concentration, using the particle density listed in Table 2
[e] included under substance specific approaches by Mihalache et al. (2016), but since the general fibres' value was attributed to nanocellulose by Stockmann-Juvala (2014) because of lack of substance-specific toxicity data, it is more appropriate to regard it as a group approach.

1.4.2.2 *Nanomaterial-specific limit values*

In principle, the specific exposure limit values listed in Table 4 are health-based, and therefore of special interest for the evaluation of the existing NRVs. An overview of the scientific justification for these values is presented in Appendix 5.8 (page 43).
For a number of OELs, the scientific justification is unclear or not based on toxicological data on the nanoparticles themselves. Katsnelson et al. (2015) have applied a multiplication factor to bulk OELs, the papers by Swidwinska et al. (2015, 2014) are written in Polish and the English abstracts do not mention the scientific basis of the reported OELs and the paper by Warheit et al. (2013) states that the OELs for nanotitanium dioxide have been derived by bridging inhalation toxicity of ultrafine TiO_2 and quartz using instillation studies, but did not describe the quantitative method used. All the above mentioned OELs without a clear or sound scientific justification were excluded from the evaluation in section 1.4.4.
The OELs for nanosilver published by Aschberger et al. (2011) were taken from a paper by Christensen et al. (2010) which is based on the final report of the ENRHES project[3] published by Stone et al. (2009). Only the original publication by Stone et al. is retained for further analysis. Stone et al. (2009) have derived three values, two based on lung effects (0.33 and 0.098 µg/m^3) and one based on liver effects (0.67 µg/m^3), using the ECHA DNEL approach. The two values derived for lung effects were based on the same Point of Departure and the same assessment factors, except for the extrapolation from LOAEC to NOAEC, for which a factor 3 and 10 were used, respectively. In view of the fact that the lung effects were only statistically significant in the high dose group, were only present with minimal severity in the low dose group and did not show a clear dose relation (see Appendix 5.9 for more details), we consider an extrapolation factor of 3 sufficient. Still, the lung effects lead to a lower OEL than the liver effects from the same study (0.33 and 0.67 µg/m^3, respectively) and therefore only the lung value is considered in the evaluation in section 1.4.4.

Table 4 Inventory of Exposure limits from scientific publications proposed for specific manufactured nanomaterials

Nanomaterials and specifications	Reference	Occupational Exposure Limit (µg/m^3)
Carbon nanotubes, MWCNT 10 nm Nanocyl NC 7000	Aschberger et al. (2011)	1
Carbon nanotubes, MWCNT	Stone et al. (2009)	0.67
Carbon nanotube group: SWCNT, DWCNT, MWCNT	Nakanishi et al. (2015)	30
Carbon nanotubes	Luizi (2009)	2.5
Nanogold	Katsnelson et al. (2015)	200
Nanosilver	Aschberger et al. (2011)	0.33
Nanosilver	Aschberger et al. (2011)	0.67
Nanosilver	Stone et al. (2009)	0.33
Nanosilver	Stone et al. (2009)	0.67
Nanosilver	Swidwinska et al. (2015)	10

[3] Engineered Nanoparticles: Review of Health and Environmental Safety (ENRHES) involving a consortium consisting of the Edinburgh Napier University (ENU), the Institute of Occupational Medicine (IOM), the Technical University of Denmark (DTU), the Institute for Health and Consumer Protection of the European Commission's Joint Research Centre (JRC), and the Institute of Nanotechnology (IoN). The project was funded under the Seventh Framework Programme of the European Commission.

Nanomaterials and specifications	Reference	Occupational Exposure Limit (µg/m^3)
Nanosilver	Katsnelson et al. (2015)	100
Nanosilver	Stone et al. (2009)	0.098
Carbon: Fullerenes, C_{60}	Shinohara (2011)	390
Carbon nanotubes: MWCNT 140 nm Baytubes ®	Aschberger et al. (2011)	2
Carbon: Fullerenes, C_{60}	Aschberger et al. (2011)	7.4
Carbon nanotubes, MWCNT Baytubes ®	Pauluhn (2010)	50
Nano copper oxide	Katsnelson et al. (2015)	50
Nano iron oxide	Katsnelson et al. (2015)	400
Nanosilica: Amorphous silica	Stockmann-Juvala et al. (2014)	300
Titanium dioxide: High surface reactivity anatase-rutile, nanoscale	Warheit et al. (2013)	1000
Titanium dioxide: Low surface reactivity, nanoscale	Warheit et al. (2013)	2000
Titanium dioxide: Pigment-grade	Warheit et al. (2013)	5000
Titanium dioxide	Aschberger et al. (2011)	17
Titanium dioxide	Ogura et al. (2011)	610
Titanium dioxide	Stockmann-Juvala et al. (2014)	100
Titanium dioxide	Swidwinska et al. (2014)	300

1.4.3 *OELs proposed by authorities (Q2)*

Only one legally binding OEL for MNMs was found: an exposure limit value of 2 fibres/cm^3 for carbon fibres with a length >5 µm, a diameter <3 µm and an aspect ratio >3, published by the Belgian federal government (ELSD, 2014). Furthermore, some proposals by governmental authorities were found. The German federal government has issued advisory exposure limits for non-entangled fibrous MNMs, nanosized granular biopersistent particles with no specific toxicity[4] as well as for nanosized granular biopersistent particles with specific toxicity[5] (BAuA, 2016). The US NIOSH has proposed two nanospecific OELs, one for titanium dioxide (NIOSH, 2011) and one for carbon nanotubes and nanofibres (NIOSH, 2013). To conclude, Katsnelson et al. (2015) cited generic a nano-OEL published by Australian Government agency “Safe Work Australia”. Only the limit values proposed by NIOSH were founded on a detailed health-based analysis of nanotoxicological literature.

[4] Defined as biopersistent nanoparticles that do not possess substance specific toxicity that goes beyond the particle driven toxicity (that is solely determined by the physicochemical particle properties). As examples the document issued by BAuA mentions carbon black, titanium dioxide, aluminium oxide and aluminium silicate, which were in the past also designated as “inert substances”.

[5] Defined as biopersistent nanoparticles that show health damaging properties or particles of which the microsized version shows health damaging properties without evidence that the nanoscale version does not possess these properties. The examples mentioned by BAuA are nanogold, nanosilver and zinc oxide.

1.4.3.1 Generic limit values

NRV class 1 (fibres)

The generic limit values listed in Table 5 for biopersistent nanofibres are all identical to the NRV value for class 1 (0.01 fibre/cm^3) except for the value published by the Belgian government: 2 fibres/cm^3.

NRV class 2 (biopersistent high-density granular particles)

No clear comparison can be made as the categories the governmental institutions have defined do not match the categories defined for the provisional NRVs. However, the OEL for globular biopersistent particles (GBP) with specific toxicity proposed by the Bundesanstalt für Arbeitsschutz und Arbeitsmedizin[6] (BAuA), leads to a limit value of 9,900/cm^3 for high density nanoparticles like nanogold, which is lower than the value of 20,000/cm^3 for this NRV class.

NRV class 3 (biopersistent low-density granular particles)

Also for this class no clear comparison can be made, for the same reasons as mentioned above. However, the OEL for insoluble GBP, expressed in particles' number/cm^3, proposed by the British Standards Institution (BSI), implies a limit value of 20,000/cm^3 also for low density globular biopersistent particles, which is lower than the value of 40,000/cm^3 for this NRV class.

[6] The German Federal Institute for Occupational Safety and Health

Table 5 Inventory of generic exposure limits using a grouping approach derived by governmental institutions

Category	Nanomaterials and specifications	Reference	Concentration		NRV equivalence		OEL/NRV
			Mass (µg/m³)	Particles/fibres (#/cm³)	Class	Part. Conc. (#/cm³)	
Carbon	Carbon fibres with a length >5 µm, a diameter <3 µm and an aspect ratio >3	Belgian Federal Government (ELSD, 2014)		2	1	0.01	200
Fibres	Fibrous nanomaterials	BSI (2007)		0.01	1	0.01	1
Fibres	Non-entangled fibrous NM	BAuA (2016)		0.01	1	0.01	1
Soluble	Soluble nanomaterials	BSI (2007)	0.5 x bulk WEL	n/a	4	undetermined	n/a
GBP	Insoluble nanomaterials	BSI (2007)	0.066 x bulk WEL	20,000	2-3	20,000 - 40,000	0.50 – 1.0
CMAR	CMAR nanomaterials	BSI (2007)	0.1 x bulk WEL	n/a	1-4	undetermined	n/a
GBP	Nanomaterials with a specific toxicity (if a substance-related OEL Is not available)	BAuA (2016)	100	9,900 – 34,000[b]	2-3	20.000-40.000	5
GBP	Nanosized, with no specific toxicity	BAuA (2016)	500	380,000[c]	2-4	undetermined	>9.5
MNM	nanocrystals, quantum dots, ceramic oxides, and metals	Katsnelson et al. (2015)[a]	0.066 x bulk WEL	n/a	2-3	20,000 - 40,000	n/a

[a] proposed in 2010 by the Australian Government agency “Safe Work Australia”

[b] calculated from mass concentration using the particle densities of gold and zinc oxide, the densest and the least dense compounds mentioned as examples of this category by BAuA

[c] calculated from mass concentration using the particle density of 2.5 g/cm^3 specified by BAuA as typical for this class of MNMs.

1.4.3.2 *Nanomaterial-specific limit values*

In principle, the specific exposure limit values listed in Table 6 are health-based, and therefore of special interest for the evaluation of the existing NRVs. For this reason, an overview of the scientific justification for these values is presented in Appendix 5.8 (page 43).

