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Scientific basis for setting a health-based occupational exposure limit

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NFA-report

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FOREWORD

In 2015, the Danish Working Environment Council made 22 recommendations to promote safe handling of nanomaterials (NMs) in the working environment, which were enforced by the Minister of Employment. One of these recommendations was 'That the Danish Working Environment Authority in cooperation with relevant scientific experts assesses whether adequate scientific documentation can be provided to use the scientific quality committee for an assessment of the scientific evidence to determine limit values for specific NMs in the work environment.' (https://www.amr.dk/nano.aspx).

On this background, The Danish Working Environment Authority asked NFAto review the scientific evidence with the aim of clarifying the possibilities for suggesting nanospecific occupational exposure limits (OELs) for three different NMs (titanium dioxide (TiO_2), carbon black and carbon nanotubes (CNT)).

The purpose of the present report is to suggest a health-based OEL for nanosized TiO_2.

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EXECUTIVE SUMMARY

In this report, a working group at the National Research Centre for the Working Environment reviews data relevant to assessing the hazard of TiO_2 nanomaterials (TiO_2 NMs), i.e. human studies (Chapter 2), toxicokinetics (Chapter 3), animal studies (Chapter 4), mechanisms of toxicity (Chapter 5), previous risk assessments of TiO_2 NMs (Chapter 6), scientific basis for setting an occupational exposure limit (OEL) (Capter 7) and finally we summarize and suggest a health based OEL for TiO_2 NM (Chapter 8). The focus of this report is only occupational exposure by inhalation.

The present working group evaluated the relevant literature on TiO_2 NM from both epidemiological and animal inhalation studies. None of the identified epidemiological studies provided information on the particle size range of the TiO_2, thus making it impossible to determine whether the exposures included TiO_2 NM. Therefore it was decided to base the suggested health-based OEL on data from experimental animal studies.

Pulmonary inflammation and carcinogenicity was observed in inhalation studies in rats. The present working group regards inflammation and carcinogenicity as the critical adverse effects and the subsequent risk assessments are conducted based on studies reporting these effects. TiO_2 NM induced cardiovascular effects were identified in animal studies. However, none of these studies were sub-chronic or chronic inhalation studies and therefore not suitable for OEL derivation. However, the present working group regards the acute phase response as a biomarker of cardiovascular effects. Due to the close association between pulmonary inflammation and the acute phase response, the present working group regards inflammation as a proxy for cardiovascular effects mediated by the acute phase response.

The present working group found strong dose response relationships for neutrophil influx as a marker of pulmonary inflammation. Neutrophil influx was related to deposited surface area. The working group considers inflammation as a threshold effect.

The present working group found that the mechanism of action of the carcinogenic effect has not been fully clarified. Secondary genotoxicity due to particle-induced inflammation is an important and well documented mechanism of action for the development of lung cancer. However, the available data did not allow ruling out that TiO_2 NM could also induce cancer through a direct genotoxic mechanism. Therefore, the present working group considers carcinogenicity as a non-threshold effect. Consequently, the present working group decided to perform the risk assessment based on both a threshold effect for inflammation and a non-threshold effect for cancer.

For an OEL based on threshold effects, the following traditional approach suggested by REACH is utilized: 1) identification of critical effect, 2) identification of the no observed adverse effect concentration (NOAEC), 3) calculation of OEL using assessment factors adjusting for inter and intra species differences. For non-threshold effects, the present working group uses two approaches. The first method uses the measured lung burden in rats exposed by inhalation and the alveolar surface area of rats and humans to estimate

the human equivalent lung burden. The second method, suggested by The European Chemicals Agency (ECHA), uses air concentrations directly.

The working group considered that data from two rat inhalation studies as the best basis for the risk assessment. The following studies were selected to be used for calculation of the derived-no-effect level (DNEL) and dose-response for excess cancer risk, respectively: A 13 week sub-chronic inhalation study in rats (0, 0.5, 2.0 and 10 mg/m^3) and a 2 year chronic cancer inhalation study in rats (0 and 10 mg/m^3). The table below shows a DNEL for pulmonary inflammation derived based on the sub-chronic inhalation study of rats, and extra lung cancer risk at 1 in 1 000, 1 in 10 000 and 1 in 100 000 derived using two different approaches.

Overview of DNEL based on a threshold based mechanism of action and exposure levels resulting in extra cancer risk levels at 1:1000, 1:10 000 and 1: 100 000 based on a non-threshold based mechanism of action.

		Suggestion of an OEL for TiO_2 NM		
Mechanism of action		**Inflammation**	**Lung cancer (method I)**	**Lung cancer (method II)**
Threshold based	DNEL	10 μg/m^3		
Non-treshold based	Extra cancer risk			
	1:1000		4 μg/m^3	47 μg/m^3
	1:10 000		0.4 μg/m^3	4.7 μg/m^3
	1:100 000		0.04 μg/m^3	0.47 μg/m^3

Both studies used for the risk assessment used P25 TiO_2 NM (15-40 nm diameter, 80% anatase/20% rutile). TiO_2 NMs differ regarding size and surface area but also coating, shape, crystal structure etc. The present working group notes that there is limited available data on the biological effects of different physico-chemical properties, but the present working group concludes that the majority of available data support that the surface area (and therefore the size) of TiO_2 is a critical driver of particle-induced inflammation and the acute phase response in the lungs. In support of this notion, The National Institute for Occupational Safety and Health (NIOSH) showed that the deposited surface area of TiO_2 particles of different sizes (fine and ultrafine) and different crystal structure (80% anatase/20% rutile and 99% rutile) can explain the observed variation in TiO_2 particle-induced pulmonary inflammation and lung cancer in rat inhalation studies. This stresses the importance of the surface area as a predictor for the inflammatory and carcinogenic response.

The present working group regards cancer as the most critical effect. The DNEL approach relies heavily on the assumption of a threshold effect on inflammation and carcinogenicity. The present working group is of the opinion that there is still uncertainly whether this is the case for TiO_2 NM–induced carcinogenicity.

Two different approaches were used for calculating excess lung cancer risk based on the same chronic inhalation study in rats. In the first approach, lung burden was used to

estimate the exposure levels. In the second approach, air concentrations were used directly. Independently of the applied method for risk assessment, the resulting OEL suggestions were all very low. These levels are all more than 100-fold lower than the current Danish OEL for titanium of 6 mg/m^3 (measured as Ti, corresponding to 10 mg/m^3 TiO_2).

The present working group recommends the risk assessment approach estimating the excess lung cancer risk based on lung burden, since this approach takes the retained pulmonary dose into account. Thus, the expected excess lung cancer risk in relation to occupational exposure to TiO_2 NMs is 1:1 000 at 4 μg/m^3, 1:10 000 at 0.4 μg/m^3 and 1:100 000 at 0.04 μg/m^3 TiO_2 NM.

DANSK SAMMENFATNING

I denne rapport vurderer en arbejdsgruppe ved Det Nationale Forskningscenter for Arbejdsmiljø data, der er relevante for at vurdere faren ved udsættelse for titanium dioxid nanomaterialer (TiO_2 NM), dvs. humane studier (kapitel 2), toksikokinetik (kapitel 3), dyreforsøg (kapitel 4), toksicitetsmekanismer (kapitel 5), tidligere risikovurderinger af TiO_2 NM (kapitel 6), det videnskabelige grundlag for fastlæggelse af en grænseværdi (kapitel 7) og endelig opsummeres og foreslås en helbredsbaseret grænseværdi for TiO_2 NM i arbejdsmiljøet (kapitel 8). Fokus i denne rapport er alene på erhvervsmæssig eksponering ved indånding.

Den nærværende arbejdsgruppe evaluerede den relevante litteratur om TiO_2 NM fra både epidemiologiske undersøgelser og inhalationsforsøg med dyr. Ingen af de identificerede epidemiologiske undersøgelser indeholdt oplysninger om TiO_2's partikelstørrelse, hvilket gør det umuligt at afgøre, om eksponeringerne omfattede TiO_2 NM. Derfor blev det besluttet at basere den foreslåede sundhedsbaserede grænseværdi i arbejdsmiljøet på data fra studier på forsøgsdyr.

Der blev observeret lungeinflammation og lungekræft i inhalationsundersøgelser af rotter. Den nærværende arbejdsgruppe anser inflammation og kræft som de vigtigste skadelige effekter. Derfor baseres de efterfølgende risikovurderinger på undersøgelser, der rapporterer om disse effekter. Der blev identificeret TiO_2 NM-inducerede kardiovaskulære effekter i dyreforsøg. Ingen af disse undersøgelser var imidlertid subkroniske eller kroniske inhalationsundersøgelser og derfor var de ikke egnede til risikovurdering. Den nærværende arbejdsgruppe anser dog akutfaseresponset som en biomarkør for kardiovaskulære effekter. På grund af den stærke sammenhæng mellem lungeinflammation og akutfasesponset betragter den nærværende arbejdsgruppe inflammation som en proxy for hjertekareffekter medieret af akutfaserespons.