NIOSH (2013) has derived OELs for two types of MWCNTs starting from benchmark doses derived from data published by Ma-Hock et al. (2009) and Pauluhn (2010) (see appendix 5.5). Since for the dose-response no doses showing intermediate toxicity were present (only highly toxic doses and minimally toxic doses), there is a high degree of uncertainty with respect to the derived benchmarks, thus rendering the OELs based on these values uncertain as well. As the lower 95% confidence level of the BMD is used as point of departure, in this case the OEL derived will be unduly conservative. In our view, in such cases it is preferable to use NOAELs as point of departure. Therefore, this OEL was excluded from the evaluation in section 1.4.4[7].

In 2006, NIOSH has derived two different tentative OELs for titanium dioxide (Kuempel et al., 2006) followed by a definitive proposal in 2011 (NIOSH, 2011). All three values were based on the same toxicological data, applying dose-response analysis to derive the Point of Departure. The only difference between the three approaches is the benchmark dose and the kinetic models used. Therefore, the latest published value (from NIOSH, 2011) is used in the evaluation in section 1.4.4.

In the same 2006 paper (Kuempel et al., 2006), NIOSH has published two different tentative OELs for carbon black, both based on the same point of departure, but applying different kinetic models: the interstitial lung sequestration model developed by NIOSH (Kuempel et al., 2001) and the MPPD model developed by CIIT and RIVM (RIVM, 2002). The difference between the values derived with both models was only a factor two. Since thereafter NIOSH has preferred the MPPD model (NIOSH, 2011) only the value derived with this model (240 µg/m^3) has been retained in the evaluation in section 1.4.4.

Table 6 Inventory of published Exposure limits proposed by governmental institutions for specific manufactured nanomaterials

Nanomaterials and specifications	Reference	Occupational Exposure Limit (µg/m^3)
Carbon black: CB ultrafine	Kuempel et al. (2006)	120
Carbon black: CB ultrafine	Kuempel et al. (2006)	240
Carbon nanotubes and nanofibres	NIOSH (2013)	<1[a]
Titanium dioxide: ultrafine	Kuempel et al. (2006)	140
Titanium dioxide: ultrafine	Kuempel et al. (2006)	73
Titanium dioxide: ultrafine	NIOSH (2011)	300

[a] Based on limit of quantification

1.4.4 *Comparison of the provisional NRVs to current scientific knowledge (Q3)*

Table 7 compares the health-based OELs retrieved from public sources with the NRV of their corresponding class.

NRV class 1 (fibres)

For NRV class 1, based on asbestos-like fibres, only OELs for carbon nanotubes were retrieved. The values derived were all based on subacute or subchronic rat studies, not

[7] It should be noted that a number of OELs based on the NOAELs from the same studies, were included in the quantitative comparison.

on chronic studies, let alone carcinogenicity studies. Since the critical effects of asbestos are mesothelioma and lung cancer, and the OELs for carbon nanotubes are based on studies that cannot detect such effects with any degree of certainty, these OELs are not considered suitable to conclude on the appropriateness of the occupational exposure level set for this NRV class. It should be noted that the NRV class 1 is based on the (former) Dutch occupational exposure limit of 0.01 fibres/cm^3 for asbestos. This limit applies to all types of asbestos and is not based on a calculated concentration corresponding to a given risk level, but is derived from (and ten times lower than) the current EU standard for chrysotile asbestos (Health Council of the Netherlands, 2010). In 2010 the Health Council of the Netherlands has published calculated limit values corresponding to a risk level of 4.10^{-5}, that are substantially lower: 0.001 fibres/cm^3 for chrysotile asbestos and 0.00042 fibres/cm^3 for amphibole asbestos[8] (Health Council of the Netherlands, 2010). In 2016 the Netherlands adopted a new legal OEL of 2,000 fibres/m^3 (= 0.002/cm^3) for all forms of asbestos (Staatsblad, 2016).

NRV class 2 (biopersistent high-density granular particles)

Only one representative nanomaterial of NRV class 2 was found with a claimed health-based OEL: nanosilver. Nanosilver is mentioned as an example of a high density in the SER document (SER, 2012). In whatever way the particles number concentration of this OEL has been derived, it is lower than the class 2 NRV value of 20,000 particles/cm^3. Especially the value calculated using the "worst case NRV-method" is quite low, but this is mainly because this method uses a 100 nm size as worst case assumption, while the geometric mean size of the nanosilver particles is 18 nm (Sung et al., 2008). Should the NRV-method be applied using 20 nm as reference size, the OEL would have been 7,500 particles/cm^3. Since the nanosilver OEL expressed in particles/cm^3 presented by Stone et al. (2009) is based on a measured particles' number concentration of 4,000 particles/cm^2, this value seems to be the most accurate one. It is a factor 5 below the NRV class 2 limit value, which might indicate that this class value should be more conservative, at least for nano-Ag. However, based on the data underlying the nanosilver OEL, a different conclusion may be drawn. The low dose in the study was considered a LOAEC (based on lung effects) by Sung et al., but based on the data we consider the high dose to be the LOAEC, since only at that dose statistically significant effects are seen (see section 5.9 (page 47)). This means that, according to us, the mid dose is a NOAEC and should be used as point of departure. This dose of 133 µg/m^3 is 2.7 times higher than the LOAEC assumed by Stone et al. (2009) (see Appendix 5.8, page 43). Consequently an extrapolation factor of 3 from LOAEC to NOAEC is no longer necessary, and using this NOAEC as a point of departure leads to an OEL of 32,400 particles/cm^3, which is above the NRV class 2 value, indicating this NRV is conservative in relation to the nanosilver OEL thus derived.

NRV class 3 (biopersistent low-density granular particles)

OELs were retrieved for five different nanoparticles in NRV class 3: carbon black, C_{60} fullerenes, Baytubes MWCNT (a non-asbestos like carbon nanotube), nanosilica and titanium dioxide. For C_{60} fullerenes two OELs are suggested, based on different studies with a different overall assessment factors (see Appendix 5.8, page 43) and highly different mass-based OELs (one approximately 50 times higher than the other). However, when the corresponding number concentrations are calculated based on the size distributions of the investigated nanoparticles, the OELs are in the same order of magnitude (differing only by a factor two). Also for the Baytubes MWCNT two values are available, both based on the same study and point of departure, but applying a different overall assessment factor. Four OELs are available for titanium dioxide, three of which (Aschberger and Christensen, 2011; Ogura et al., 2011; Stockmann-Juvala et al., 2014) are based on the same study by Bermudez et al. (2004), but using different overall assessment factors or even points of departure (see Appendix 5.8, page 43). The fourth value for titanium dioxide, derived by NIOSH (2011) is the only OEL found based on a

[8] Numbers as measured by phase contrast microscope. When measured by transmission electron microscopy (TEM) these values are twice as high.

chronic study. It should be noted that this value was derived by linear extrapolation from the $BMDL_{10}$ to a 1:1000 extra cancer risk, an approach usually reserved for (directly) genotoxic carcinogens. As the mechanism underlying the carcinogenic effects of titanium dioxide is most likely related to macrophage overload, a threshold approach is more appropriate. When using a benchmark dose approach to dose-response, commonly the $BMDL_{10}$ is considered to be equivalent to a NOAEC, meaning that in this case the OEL based on carcinogenic effects would be 10 times as high and equal to 3,000 µg/m^3 (equivalent to 43,000 particles/cm^3, as calculated using the size distribution in the study). Considering this value, it appears carcinogenicity is not the critical effect for titanium dioxide.

Table 7 Comparison of nanoparticle specific OELs from public sources and NRVs

Nanomaterials and specifications	Particle characteristics in toxicity study used for OEL: Primary particle size (nm)	Particle size distribution (airborne) (nm/GSD)[n]	Source	Concentration: Mass (µg/m³)	Particles/fibres (#/cm³): Measured	Calc.: NRV method	Calc. based on size distr.[p]	NRV equivalence: Class	Part. Conc. (#/cm³)
Proposals from peer-reviewed scientific literature									
Carbon nanotube group: SWCNT, DWCNT, MWCNT	2.8	no data	Nakanishi et al. (2015)	30	--	86,000 – 6,478,000[b]	n/a	1	0.01
Carbon nanotubes, MWCNT	10-20	350-400[o]/2.0	Stone et al. (2009)	0.67	--	3,700[a]	n/a	1	0.01
Carbon nanotubes, MWCNT 10 nm Nanocyl NC 7000	10	500-1,300/3.1-5.4	Aschberger et al. (2011)	1	--	5,600[a]	n/a	1	0.01
Carbon nanotubes, MWCNT 10 nm Nanocyl NC 7000	10	500-1,300/3.1-5.4	Luizi (2009)	2.5	--	14,000[a]	n/a	1	0.01
Nanosilver	no data	18-19[o]/1.1-1.6	Stone et al. (2009)	0.33	4,000[m]	*60*[c]*	*4,500*[i]*	2	20,000
Carbon black: CB ultrafine	no data	1,950/1.84	Kuempel et. (2006)	240	--	250,000[c]	440[d]	3	40,000
Carbon nanotubes: MWCNT 140 nm Baytubes®	140	1,700-3,400/1.7-2.1	Aschberger et al. (2011)	2	--	12,000[c]	4- 44[f]	3	40,000
Carbon nanotubes: MWCNT 140 nm Baytubes®	140	1,700-3,400/1.7-2.1	Pauluhn (2010)	50	--	310,000[c]	93- 1110[f]	3	40,000
Carbon: Fullerenes, C_{60}	no data	96[o]/2.0	Shinohara et al. (2011)	390	--	430,000[c]	56,000[e]	3	40,000
Carbon: Fullerenes, C_{60}	no data	55[o]/1.48	Aschberger et al. (2011)	7.4	3,400[g]	*8,200*[c]	*25,000*[k]	3	40,000
Nanosilica: Amorphous silica	12	not measured	Stockmann-Juvala et al. (2014)	300	--	260,000[c]	no data	3	40,000
Titanium dioxide	21	1440/2.6	Aschberger et al. (2011)	17	--	7,700[c]*	18[h]*	3	40,000
Titanium dioxide	21	1440/2.6	Ogura et al. (2011)	610	--	280,000[c]*	630[h]*	3	40,000
Titanium dioxide	21	1440/2.6	Stockmann-Juvala et al. (2014)	100	--	45,000[c]*	100[h]*	3	40,000
Titanium dioxide: ultrafine	no data	800/1.8	NIOSH (2011)	300	--	140,000[c]*	4,300[i]*	3	40,000