Den nærværende arbejdsgruppe fandt stærk dosis-respons-sammenhæng for neutrofilt influx som markør for lungeinflammation. Det samlede lungedeponerede specifikke overfladeareal prædikterede neutrofilt influx. Arbejdsgruppen anser inflammation for at være en tærskeleffekt.

Den nærværende arbejdsgruppe fandt, at virkningsmekanismen for den kræftfremkaldende effekt ikke er blevet fuldstændigt afklaret. Sekundær genotoksicitet forårsaget af partikelinduceret inflammation er en vigtig og veldokumenteret virkningsmekanisme for udvikling af lungekræft. De tilgængelige data tillod dog ikke at udelukke at TiO_2 NM også kunne inducere kræft gennem en direkte genotoksisk mekanisme. Derfor anser den nærværende arbejdsgruppe kræft som ikke-tærskel effekt. Det blev derfor besluttet at udføre risikovurderingen baseret på både en tærskeleffekt for inflammation og en ikke-tærskeleffekt for kræft.

For en grænseværdi i arbejdsmiljøet baseret på tærskeleffekt anvendes følgende traditionelle tilgang, som anbefalet af REACH: 1) identifikation af kritisk effekt, 2) identifikation af NOAEC, og 3) beregning af grænseværdi ved anvendelse af vurderingsfaktorer, der justerer for inter- og intraspecies forskelle. For ikke-

tærskeleffekter anvender den nærværende arbejdsgruppe to metoder. Ved den første metode anvendes den målte lungedeponerede dosis hos rotter til at estimere den tilsvarende eksponering i arbejdsmiljøet. Ved den anden metode anvendes luftkoncentrationer direkte.

Arbejdsgruppen fandt, at data fra to inhalationsundersøgelser i rotter var det bedste grundlag for risikovurderingen. Følgende undersøgelser blev udvalgt til beregning af henholdsvis DNEL og kræftrisiko: En 13-ugers subkronisk inhalationsundersøgelse af rotter (0, 0,5, 2,0 og 10 mg/m^3) og en 2-årig kronisk kræftinhalationsundersøgelse af rotter (0 og 10 mg/m^3). Tabellen nedenfor viser en DNEL for lungeinflammation beregnet på basis af det subkroniske inhalationsstudie af rotter og ekstra lungekræftrisiko hos 1 ud af 1.000, 1 ud af 10.000 og 1 ud af 100.000 beregnet på to forskellige måder.

Oversigt over DNEL baseret på en tærskelbaseret virkningsmekanisme og eksponerings-niveauer, der resulterer i ekstra kræftrisikoniveauer på 1: 1000, 1:10 000 og 1: 100 000 baseret på en ikke-tærskelbaseret virkningsmekanisme.

		Forslag til grænseværdi for TiO_2 NM		
Virkningsmekanisme		**Inflammation**	**Lungekræft (metode I)**	**Lungekræft (metode II)**
Tærskel-baseret	DNEL	10 μg/m^3		
Ikke tærskel-baseret	Ekstra kræftrisiko			
	1:1 000		4 μg/m^3	47 μg/m^3
	1:10 000		0.4 μg/m^3	4.7 μg/m^3
	1:100 000		0.04 μg/m^3	0.47 μg/m^3

Begge undersøgelser, som blev anvendt til risikovurderingen, benyttede P25 TiO_2 NM (15-40 nm diameter, 80% anatase / 20% rutil). TiO_2 NM'er er forskellige med hensyn til størrelse og overflade, men også coating, form, krystalstruktur mv. Den nærværende arbejdsgruppe bemærker, at der er begrænsede tilgængelige data om de biologiske effekter af forskellige fysisk-kemiske egenskaber, men arbejdsgruppen konkluderer, at størstedelen af de tilgængelige data støtter, at overfladearealet (og derfor også størrelsen) af TiO_2 er en prædiktor for partikelinduceret inflammation og akutfaserespons i lungerne. NIOSH har vist, at partikeloverfladearealet af TiO_2-partikler af forskellige størrelser (fin og ultrafin) og forskellige krystalstrukturer (80% anatase/20% rutil og 99% rutil) kan forklare den observerede variation i TiO_2-partikelinduceret lungeinflammation og lungekræft i rotteinhalationsundersøgelser. Dette understreger vigtigheden af overfladearealet som en prædiktor for det inflammatoriske respons og kræftfremkaldende effekt.