* Calculated using the density of the chemical compound instead of the air-borne particles' density

[a] calculated using the single fibre and the bundles fibre masses for SWCNT(A) listed in Table 2

[b] calculated using the fibre mass for MWCNT Nanocyl NC7000 listed in Table 2

[c] calculated using the density listed in Table 1 and assuming a spherical particle of 100 nm

[d] calculated using the particle size distribution specified in the study that served as point of departure as reported by Mauderly et al. (1994) and NIkula et al. (1995) and the density listed in Table 2

[e] calculated using the particle size distribution and the density reported by Shinohara et al. (2015)

[f] calculated using the particle size distribution reported in the study by Pauluhn (2010) from which the point of departure was derived and the density listed in Table 2

[g] calculated using the number concentrations reported in the study used for the point of departure reported by Baker et al. (2008)

[h] calculated using the particle size distribution reported in the study by Bermudez et al. (2004) from which the point of departure was derived, and the density listed in Table 2

[i] calculated using the particle size distribution reported in the study from which the point of departure was derived, described by Heinrich et al. (1995) and Muhle et al. (1994), and the density listed in Table 2.

[j] calculated using the density listed in Table 2 and the particle size distribution specified in the study by Sung et al. (2008) that served as point of departure

[k] calculated using the density listed in Table 2 and the particle size distribution specified in the study by Baker et al. (2008). It should be noted this calculated value is approx. 10 times higher than the measured value.

[m] based on measured values from the study by Sung et al. (2009, 2008) as calculated by Stone et al. (2009). Note: The selection of the lowest dose in this study as a LOAEC and Point of Departure for the derivation of the OEL is debatable (see page 17). Selecting the mid dose as a NOAEL would lead to an OEL of 32,400 particles/cm^3

[n] MMAD, unless otherwise indicated

[o] CMD

[p] Values lower than the value of the corresponding NRV class are printed in red

2 Evaluation of the necessity to update the NRVs for NNMs(Q4a)

2.1 Introduction

This chapter addresses the first part of question 4: Is it necessary to adapt the NRVs? Since the conclusion of the evaluation reported below is that they should be adapted, a proposal for an approach on how to decide on the nature and extent of modifications of NRVs is presented in this chapter.

2.2 NRV Class 1 - Nanofibres

In the present NRV approach, nanofibres are assigned either to class 1 of the asbestos-like fibres or to class 3. The decision is based on the whether the manufacturer has or has not excluded that its nanosized carbon or metal oxide fibres possess asbestos-like effects.
The OEL limit values proposed for nanofibrous materials do not constitute a proper yardstick to evaluate NRV class 1 as the main suspected effect in this class, asbestos-like carcinogenicity, has not been included in the evidence-base from which these values were derived.
For one type of carbon nanotubes for which asbestos-like effects can be excluded, Baytubes, two OELs were found in public literature, both based on the same inhalation repeated dose study (by Pauluhn, 2010). Depending on the assessment factors used and/or the method to calculate particle concentrations, the corresponding class 3 NRV is in the same order of magnitude or much higher. This is discussed further in section 2.4.
NRV class 1 is based on the former Dutch occupational exposure limit for asbestos of 0.01 fibres/cm^3. This limit applies to all types of asbestos and is not health-based. It is derived from (and ten times lower than) the current EU standard for chrysotile asbestos (Health Council of the Netherlands, 2010). In 2010 the Health Council of the Netherlands has published health-based limit values that are substantially lower: 0.001 fibres/cm^3 for chrysotile asbestos and 0.00042 fibres/cm^3 for amphibole asbestos[9] (Health Council of the Netherlands, 2010)[10]. Based on this advice, the Dutch Ministry of Social Affairs and Employment has recently reduced the occupational limit value for asbestos to 0.002 fibres/cm^3 (Staatsblad, 2016).
In 2014, it was announced that IARC will classify a certain type of MWCNT (MWCNT-7) as a group 2B carcinogen, implying it is possibly a human carcinogen based on animal test results, while MWCNT other than MWCNT-7 are assigned to group 3, meaning the available evidence is inconclusive with respect to carcinogenicity (Grosse et al., 2014). The scientific basis for these conclusions will be published in the IARC monograph series on the evaluation of carcinogenic risks to humans volume 111, but until now this volume has not yet been released by IARC[11].
At the moment, it is not clear whether MWCNTs cause carcinogenicity via the same mechanism as asbestos nor whether they have a comparable potency. Furthermore, it is debatable whether the mechanism of carcinogenicity of asbestos justifies the linear extrapolation of observed dose-response relationships to low doses, and if so, whether this also would be valid for MWCNT-7. Therefore, it is not yet clear whether a lowering of the class 1 NRV is scientifically justifiable. Also it is not clear whether the present NRV class 1 is conservative or not. Therefore, it is advised not to decide on the possible

[9] Numbers as measured by phase contrast microscope. When measured by transmission electron microscopy (TEM) these values are twice as high.
[10] The new limit value for all asbestos fibres of 0,002 fibres/cm^3 is based on the findings of this report.
[11] IARC website (http://monographs.iarc.fr/ENG/Classification/latest_classif.php) last consulted on 27-12-2016

modification of the present class 1 NRV until this publication is available for inclusion in the considerations.
Alternatively, NRV class 1 could be lowered to the current asbestos norm of 0.002 fibres/cm^3, applying the precautionary principle. However, this would be a policy decision whose necessity does not follow from the scientific evidence evaluated in this report.

2.3 NRV class 2 - Biopersistent high-density granular particles

This class is only represented by a single OEL, for nanosilver. The single OEL for the class 2 NRV is, although being a factor of 5 lower than the provisional NRV, insufficient to draw conclusions regarding a potential adjustment of the current NRV, also because its value is debatable and may be an order of magnitude higher (see page 17).

2.4 NRV class 3 - Biopersistent low-density granular particles

The number of health-based, nanomaterial-specific OELs for this class is also quite low: it is only represented by OELs for five different nanomaterials. For a number of particles in class 3 more than one OEL was retrieved. Although the toxicity database available was essentially the same for each same-substance and sometimes even the same toxicity study was used as point of departure for derivation of the OEL (see Appendix 5.8, page 43), their values differ one or even nearly two orders of magnitude. For instance, on a mass basis, the OELs derived for titanium dioxide range from 17 to 610 µg/m^3, equivalent to 18 to 4,300 particles/cm^3, calculated based on the size distribution in the study serving as point of departure (see Table 5, page 19). This implies that all values are below the current provisional NRV. The same trend is seen for carbon fullerenes, C_{60} and MWCNT 140 nm Baytubes.
In order compare the OELs retrieved with the corresponding NRV, most of them had to be converted from mass concentration to particles' number concentration (PNC), as the NRVs are expressed in the latter metric. Two methods were used for this conversion, one based on the assumption that all particles present have a diameter of 100 nm and are spherical, the other based on the MMAD or CMD and GSD measured in the study used as point of departure in the OEL derivation, and the assumption of sphericity (see sections 1.3.1 and). The results of both methods can be very different, up to 3 orders of magnitude. This is mainly due to the fact that in practice particle sizes may be very different from an assumed size of 100 nm, e.g. by association of primary particles. As the method using actually measured size distributions of toxicologically relevant particles is of higher practical relevance, this result of conversion will be leading in the comparison between proposed OELs and NRVs. Based on these values, eight of the nine proposed OELs are lower than the corresponding NRVs (see Table 7), meaning the NRVs are less conservative than these OELs. Whether or not this conclusion will hold in general for all GBPs cannot be decided in view of the very low number of OELs available. Still these data give clear indications that the current NRV may not be as conservative as assumed, and therefore the value of the class 3 NRVs should be updated.

2.5 NRV Class 4 - Soluble MNMs

No data on soluble MNMs (class 4) were encountered in public literature and consequently there is no basis for the re-evaluation of this class of MNMs. Therefore the class 4 NRV is not further discussed here. Monitoring using the regular OEL for the substance is considered applicable.

2.6 General issues

Another issue to consider is whether particles' number concentration is the best metric to express the class 2 and 3 NRVs in. Except for fibres, regulatory practice is to always

use mass based OELs. While there seems to be consensus that mass is not the most relevant dose metric to express toxic potency of nanoparticles, there is no consensus on what is the most relevant metric, e.g. particle number or surface area. It might also depend on the mechanism of toxicity of a particular nanoparticle, e.g. particles' number when macrophage overload is the mechanism and surface area when release of toxic ions or surface reactivity is the cause of toxicity.

Toxicity of ultrafine and fine carbon black in instillation toxicity experiments in rats proved to be comparable on the basis of surface area, but not on the basis of mass (Sager and Castranova, 2009). The same conclusion can be drawn based on inhalation toxicity experiments with rats using pigmentary and ultrafine titanium dioxide (Bermudez et al., 2004, 2002). However, an inhalation study by Ho et al. (2011) on exposure of rats to low, moderate, or high dose of 35 and 250 nm ZnO particles, which induced lung inflammation, provided a more complex picture. Mass concentration was significantly correlated with the percentage of neutrophils (R^2=0.84), number of neutrophils (R^2=0.84) and total cells (R^2=0.73) in BALF. Also surface area concentration was significantly correlated with the percentage of neutrophils (R^2=0.94), number of neutrophils (R^2=0.81) and total cells (R^2=0.76), but there was no correlation between the particles' number and lung inflammation. NIOSH demonstrated that the dose-response lung tumours caused by a number of so called poorly soluble of low toxicity particles (talc, titanium dioxide, carbon black, diesel soot and toner) is best described based on surface area (NIOSH, 2011).

Based on this short overview, the use of surface area instead of particle number to define class 2 and 3 NRVs should be seriously considered, although it is recognized it is difficult to measure in an occupational setting, where mass and number based methods are easier to apply. Since in most comparisons only mass and surface area were set off against each other and particles' number was not considered, it is too early to draw a definitive conclusion.

Concluding, to bring the on-going discussion on the relevant dose metric to a conclusion, a number of issues need to be resolved, of which toxicological relevance and applicability in day-to-day occupational hygienic practice are the most important.

3 Proposal for an approach to modify the NRVs for biopersistent granular MNMs (Q4b)

The two main issues to be addressed are selection of the appropriate metric to express the NRV in, which should be based on its toxicological relevance, and the establishment of NRVs that are as much as possible based on health-based evidence.
The current discrimination between class 2 and 3 NRVs is based on differences in density between nanoparticles, motivated by the expression of NRVs in particle concentrations rather than mass. The values of these classes only differ by a factor 2, which is negligible in view of the uncertainties surrounding the NRV values. Therefore, density is not an a priori useful criterion to discriminate between toxic and less toxic granular biopersistent nanoparticles.
BAuA has applied a criterion different from density to discriminate between more and less toxic granular nanoparticles, the criterion “specific” toxicity (see section1.4.2, page 10). They defined nanoparticles not possessing specific toxicity as biopersistent nanoparticles that do not possess substance specific toxicity that goes beyond the particle driven toxicity (that is solely determined by the physicochemical particle properties). As examples the document issued by BAuA mentions carbon black, titanium dioxide, aluminium oxide and aluminium silicate, which were in the past also designated as “inert substances”. The scientific basis of this discrimination is rather unclear and arbitrary. E.g. elemental gold is generally considered to be an inert material and is not classified for toxicity, yet BAuA mentions nanogold as an example of a nanoparticle that does possess specific toxicity (see section 1.4, footnote 5). Therefore, this approach is not considered to be applicable in establishing (revised) temporary nano reference values.
Consequently, it has been decided to treat these nanoparticle classes as one group in the approach outlined below, until a (new) relevant metric has been selected to express them in, and data have been gathered that could support the establishment of one or more classes of NRVs based on the chosen metric or another physicochemical property of the nanoparticles.
Below a stepwise approach is proposed to establish new NRVs for biopersistent granular MNMs. The rationale behind this approach is to derive health-based, consistent OELs expressed in a relevant metric for those nanoparticles for which sufficient toxicity data are available. Subsequently, these OELs are used to establish new temporary NRVs to be able to control occupational exposure to nanoparticles for which not enough toxicity data are available to derive a specific OEL. The steps proposed are the following:

1. Collect a database of *in vivo* inhalation toxicity studies with well-characterised nanoparticles for which the dose can be expressed in terms of mass, particle number and surface area, e.g. using the data that is being collected in the NANoREG[12], NanoReg2[13] and caLIBRAte[14] projects.

Chronic *in vivo* inhalation studies are preferred because inhalation is the major relevant route of occupational exposure to nanoparticles and lifetime occupational exposure should be covered. The often executed *in vivo* instillation studies are not representative of occupational exposure due to the high concentrations used and the form in which they are administered: liquid instead of aerosol. Furthermore, at the moment there is no reliable method to extrapolate *in vitro* toxicity test results to relevant quantitative *in vivo* toxicity parameters.

2. For specific nanoparticles, select a combination of studies that allow analysis of dose-response relationships in term of mass, particle number and surface area.

[12] http://www.nanoreg.eu/
[13] http://www.nanoreg2.eu/
[14] http://www.nanocalibrate.eu/home

This means that for a particular nanoparticle, experiments should be available that test different sizes, masses and specific surface-areas.

3. Perform dose-response analyses for each suitable set of specific nanoparticles using e.g. a software package like the USEPA BMDS[15], that provides statistical measures of 'goodness of fit', which may be used to rank the different dose metrics used.
4. Based on the results thus obtained, draw conclusions on the most appropriate dose metric to express nanoparticle toxicity, based on which metric produces the best-fitting dose response model in the previous step. If no unequivocal choice can be made, investigate whether the most appropriate dose metric for a given nanoparticle can be linked to a specific physicochemical property.

The results of the investigations into the appropriate dose metric may also serve as input for international consensus building on this issue.
In order to establish NRVs which are designed to avoid possible health risks from nanoparticles, it is crucial to have a good overview of the health risks that are already known, preferably expressed in a health-based occupation limit value. From the OELs collected so far, it has become quite clear that very different limit values can be derived based on the same toxicity data. Therefore, a uniform approach, with harmonized assessment factors and selection criteria for critical toxic effects is essential. To achieve this, the following next steps are proposed:

5. Extend the database collected under point 1 with other inhalation studies with well-characterised nanoparticles that did not meet all the criteria mentioned there, but do provided toxicity data for doses that can be expressed in the dose metric(s) selected under point 4.
6. Based on the collected database, derive limit values for as many specific nanoparticles as possible using the methods described by ECHA to derive long-term occupational DNELs or DMELs, expressed in the metric(s) selected under point 4.

When selecting the critical toxic effect, care should be taken to choose effects that are clearly adverse and not adaptive in nature. E.g. an increase in inflammation parameters in the lungs in a short-term study can be considered an adaptive response, while interstitial fibrosis that has arisen as a consequence of inflammation is a clear adverse effect.

7. Based on the results obtained under points 4 and 6, identify classes of MNMs and propose NRVs for these classes.
8. Develop a practical method to translate the proposed NRVs to practical measures for use in an occupational hygiene setting.

Modifications of simple, pristine nanoparticles like coatings, shells, addition of additional chemical compounds to the core, etc. may influence their toxicity. Clearly, for many of those modified nanoparticles no sufficient data will be available to derive health-based OELs. Therefore it may be considered to investigate whether e.g. comparative in vitro data would justify specific NRVs for specific modifications of pristine nanomaterials and serve as a basis to derive such specific NRVs.
In view of the role of the Dutch Health Council in establishing occupational reference values, it is suggested to discuss the proposals set out in this chapter with the Council establishing the definite strategy to come to adapted NRVs. As this is outside the scope of the present project, it should be part of a follow-up project.
Furthermore, it is proposed to include KIR-nano occupational hygienists platform as a mirror-group in the modification process.

[15] https://www.epa.gov/bmds

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5 List of abbreviations

ABS	Acrylonitrile Butadiene Styrene
BAuA	Bundesanstalt für Arbeitsschutz und Arbeitsmedizin
BGNP	Background NanoParticle
BMDS	BenchMark Dose Software
BSI	British Standards Institute
CB	Carbon Black
CDNP	Combustion Derived Nanoparticle
CMAR	Carcinogenic, Mutagenic, Asthmagenic and Reprotoxic (substances/nanoparticles)
CMD	Count Median Diameter
DEP	Diesel Exhaust Particulates
DNEL	Derived No Effect Level
ECHA	European Chemicals Agency
FCAW	Flux Cord Arc Welding
FCNP	Fraction of NanoParticles in Conventional compounds
GBP	Globular Biopersistent Particle
GESTIS	Dangerous Substances Information System (Gefahrstoffinformationssystem)
GMAW	Gas Metal Arc Welding
GMD	Geometric Mean Diameter
GSD	Geometric Standard Deviation
GTAW	Gas Tungsten Arc Welding
GTAW	Tungsten Arc Welding
HDPE	High Density Polypropylene
IARC	International Agency for Research on Cancer
KIR-nano	Knowledge and Information Point for Nanotechnology (Kennis- en informatiepunt risico's (KIR) Nanotechnologie)
LOAEC	Lowest Observed Adverse Effect Concentration
MAG	Metal Active Gas Welding
MIG aluminum	Metal Inert Gas Welding on aluminum
MIG soldering	Metal Inert Gas Soldering on zinc plated base material
MMAD	Mass Median Aerodynamic Diameter
MMAW	Manual Metal Arc Welding
MMD	Mass Median Diameter
MNM	Manufactured Nano Material
MWCNT	Multiple Walled Carbon Nanotube
n	number
NANoREG	A common European approach to the regulatory testing of nanomaterials
NanoReg2	A common European approach to the regulatory testing of nanomaterials, second project
Nd:YAG	Neodymium doped YAG-crystal
NIOSH	National Institute for Occupational Safety and Health
NOAEC	No Observed Adverse Effect Concentration

NRV	Nano Reference Value
OEL	Occupational Exposure Limit
PAPR	Powered Air Purified Respirators
PGNP	Process Generated NanoParticle
PLA	PolyLactic Acid
PM	Particulate Matter
PNC	particles' number concentration
RIVM	Netherlands National Institute for Public Health and the Environment
ROS	Reactive Oxygen Species
RSW	Resistance Spot Welding
SCOEL	Scientific Committee on Occupational Exposure Limits
SER	Dutch Social and Economic Council
SMAW	Shielded Metal Arc Welding
SSA	Specific Surface Area
SWCNT	Single Walled Carbon Nanotube
SZW	Dutch Ministry of Social Affairs and Employment
TEM	Transmission Electron Microscopy
TIG	Tungsten Inert Gas Welding
TNO	Netherlands Organisation for applied scientific research
TWA	Time Weighted Average
UFP	UltraFine Particle
US	United States
USEPA	United States Environmental Protection Agency
WF	Welding Fumes