Den nærværende arbejdsgruppe betragter kræft som den vigtigste effekt. DNEL-tilgangen er stærkt afhængig af antagelsen om en tærskeleffekt for inflammation og kræft. Den nuværende arbejdsgruppe er af den opfattelse, at der stadig er usikkerhed om, hvorvidt dette er tilfældet for TiO_2 NM induceret kræft.

Der blev anvendt to forskellige metoder til beregning af den overskydende risiko for lungekræft baseret på den samme kroniske inhalationsundersøgelse. Ved den første

metode blev den lungedeponerede dosis brugt til at estimere eksponeringsniveauerne. Ved den anden metode blev luftkoncentrationerne anvendt direkte. Uafhængigt af den anvendte metode til risikovurderingen, er de beregnede forslag til grænseværdier alle meget lave. Disse niveauer er mere end 100 gange lavere end den nuværende danske grænseværdi i arbejdsmiljøet for titanium på 6 mg/m^3 (målt som Ti svarende til 10 mg/m^3 TiO_2).

Den nærværende arbejdsgruppe anbefaler metoden, hvor den overskydende risiko for lungekræft baseres på lungedeponeret dosis, da denne tilgang tager højde for den faktiske lungedeponering. Således er den forventede overskydende lungekræftrisiko i forbindelse med erhvervsmæssig udsættelse for TiO_2 NM 1: 1 000 ved 4 µg/m^3, 1:10 000 ved 0,4 µg/m^3 og 1: 100 000 ved 0,04 µg/m^3 TiO_2 NM.

CONTENTS

ABBREVIATIONS

3-ClTyr	3-chlorotyrosine
3-NOTyr	3-nitrotyrosine
5-OHMeu	5-hydroxymethyl uracil
8-OHdG	8-hydroxy-2-deoxyguanosine
8-OHG	8-hydroxyguanosine
AF	Assessment factor
Apo-A1	Apolipoprotein A1
ApoE	Apolipoprotein E
BAL	Broncho alveolar lavage
BMD	Benchmark dose
BMDL	Benchmark dose lower bound
C3	Complement factor 3
CI	Confidence interval
CNT	Carbon nanotube
CRP	C reactive protein
DNEL	Derived-no-Effect Level
ECHA	European Chemicals Agency
EBC	Exhaled breath condensate
eNOS	Endothelial nitric oxide synthase
HDL	High density lipoprotein
Hs-CRP	High-sensitivity C reactive protein
IARC	The International Agency for Research on Cancer
ICAM-1	Intercellular cell adhesion molecule-1
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IL	Interleukine
INEL	Human indicative no-effect levels
IP	Intraperitoneal
LDL	Low-density lipoproteins
LOAEC	Lowest observed adverse effect concentration
MAD	Malondialdehyde
MMAD	Mass median aerodynamic diameter
MWCNT	Multi-walled carbon nanotube
NIOSH	National Institute for Occupational Safety and Health
NM	Nanomaterial
NO	Nitrogen oxide
NOAEC	No observed adverse effect concentration
NFA	National Research Centre for the Working Environment
OEL	Occupational exposure limit
o-Tyr	o-tyrosine
RAC	ECHA's Committee for Risk Assessment
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
REL	Recommended exposure limit
ROS	Reactive oxygen species
RR	Relative risk
SAA	Serum amyloid A

SCCS	Scientific Committee on Consumer Safety
SMR	Standardized mortality ratio
SOD	Superoxide dismutase
SP-D	Surfactant protein D
TiO_2	Titanium dioxide
TNF	Tumour necrosis factor
TWA	Time-weighted average
VCAM-1	Vascular cell adhesion molecule-1

INTRODUCTION

Titanium dioxide (TiO_2) is a white solid inorganic and poorly soluble compound. TiO_2 in various particle sizes including nanosizes have been used for almost 100 years in a diverse range of industrial and consumer products. TiO_2 is used as white pigment in e.g paints and as food colorant. Traditionally, TiO_2 has been considered a low toxicity particles (Oberdorster et al. 2005). For that reason TiO_2 has previously been used as a negative control particle in many animal studies. However, this view was changed with studies showing lung cancer in rats following chronic exposure to a high dose of fine TiO_2 (Lee et al. 1985) and a lower dose of TiO_2 nanomaterial (TiO_2 NM)(Heinrich et al. 1995). At the same time, toxicological studies showed that smaller TiO_2 particles induced more inflammation than larger TiO_2 particles (reviewed by (Stone et al. 2017)). This observation was followed up by toxicological studies of other types of low solubility particles showing that more inflammation was induced by smaller particles compared to larger particles with the same chemistry (Oberdorster et al. 2005).