6 Appendices

1.1 Scopus Search

Scopus was searched using the following search string:
((TITLE-ABS-KEY (zno OR "zinc oxide") OR TITLE-ABS-KEY (coo OR "cobaltous oxide" OR "cobalt oxide") OR TITLE-ABS-KEY ("ferric oxide" OR fe2o3 OR "ferrosoferric oxide" OR fe3o4 OR "triiron tettraoxide" OR "iron oxide" OR "ferrous oxide" OR feo) OR TITLE-ABS-KEY (lead OR pb) OR TITLE-ABS-KEY (lanthanum) OR TITLE-ABS-KEY (sb2o5 OR "antimony pentoxide" OR "diantimony pentoxide" OR "antimony oxide") OR TITLE-ABS-KEY (sno2 OR "cassiterite" OR "tin oxide") OR TITLE-ABS-KEY ("carbon black")) AND SUBJAREA (mult OR agri OR bioc OR immu OR neur OR phar OR mult OR medi OR nurs OR vete OR dent OR heal)) AND ((TITLE-ABS-KEY (nano**) AND SUBJAREA (mult OR agri OR bioc OR immu OR neur OR phar OR mult OR medi OR nurs OR vete OR dent OR heal)) AND (oel OR o els OR wel OR wels OR tlv OR tlvs OR "threshold limit value" OR "threshold limit values" OR "occupational consideration" OR "occupational considerations" OR "exposure limit" OR "exposure limits" OR "occupationally relevant dose" OR "occupationally relevant doses" OR "maximum acceptable concentration" OR "maximum acceptable concentrations" OR "number concentration" OR "number concentrations" OR "number value" OR "number values" OR nrv OR NRVs OR "maximum allowable concentration" OR "maximum allowable concentrations" OR "threshold limit value")) AND (LIMIT-TO (SUBJAREA , "MEDI") OR LIMIT-TO (SUBJAREA , "ENVI") OR LIMIT-TO (SUBJAREA , "PHAR") OR LIMIT-TO (SUBJAREA , "CHEM") OR LIMIT-TO (SUBJAREA , "AGRI") OR LIMIT-TO (SUBJAREA , "MULT") OR LIMIT-TO (SUBJAREA , "Undefined"))
Scopus results
The search performed on October 27, 2016 yielded 396 references. Based on title the publications listed in Table 6 were selected for further scrutiny. Based on abstract and/or complete paper, in the end only one paper was added to the papers already discussed by Mihalache et al. (2016). The reasons for rejecting or accepting specific papers are listed in Table 6.

Table 8 Selection of papers from SCOPUS search

Authors	Title	Year	Source title	Selected	Motivation
Liu, J., Feng, X., Wei, L., Chen, L., Song, B., Shao, L.	The toxicology of ion-shedding zinc oxide nanoparticles	2016	Critical Reviews in Toxicology	No	no OELs
Pease, C., Rücker, T., Birk, T.	Review of the Evidence from Epidemiology, Toxicology, and Lung Bioavailability on the Carcinogenicity of Inhaled Iron Oxide Particulates	2016	Chemical Research in Toxicology	No	no OELs
Kim, S.-H., Heo, Y., Choi, S.-J., Kim, Y.-J., Kim, M.-S., Kim, H., Jo, E., Song, C.-W., Lee, K.	Safety evaluation of zinc oxide nanoparticles in terms of acute dermal toxicity, dermal irritation and corrosion, and skin sensitization	2016	Molecular and Cellular Toxicology	No	no OELs

Authors	Title	Year	Source title	Selected	Motivation
Dekkers, S., Oomen, A.G., Bleeker, E.A.J., Vandebriel, R.J., Micheletti, C., Cabellos, J., Janer, G., Fuentes, N., Vázquez-Campos, S., Borges, T., Silva, M.J., Prina-Mello, A., Movia, D., Nesslany, F., Ribeiro, A.R., Leite, P.E., Groenewold, M., Cassee, F.R., Sips, A.J.A.M., Dijkzeul, A., van Teunenbroek, T., Wijnhoven, S.W.P.	Towards a nanospecific approach for risk assessment	2016	Regulatory Toxicology and Pharmacology	No	no OELs
Zou, H., Zhang, Q., Xing, M., Gao, X., Zhou, L., Tollerud, D.J., Tang, S., Zhang, M.	Relationships between number, surface area, and mass concentrations of different nanoparticles in workplaces	2015	Environmental Sciences: Processes and Impacts	No	no OELs
Katsnelson, B.A., Privalova, L.I., Sutunkova, M.P., Gurvich, V.B., Loginova, N.V., Minigalieva, I.A., Kireyeva, E.P., Shur, V.Y., Shishkina, E.V., Beikin, Y.B., Makeyev, O.H., Valamina, I.E.	Some inferences from in vivo experiments with metal and metal oxide nanoparticles: The pulmonary phagocytosis response, subchronic systemic toxicity and genotoxicity, regulatory proposals, searching for bioprotectors (a self-overview)	2015	International Journal of Nanomedicine	Yes	mentions safe exposure limits
Arts, J.H.E., Hadi, M., Keene, A.M., Kreiling, R., Lyon, D., Maier, M., Michel, K., Petry, T., Sauer, U.G., Warheit, D., Wiench, K., Landsiedel, R.	A critical appraisal of existing concepts for the grouping of nanomaterials	2015	Regulatory Toxicology and Pharmacology	No	no OELs
Lach, K., Steer, B., Gorbunov, B., Mička, V., Muir, R.B.	Evaluation of exposure to airborne heavy metals at gun shooting ranges	2015	Annals of Occupational Hygiene	No	no OELs
Pauluhn, J.	Derivation of occupational exposure levels (OELs) of Low-toxicity isometric biopersistent particles: How can the kinetic lung overload paradigm be used for improved inhalation toxicity study design and OEL-derivation?	2014	Particle and Fibre Toxicology	No	Mentions OELs. However, only deals with the methodology to derive OELs for poorly soluble particles (PSPs)
Chuang, H.-C., Juan, H.-T., Chang, C.-N., Yan, Y.-H., Yuan, T.-H., Wang, J.-S., Chen, H.-C., Hwang, Y.-H., Lee, C.-H., Cheng, T.-J.	Cardiopulmonary toxicity of pulmonary exposure to occupationally relevant zinc oxide nanoparticles	2014	Nanotoxicology	No	no OELs
Kreider, M.L., Cyrs, W.D., Tosiano, M.A., Panko, J.M.	Evaluation of Quantitative Exposure Assessment Method for Nanomaterials in Mixed Dust Environments: Application in Tire Manufacturing Facilities	2014	Annals of Occupational Hygiene	No	no OELs
Kim, B., Lee, J.S., Choi, B.-S., Park, S.-Y., Yoon, J.-H., Kim, H.	Ultrafine particle characteristics in a rubber manufacturing factory	2013	Annals of Occupational Hygiene	No	no OELs

Authors	Title	Year	Source title	Selected	Motivation
Vandebriel, R.J., De Jong, W.H.	A review of mammalian toxicity of ZnO nanoparticles	2012	Nanotechnology, Science and Applications	No	no OELs
Ho, M., Wu, K.-Y., Chein, H.-M., Chen, L.-C., Cheng, T.-J.	Pulmonary toxicity of inhaled nanoscale and fine zinc oxide particles: Mass and surface area as an exposure metric	2011	Inhalation Toxicology	No	no OELs
Pauluhn, J.	Poorly soluble particulates: Searching for a unifying denominator of nanoparticles and fine particles for DNEL estimation	2011	Toxicology	No	no OELs
Miller, A., Drake, P.L., Hintz, P., Habjan, M.	Characterizing exposures to airborne metals and nanoparticle emissions in a refinery.	2010	The Annals of occupational hygiene	No	no OELs
Osmond, M.J., McCall, M.J.	Zinc oxide nanoparticles in modern sunscreens: An analysis of potential exposure and hazard	2010	Nanotoxicology	No	no OELs
Sager, T.M., Castranova, V.	Surface area of particle administered versus mass in determining the pulmonary toxicity of ultrafine and fine carbon black: Comparison to ultrafine titanium dioxide	2009	Particle and Fibre Toxicology	No	no OELs
Kuhlbusch, T.A.J., Neumann, S., Fissan, H.	Number size distribution, mass concentration, and particle composition of PM1 PM2.5, and PM10 in bag filling areas of carbon black production	2004	Journal of Occupational and Environmental Hygiene	No	no OELs

1.2 Search terms Toxline/Medline

After completing the searches in Scopus, also a search in Toxline/Medline was performed using following (simplified) search string:
(zno OR "zinc oxide" OR coo OR "cobaltous oxide" OR "cobalt oxide" OR "ferric oxide" OR fe2o3 OR "ferrosoferric oxide" OR fe3o4 OR "triiron tettraoxide" OR "iron oxide" OR "ferrous oxide" OR feo OR lead OR pb OR lanthanum OR sb2o5 OR "antimony pentoxide" OR "diantimony pentoxide" OR "antimony oxide" OR sno2 OR "cassiterite" OR "tin oxide" OR "carbon black") AND (oel OR oels OR wel OR wels OR tlv OR tlvs OR "threshold limit value" OR "threshold limit values" OR "occupational consideration" OR "occupational considerations" OR "exposure limit" OR "exposure limits" OR "occupationally relevant dose" OR "occupationally relevant doses" OR "maximum acceptable concentration" OR "maximum acceptable concentrations" OR "number concentration" OR "number concentrations" OR "number value" OR "number values" OR nrv OR NRVs OR "maximum allowable concentration" OR "maximum allowable concentrations" OR "threshold limit value") AND (nano**)

Toxline results

Search	Database	Query	Time	Result
# 5	toxline	#1 #2	09:25:31	9
# 2	toxline	nano	09:23:59	31204
# 1	toxline	((zno OR "zinc oxide" OR coo OR "cobaltous oxide" OR "cobalt oxide" OR "ferric (oxide" OR 16833-27-5 [rn]) OR fe2o3 OR "ferrosoferric oxide" OR fe3o4 OR "triiron tettraoxide" OR "iron oxide" OR "ferrous oxide" OR feo OR (lead OR "olow polish " OR "lead s2" OR "lead flake" OR "ks 4" OR 7439-92-1 [rn]) OR (pb OR "piperonyl butoxide" OR "nia 5273" OR "fmc 5273" OR butoxide OR butocide OR butacide OR 51-03-6 [rn]) OR (lanthanum OR "unii 6i3k30563s" OR "ec 231 099 0" OR 7439-91-0 [rn]) OR sb2o5 OR "antimony pentoxide" OR "diantimony pentoxide" OR "antimony oxide" OR sno2 OR "cassiterite" OR "tin oxide" OR "carbon black") AND (oel OR oels OR wel OR wels OR tlv OR tlvs OR "threshold limit value" OR "threshold limit values" OR "occupational consideration" OR "occupational considerations" OR "exposure limit" OR "exposure limits" OR "occupationally relevant dose" OR "occupationally relevant doses" OR "maximum acceptable concentration" OR "maximum acceptable concentrations" OR "number concentration" OR "number concentrations" OR "number value" OR "number values" OR nrv OR NRVs OR "maximum allowable concentration" OR "maximum allowable concentrations" OR "threshold limit value"))	09:19:50	1346

None of the nine retrieved papers was relevant, if not already retrieved by the Scopus search.

1.3 List of MNMs in OECD testing programme

(downloaded from www.oecd.org on 27-10-2016)

Nanomaterials
Cerium oxide
Dendrimers
Fullerenes (C60)
Gold nanoparticles
Multi-walled carbon nanotubes (MWCNTs)
Nanoclays
Silicon dioxide
Silver nanoparticles
Single-walled carbon nanotubes (SWCNTs)
Titanium dioxide (NM100-NM105)
Zinc oxide

1.4 List of exemplary MNMs from SER NRV publication, NOT in OECD programme

Nanomaterials
CoO
Fe
Fe_xO_y
La
Pb
Sb_2O_5
SnO_2
carbon black
polystyrene nanofibres

1.5 Mass to number conversion based on size distribution assuming spherical particles

Often, the concentration of nanoparticles in the air is composed of agglomerates or even aggregates of primary particles, instead of solely primary particles. The measured mass concentration will reflect the entire distribution of these primary particles, agglomerates and aggregates within the measured particle size range. Therefore, it could be argued that the entire size distribution should be taken into account when calculating number concentrations. Many respiratory studies provide descriptors of the size distributions of the particles dispersed into air, mostly Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD). Therefore, in the current report number concentrations were also calculated based on the size distribution of the nanoparticles concerned.

The basic assumption for these calculations is that particle size of particles dispersed into air is log-normally distributed. When this assumption can be reasonably made the following formula describes the relationship between number (C_N) and mass (C_m) concentrations, according to Hinds (1999) (equation 4.20 on page 83):

$$C_m = C_N \frac{\rho \pi}{6} d_{\bar{m}}^3 \quad \text{....(6.1.1)}$$

in which ρ is the density of the particle and $d_{\bar{m}}$ the diameter of the average mass. Rewriting of equation (1) gives:

$$C_N = C_m \frac{6}{\rho \pi d_{\bar{m}}^3} \quad \text{....(6.1.2)}$$

Equation 4.53 on page 99 of Hinds (1999) gives the relation between $d_{\bar{m}}$ and the Count Median Diameter (CMD):

$$d_{\bar{m}} = CMD\, e^{1.5\, ln^2\, \sigma_g} \quad \text{....(6.1.3)}$$

In which σ_g is the Geometric Standard Deviation (GSD). Substituting equation (3) into equation (2) yields:

$$C_N = C_m \frac{6}{\rho \pi CMD^3 e^{4.5\, ln^2\, \sigma_g}} \quad \text{....(6.1.4)}$$

Since most often in respiratory toxicity studies, particle size distribution is described by the Mass Median Aerodynamic Diameter (MMAD), CMD needs to be converted into MMAD. Since Hinds (1999) only provides a formula for conversion of CMD to mass median diameter (MMD), first the MMD is written into formula (4) using equation 4.49 (page 98): $MMD = CMD\, e^{3\, ln^2\, \sigma_g}$, which can be rewritten as:

$$CMD = MMD\, e^{-3\, ln^2\, \sigma_g} \quad \text{....(6.1.5)}$$

Substituting equation (5) into equation (4) yields:

$$C_N = C_m \frac{6}{\rho \pi MMD^3 e^{-4.5\, ln^2\, \sigma_g}} \quad \text{....(6.1.6)}$$

According to Hinds (1999), the aerodynamic diameter d_a is defined as the diameter of the spherical particle with a density of 1 g/cm^3 that has the same setting velocity (V_{TS}) as the particle. This is expressed in the following equation (3.26 on page 53):

$$V_{TS} = \frac{\rho \pi d_e^2 g}{18 \eta \chi} = \frac{\rho_0 \pi d_a^2 g}{18 \eta} \quad \text{....(6.1.7),}$$

in which d_e is the equivalent volume diameter (that is the diameter of the sphere having the same volume as the irregular particle), ρ_0 unit density (1 g/cm^3), g the acceleration constant of gravity, η viscosity and χ the slip correction factor, which is a unitless number and equal to 1 for spheres. Thus, assuming the dispersed particles to be spheres, the following relation can be derived between "true" particle diameter d and d_a: $\rho\, d^2 = \rho_0\, d_a^2$, which can be rewritten as:

$$d = d_a \sqrt{\frac{\rho_0}{\rho}} \text{ or } MMD = MMAD \sqrt{\frac{\rho_0}{\rho}} \quad \text{....(6.1.8)}$$

Substituting equation (8) into equation (6) yields:

$$C_N = C_m \frac{6}{\rho\, \pi\, MMAD^3 \left(\sqrt{\frac{\rho_0}{\rho}}\right)^3 e^{-4.5\, ln^2\, \sigma_g}} \quad \text{....(6.1.9)}$$

When C_N is expressed in number/cm^3, C_m in µg/m^3, density in g/cm^3 and MMAD in nm, equation (9) converts into:

$$C_N = 10^9 C_m \frac{6}{\rho\, \pi\, MMAD^3 \left(\sqrt{\frac{\rho_0}{\rho}}\right)^3 e^{-4.5\, ln^2\, \sigma_g}} \quad \text{....(6.1.10)}$$

Equation (10) is used to convert exposure limit values expressed in µg/m^3 into number concentrations in particles/cm^3, assuming the dispersed particles are spherical.
When CMD (equal to the geometric mean diameter, see Hinds (1999), page 102) has been measured instead of MMAD, the following adaptation of equation (6) is used to correct for the units used (C_N in number/cm^3, C_m in µg/m^3, density in g/cm^3 and CMD in nm):

$$C_N = 10^9 C_m \frac{6}{\rho\, \pi\, CMD^3 e^{4.5\, ln^2\, \sigma_g}} \quad \text{....(6.1.11)}$$

Sung et al. (2008) exposed rats to nanosilver and measured the particle size distributions, the particle concentrations and the mass concentrations of the nanosilver particles dispersed into the inhaled air. These data were used to confirm the validity of formula (11). The calculated values are somewhat higher than the measured ones, but in the same order of magnitude, being approximately twice as high as the measured values (see Table 9).

Table 9 Comparison of measured number concentrations and calculated number concentrations of nanosilver in the experiments conducted by Sung et al. (2008)

Group	Diameter-Ag (nm)		Number (particles/cm^3) Measured	Mass-Ag (µg/m^3)	Number (particles/cm^3) Calculated with formula (4)
	GM	GSD			
Low	18.12	1.42	6.64E+05	48.94	8.6E+05
Middle	18.33	1.12	1.43E+06	133.19	3.7E+06
High	18.93	1.59	2.85E+06	514.78	5.2E+06

It should be noted that in these calculations the number of particles is inversely proportional to $\sqrt[3]{density^2}$. When in these calculations, the density of the chemical compound is used instead of the density of the particles dispersed into the air, which may overestimate the particle density, and the particle density would be a factor ten lower, the real particle number diameter would be approximately 5 times higher. In the tables presenting calculated particles numbers based on chemical compound density instead of particle density are marked with an asterisk.

1.6 Mass to number conversion for fibres

Two different approaches were used to calculate fibre weight for carbon nanotubes, depending on the available data. In both cases the fibres were assumed to be cylindrical in shape. When data on the specific surface are were available in combination with data on fibre dimensions, the following formula was applied:

$$M_{CNT} = 10^{-12} \times \frac{\pi\ \mathrm{d}\ \mathrm{L}}{\mathrm{SSA}} \quad \text{....(6.2.1)}$$

in which W = fibre weight (µg/fibre), SSA = specific surface area in m^2/g, d = fibre diameter (nm), L = fibre length in nm. The mass concentration expressed in µg/m^3 was then divided by M_{CNT} and multiplied with 10^{-6} (m^3/cm^3) to obtain the number of (nano)fibres/cm^3, leading to the following overall formula:

$$C_N = 10^6 C_m \frac{SSA}{\pi\ d\ L} \quad \text{....(6.2.2)}$$

When no data on SSA were available, the density of the fibre was calculated using the following formula adapted from Laurent et al. (2010):

$\rho_{CNT} = \frac{4000}{1315}[n/d_{out} - (0.34\, n(n-1)/d_{out}^2)]$ ….(6.2.3)
in which ρ_{CNT} is the density of the CNT fibre in g/cm^3, n the number walls of the CNT and d_{out} the outer diameter in nm. The mass of one fibre (in µg) was then calculated by applying the formula $M_{CNT} = 2.5\ 10^{-16}\ \rho_{CNT}\ \pi\ d_{out}^2\ \mathrm{L}$. The mass concentration expressed in µg/m^3 was then divided by M_{CNT} and multiplied with 10^{-6} (m^3/cm^3) to obtain the number of (nano)particles/cm^3, leading to the following overall formula:
$C_N = 1.315\ 10^9 C_m/\{[n\ d_{out} - (0.34\ n\ (n-1)]\ \pi\ \mathrm{L}\}$ ….(6.2.4)
The result of the fibre mass calculations for two types of CNTs are presented in Table 10.