The EU has adopted the following definition of a NM "A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm."(European Commission 2017). TiO_2 NMs differ regarding size and surface area but also coating, shape, crystal structure etc. (OECD Environment 2016;NIOSH 2011).

In 2006, the International Agency for Research on Carcinogenicity (IARC) classified TiO_2 as possibly carcinogenic to humans (group 2B). This classification was based on sufficient evidence of carcinogenicity in experimental animals and insufficient evidence in humans. IARC does not differentiate between nano- and fine particles in their classification (IARC 2010).

In 2011, the National Institute for Occupational Safety and Health (NIOSH) recommended that exposure limits for TiO_2 are set based on their size: NIOSH recommends exposure limits (RELs) of 2.4 mg/m^3 for fine TiO_2 and 0.3 mg/m^3 for ultrafine TiO_2. These RELs are estimated to equal lung cancer risk below 1 in 1,000 during working lifetime. Due to the lack of epidemiological studies of TiO_2, NIOSH choses to base the RELs on chronic rat inhalation studies and extrapolation to human risk (NIOSH 2011).

To our knowledge, there are no legally binding NM-specific occupational exposure limits (OELs) for TiO_2. The present Danish OEL for titanium is 6 mg/m^3 (as Ti, corresponding to 10 mg/m^3 for TiO_2), and is regulated by the Danish Working Environment.

The aim of the present report is to investigate if the present knowledge allows for a suggestion of a health-based nanospecific OEL for TiO_2 NM. In this document we review the relevant literature on the adverse effects of TiO_2 NM. The risk assessment methodology of this report will follow the guidelines suggested by REACH (ECHA 2012). First, threshold or non-threshold effects are determined. Threshold effect assumes

that the organism can withstand a certain dose before adverse effects occur, whereas non-threshold effects assume that any exposure to the substance can result in adverse effects. For an OEL based on threshold effects, the following traditional approach is utilized: 1) identification of critical effect, 2) identification of the NOAEC, 3) calculation of OEL using assessment factors adjusting for inter and intra species differences. For non-threshold effects, the present working group will use two different approaches for calculating excess lung cancer risk. In the first approach lung burden will be used to estimate the exposure levels. In the second approach, air concentrations were used directly. Conclusively, the calculated OELs will be compared and lastly, a recommended OEL for TiO_2 NM exposure will be proposed.

HUMAN STUDIES

Human exposure

There are limited data on occupational exposure TiO_2 NM. A review of reported exposure to different types of engineered NMs included 29 exposure scenarios of TiO_2 NM exposure. TiO_2 NM exposure occurred in research laboratories, industrial-scale synthesis units, in a pilot-scale synthesis unit, in a laboratory-scale production unit, and in a university research laboratory. The mass concentrations of respirable TiO_2 in the workers' breathing zone ranged from 10 to 150 $\mu g/m^3$ during bag filling. TiO_2 NM was mainly detected as aggregated structures in a size range spanning from nanometer to micrometer (Debia et al. 2016).

A recent exposure assessment, which was not included in the review by Debia et al., was performed at a Chinese TiO_2 manufacturing plant. Mass concentrations were assessed in two different worksites at the plant: In the packaging workshop, total dust concentrations were 3.17 mg/m^3 of which 1.22 mg/m^3 was dust in the nanosize range. In the milling workshop, total dust concentrations were 0.79 mg/m^3 of which 0.31 mg/m^3 was dust in nanosize range. ICP-MS analysis showed that a rather small part of the dust was TiO_2: TiO_2 content in total dust was 46.4 $\mu g/m^3$ at the packaging workshop and 39.4 $\mu g/m^3$ at the milling workshop. TiO_2 NM constituted 16.7 and 19.4 $\mu g/m^3$, respectively (Xu et al. 2016).