Table 10 Mass calculations for two types of CNTs

Nanocyl NC7000		
SSA m^2/g	250 - 300	Data from technical datasheet (Nanocyl, 2016)
d(MWCNT) nm	9.5	
L nm	1500	
Mass fibre (µg)	1.5 - 1.8 10^{-10}	Calculated
SWCNT (A)	Description (from Nakanishi et al., 2015) : bundles 0.19 µm (1.6)-0.21 µm (1.7) X 0.66 µm (1.6)-0.69 µm (1.7) Primary Particle Size (SD) 2.8 nm (1.5)	
density SWCNT g/cm^3	1.09	Calculated with formula from Laurent et al. (2010)
L SWCNT nm	690	Highest numbers selected for calculation
d SWCNT nm	2.8	
d SWCNT bundle nm	210	
n SWCNT/bundle	75	Calculated by dividing bundle diameter by SWCNT fibre diameter
Mass SWCNT (µg)	4.6 10^{-12}	
Mass SWCNT bundle (µg)	3.5 10^{-10}	

1.7 Benchmark dose models of MWCNT toxicity data by NIOSH (2013)

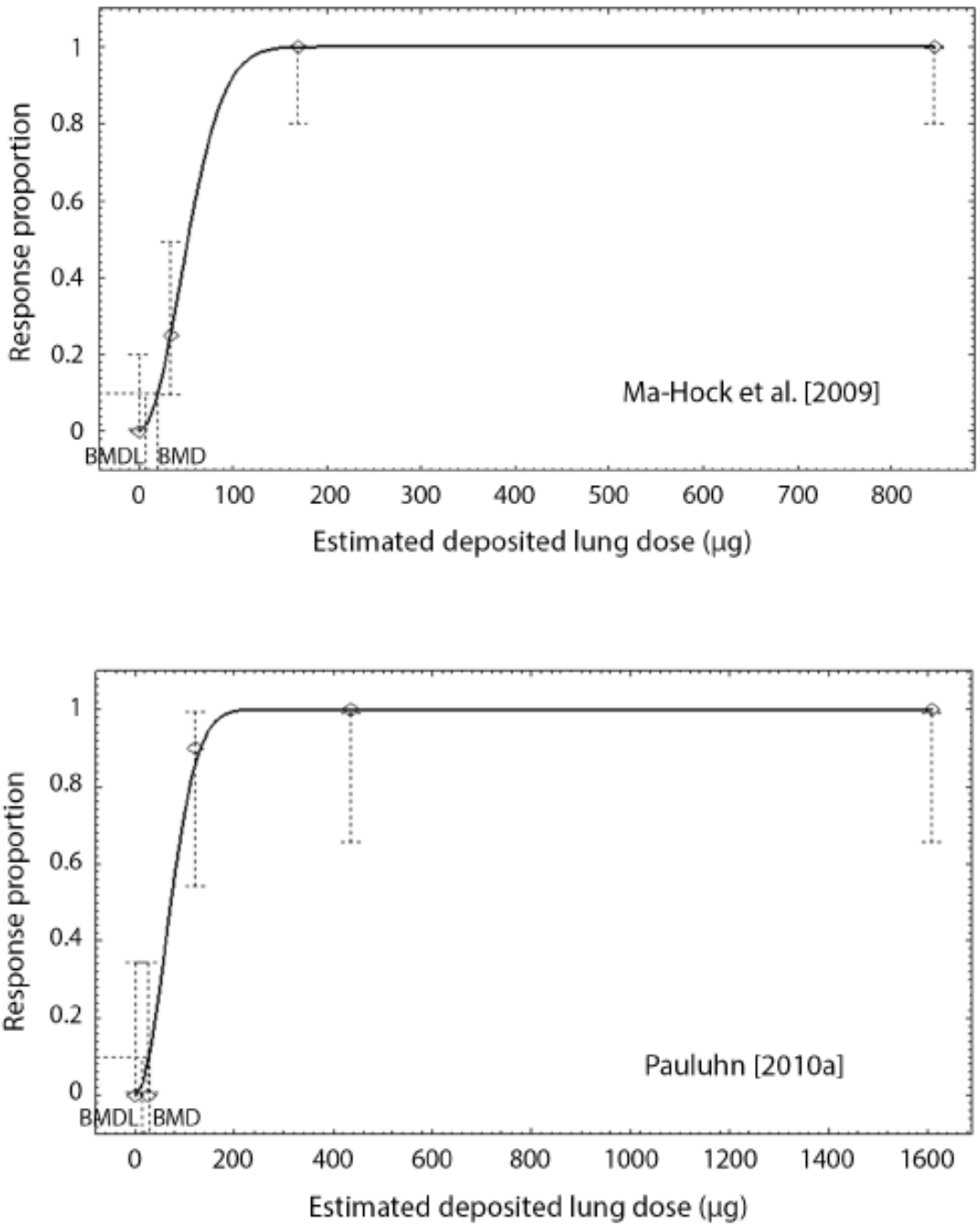


Figure A–1. Benchmark dose model (multistage, polynomial degree 2) fit to rodent dose-response data from the two subchronic inhalation studies of MWCNT in rats: Ma-Hock et al. [2009], response: granulomatous inflammation, Pauluhn [2010a], response: alveolar septal thickening, minimal or greater. *P* values are 0.99 for Ma-Hock et al. [2009] and 0.88 for Pauluhn [2010a].

1.8 Scientific basis for the specific OELs

Nanomaterials and specifications	Source reference	Limit value (µg/m³)	Point of departure			Extra-polation/ assess-ment factor	Study type - species	Critical effects	Size distribution (value (nm) (type) ± GSD)	Study reference(s)
			Type	Value	Unit					
Proposals by governmental institutions										
All carbon nanotubes and nanofibres	NIOSH (2013)	< 1	BMD_{10}	24	µg/lung	n/a	90-day inhalation - rat	slight/mild lung effects (granulomatous inflammation)	500-1,300 (MMAD) ± 3.1-5.4[16]	Ma-Hock et al. (2009)
			BMD_{10}	150	µg/lung	n/a	90-day inhalation - rat	slight/mild lung effects (alveolar septal thickening)	1,700-3,400 (MMAD) ± 1.7-2.1[17]	Pauluhn (2010)
TiO_2 ultrafine	NIOSH (2011)	300	$BMDL_{0.1}$[b]	0.18	mg/g lung	n/a[e]	2 year inhalation carcinogenicity study - rat	Lung tumours	800 (MMAD) ± 1.8	Heinrich et al. (1995), Lee et al. (1985), Muhle et al. (1991)
Proposals from peer-reviewed scientific literature										
Carbon nanotube group, SWCNT, DWCNT, MWCNT	Nakanishi et al. (2015)	30	NOAEC[a]	130	µg/m³	4	28-d inhalation	no adverse effects observed	no size distribution (see Table 10 page 41 for dimensions)	
Multi-walled carbon nanotubes, MWCNT 10 nm Nanocyl NC 7000	Aschberger et al. (2011)	1	LOAEC	100	µg/m³	100	90-day inhalation - rat	slight/mild lung effects (granulomatous inflammation)	500-1,300 (MMAD) ± 3.1-5.4[16]	Ma-Hock et al. (2009)
Multi-walled carbon nanotubes, MWCNT 10 nm Nanocyl NC 7000	Luizi (2009)	2.5		100	µg/m³	40	90-day inhalation - rat	slight/mild lung effects (granulomatous inflammation)	500-1,300 (MMAD) ± 3.1-5.4[16]	Ma-Hock et al. (2009)
Multi-walled carbon nanotubes, MWCNT	Stone et al. (2009)	0.67	LOAEC	300	µg/m³	450	14-day inhalation - mouse	systemic immune effects	350–400 (CMD) ± 2.0	Mitchell et al. (2007)
nanogold	Katsnelson et al. (2015)	200	bulk OEL	3000	µg/m³	15	n/a	not provided	not provided	not provided

[16] Other parameters used in fibre number calculations: Nanotube diameters of 5–15 nm and length 0.1–10 µm, SSA = 250-300 m^2/g.
[17] Lowest lower level and highest upper level of two measurement methods (cascade impactor and TSI APS 3321 (laser velocimetry))

Nanomaterials and specifications	Source reference	Limit value (µg/m³)	Point of departure			Extra-polation/ assess-ment factor	Study type - species	Critical effects	Size distribution (value (nm) (type) ± GSD)	Study reference(s)
			Type	Value	Unit					
Nano Ag	Aschberger et al. (2011)	0.33	LOAEC	49	µg/m³	150	90-day inhalation - rat	reduced lung function (inflammatory response and alterations in the lung function)	18-19 (CMD) ± 1.1-1.6	Sung et al. (2009)
Nano Ag	Aschberger et al. (2011)	0.67	NOAEC	133	µg/m³	200	90-day inhalation - rat	liver effects: bile duct hyperplasia	18-19 (CMD) ± 1.1-1.6	Sung et al. (2009)
Nano Ag	Stone et al. (2009)	0.33	LOAEC	49	µg/m³	150	90-day inhalation - rat	reduced lung function (inflammatory response and alterations in the lung function)	18-19 (CMD) ± 1.1-1.6	Sung et al. (2009)
Nano Ag	Stone et al. (2009)	0.67	NOAEC	133	µg/m³	200	90-day inhalation - rat	liver effects: bile duct hyperplasia	18-19 (CMD) ± 1.1-1.6	Sung et al. (2009)
Nano Ag	Swidwinska et al. (2015)	10	not clear: only abstract in English, paper in Polish and abstract does not specify the scientific basis for this value							
Nanosilver	Katsnelson et al. (2015)	100	bulk OEL	1500	µg/m³	15	n/a	not provided	not provided	not provided
Nano Ag	Stone et al. (2009)	0.098	LOAEC	49	µg/m³	500	90-day inhalation - rat	reduced lung function (inflammatory response and alterations in the lung function)	18-19 (CMD) ± 1.1-1.6	Sung et al. (2009)
Carbon black, CB ultrafine	Kuempel et al. (2006)	120	$BMDL_{0.1}$**	0.19	mg/g lung	n/a	2 year inhalation carcinogenicity study - rat	lung tumours	1,950 (MMAD) ± 1.84	Heinrich et al. (1995), Muhle et al. (1991), Nikula et al. (1995)
Carbon black, CB ultrafine	Kuempel et al. (2006)	240	$BMDL_{0.1}$**	0.19	mg/g lung	n/a	2 year inhalation carcinogenicity study - rat	lung tumours	1,950 (MMAD) ± 1.84	Heinrich et al. (1995), Muhle et al. (1991), Nikula et al. (1995)
Fullerenes, C60	Shinohara (2011)	390	NOAEC	3100	µg/m³	8	instillation test - rat	increased neutrophils in BALF	96 (CMD) ± 2.0	Sayes et al. (2009)
Fullerenes, C60	Aschberger et al. (2011)	7.4	NOAEC	2220	µg/m³	300	10-day inhalation - rat	not observed (inflammatory responses investigated in BALF)	55 (CMD) ± 1.48	Baker et al. (2008)

Nanomaterials and specifications	Source reference	Limit value (µg/m³)	Point of departure			Extra-polation/ assess-ment factor	Study type - species	Critical effects	Size distribution (value (nm) (type) ± GSD)	Study reference(s)
			Type	Value	Unit					
Multi-walled carbon nanotubes, MWCNT 140 nm Baytubes ®	Aschberger et al. (2011)	2	NOAEC	100	µg/m³	50	90-day inhalation - rat	slight/mild lung effects (alveolar septal thickening)	1,700-3,400 (MMAD) ± 1.7-2.1[18]	Pauluhn (2010)
Multi-walled carbon nanotubes, MWCNT Baytubes ®	Pauluhn (2010)	50	NOAEC	100	µg/m³	2	90-day inhalation - rat	slight/mild lung effects (alveolar septal thickening)	1,700-3,400 (MMAD) ± 1.7-2.1[19]	Pauluhn (2010)
copper oxide	Katsnelson et al. (2015)	50	bulk OEL	750	µg/m³	15	n/a	not provided	not provided	not provided
iron oxide	Katsnelson et al. (2015)	400	Russian bulk MAC	6000	µg/m³	15	n/a	not provided	not provided	not provided
Amorphous silica, SiO_2	Stockmann-Juvala et al. (2014)	300	NOAEC	1300	µg/m³	4.3	90-day inhalation - rat	local lung effects: progressive epithelial and fibroproliferative changes	not measured	Reuzel et al. (1991)
High surface reactivity anatase-rutile nanoscale TiO2	Warheit et al. (2013)	1000	unclear	unclear			bridging inhalation toxicity of ultrafine TiO2 and quartz using instillation studies, however quantitative method not specified			
Low surface reactivity nanoscale TiO_2	Warheit et al. (2013)	2000	unclear	unclear			bridging inhalation toxicity of ultrafine TiO2 and quartz using instillation studies, however quantitative method not specified			
Pigment-grade TiO_2 particle types	Warheit et al. (2013)	5000	unclear	unclear			bridging inhalation toxicity of ultrafine TiO2 and quartz using instillation studies, however quantitative method not specified			
TiO_2	Aschberger et al. (2011)	17	NOAEC	500	µg/m³	30	90-day inhalation - rat	local lung effects: progressive epithelial and fibroproliferative changes and inflammatory responses	1,440 (MMAD) ± 2.6	Bermudez et al. (2004)

[18] Lowest lower level and highest upper level of two measurement methods (cascade impactor and TSI APS 3321 (laser velocimetry))
[19] Lowest lower level and highest upper level of two measurement methods (cascade impactor and TSI APS 3321 (laser velocimetry))

Nanomaterials and specifications	Source reference	Limit value (µg/m³)	Point of departure			Extra-polation/ assess-ment factor	Study type - species	Critical effects	Size distribution (value (nm) (type) ± GSD)	Study reference(s)
			Type	Value	Unit					
TiO_2	Ogura et al. (2011)	610	NOAEC	2000	µg/m³	3.3	90-day inhalation - rat	local lung effects: progressive epithelial and fibroproliferative changes and inflammatory responses	1,440 (MMAD) ± 2.6	Bermudez et al. (2004)
TiO_2	Stockmann-Juvala et al. (2014)	100	NOAEC	500	µg/m³	5	90-day inhalation - rat	local lung effects: inflammatory responses	1,440 (MMAD) ± 2.6	Bermudez et al. (2004)
TiO_2	Swidwinska et al. (2014)	300	not clear: only abstract in English, paper in Polish and abstract does not specify the scientific basis for this value							
TiO_2 ultrafine	Kuempel et al. (2006)	73	$BMDL_{0.1}$[b]	0.18	mg/g lung	n/a[c]	2 year inhalation carcinogenicity study - rat	Lung tumours	800 (MMAD) ± 1.8	Heinrich et al. (1995), Lee et al. (1985), Muhle et al. (1991)
TiO_2 ultrafine	Kuempel et al. (2006)	140	$BMDL_{0.1}$[b]	0.18	mg/g lung	n/a[d]	2 year inhalation carcinogenicity study - rat	Lung tumours	800 (MMAD) ± 1.8	Heinrich et al. (1995), Lee et al. (1985), Muhle et al. (1991)

[a] Group assessment using a biaxial approach based inhalation and instillation studies with extrapolation from instillation to inhalation based inflammation parameters. The relative neutrophil count in BALF one month after instillation of 1 mg/kg CNT was used as an indicator: this is the ratio of increase rate BALF neutrophil count of CNT to that of SWCNT (A). Based on this comparison SWCNT (A) selected as representative of the group.

[b] BMDL at 10% excess risk of lung cancer, with linear extrapolation to 0.1% excess risk

[c] Using the Interstitial/sequestration model to extrapolate mass retained in lungs to air concentrations

[d] Using the MPPD model to extrapolate mass retained in lungs to air concentrations

[e] Based on averaged BMD models, using MPPD model to extrapolate mass retained in lungs to air concentrations

1.9 Summary of lung and liver effects of nanosilver reported by Sung et al. (2009, 2008)

In a 90-day whole body inhalation study rats were exposed 6 h day/ 5 days a week to nano-silver (18–19 nm), at low (49 µg/m^3, equivalent to 0.6 × 10^6 particles/cm^3 and 1.08 × 10^9 nm^2/cm^3), medium (133 µg/m^3, equivalent to, 1.4 × 10^6 particles/cm^3 and 2.39 × 10^9 nm^2/cm^3) and high (515 µg/m^3, equivalent to 3.0 × 10^6 particles/cm^3 and 6.78 × 10^9 nm^2/cm^3) concentrations (Sung et al., 2008; Sung et al., 2009). The main targets of accumulation and toxicity were the lungs and liver. In the liver, minimal bile-duct hyperplasia was identified in 0/10, 0/10, 1/10, and 4/9 of the control, low, middle, and high-dose males, respectively. The higher incidence of bile-duct hyperplasia in the high-dose males suggests a minimal test article–related effect at high dose. Minimal bile-duct hyperplasia was also present in 3/10, 2/10, 4/10, and 8/10 of the control, low, middle, and high-dose females, respectively. Single-cell hepatocellular necrosis, characterized by increased cellular eosinophilia and shrunken condensed nuclei, was noted in 3/10 of the high-dose females. Animals exhibited lung inflammation at the highest dose, but in a minimal grade (see Table 9). The lung function markers tidal volume, minute volume and peak inflammatory flow were measured at seven day intervals (Sung et al., 2008). Peak inflammatory flow was not clearly influenced by nanosilver exposure, while minute volume was reduced in high dose males and all exposed females, more or less in a dose-related manner. Tidal volume increase over time was reduced in all exposed males, more or less in a dose related manner, while in females it was only reduced at mid and high dose, but not in a dose related manner. At high dose, if any, the reductions were around 20% in size. In view of the considerable variation in these parameters as demonstrated in this study, it is not clear whether this size of effect should already be considered adverse.

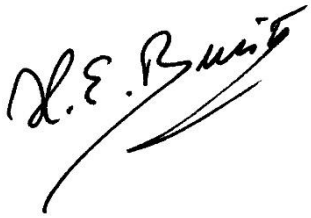
Table 11 Lung histopathology after subchronic inhalation in rats (Sung et al., 2009)

Histopathology	**Nanosilver concentration (µg/m^3)**			
	0	**49**	**133**	**515**
Males	% (n=10)	% (n=10)	% (n=10)	% (n=9)
No microscopic findings	50	30	30	0
Abnormality	50	70	70	100
Accumulation Macrophage, alveolar Minimum	30	50	50	88.9
Inflammation, Chronic, alveolar Minimum	20	30	20	88.9**
Infiltrate Mixed cell perivascular Minimum	30	40	60	77.8
Haemorrhage Alveolar Minimum	10	0	0	0
Osseous foreign body	0	0	0	11.1
Hyperplasia level I Respiratory epithelium	0	0	0	11.1
Females	% (n=10)	% (n=10)	% (n=10)	% (n=10)
Lungs No microscopic findings	30	50	60	20
Abnormality	70	50	40	80
Accumulation Macrophage, alveolar Minimum	70	40	40	60
Inflammation, Chronic, alveolar Minimum	30	20	0	80**
Infiltrate, Mixed cell perivascular Minimum	0	0	10	70**

**p < 0.01, compared with control

7 Signature

Zeist, 29 June 2017 TNO



M.A.J. Rennen
Research Manager RAPID

H.E. Buist
Author