Epidemiological studies

A few epidemiological studies have been performed to evaluate the adverse effects of inhalation exposure to TiO_2 in humans. None of the epidemiological studies included information on the size range of the TiO_2 particles. Thus, it is not possible to determine whether the exposures included TiO_2 NM. In total eight studies were identified of which five are cohort studies (Chen and Fayerweather 1988;Fryzek et al. 2003;Boffetta et al. 2004;Ellis et al. 2010;Ellis et al. 2013) and three are case-control studies (Siemiatycki, 1991 (as referred by (IARC 2010) 2010 and (NIOSH 2011); (Boffetta et al. 2001),(Ramanakumar et al. 2008).

Except for the newest studies by Ellis et al. (Ellis et al. 2010;Ellis et al. 2013), the studies are included in the evaluations of TiO_2 performed by IARC (IARC 2010) and/or NIOSH (NIOSH 2011). The present working group refers to these publications for a more detailed description of these studies. The evaluation of TiO_2 performed in 2006 by IARC concluded that there was inadequate evidence of carcinogenicity in humans (IARC 2010). The evaluation of TiO_2 by NIOSH in 2011 concludes that "these studies provide no clear evidence of elevated risks of lung cancer mortality or morbidity among those workers exposed to TiO_2 dust"(NIOSH 2011).

Among the studies included in NIOSH and/or IARC, Chen and Fayerweather (Chen and Fayerweather 1988), Fryzek et al. (Fryzek et al. 2003) and Boffetta et al. (Boffetta et al. 2004) in addition to all cause and lung cancer also assessed death caused by cardiovascular diseases.

The number of deaths from ischemic heart disease and cerebrovascular disease was numerically increased among employees with TiO_2 exposure (n = 1 576) as compared with employees without TiO_2 exposure (n=901) at two TiO_2 producing plants (Dupont, US). However, this increase was not statistically significant at $p<0.10$. When the TiO_2 exposed workers were compared with the US reference group, the number of deaths from diseases of the circulatory system was slightly decreased (Chen and Fayerweather 1988).

In the cohort study by Fryzek et al. (Fryzek et al. 2003) of 4 241 TiO_2 exposed workers at four US TiO_2 plants, no significantly increased standardized mortality ratio (SMR) was found for heart disease or cerebrovascular disease or for any other specific cause of death as compared to the general background population.

In the European cohort study by Boffetta et al. (Boffetta et al. 2004) of 15 017 workers employed at factories producing TiO_2, no statistically significant increase in number of deaths from cerebrovascular disease was found as compared with the general background population.

The study by Ellis et al. (Ellis et al. 2010) investigated the mortality among workers employed for at least 6 months in three DuPont TiO_2 plants in the United States (n=5054). The general US population was used as reference. The mortality from all causes, lung and larynx cancer, non-malignant respiratory disease and all heart disease was statistically significantly decreased compared to the general population. The number of cancers belonging to the category "Other respiratory cancers" was increased 2.5 fold (95% CI: 0.62-6.46) compared to the general population. However, this was based on only 3 cases and was not statistically significant. The very low standardized mortality rates are characteristic when occupational active persons are compared with the general population due to the healthy worker effect.

A second study by Ellis et al. (Ellis et al. 2013), sponsored by E.I. du Pont de Nemours and Company, investigated the mortality among 3607 workers employed in the same three DuPont TiO_2 plants as in the previous study by Ellis et al. (Ellis et al. 2010). The study cohort overlapped with the 5054 workers in the previous study. Compared to the previous study, stricter inclusion criteria were applied: In addition to having been employed for at least 6 months, the job held had to have potential TiO_2 exposure, and no more than 25% or 5 years missing job history was accepted. The outcomes were death from all causes, all cancer, lung cancer, non-malignant respiratory disease, and all heart disease. The number of employees and the age of the study group differed vastly between the three factories which were included in the study. The employees at the Edgemoor plant contributed with 56% of the person-years for follow-up, and 85% of the deceased. Employees at Edgemoor plant were on average born in 1935, whereas employees were on average born in 1952 and 1958, respectively, on the two other plants. Consequently, the observed associations were driven by the Edgemoor plant, which contributed with the largest study group and the most cases. The study used two different reference groups, the US population and a control group of other Dupont workers who were not exposed to TiO_2. The present working group is of the opinion that the reference group of non-exposed Dupont workers is the most appropriate reference

group, since it is most comparable to the TiO_2 exposed Dupont workers regarding health worker effects, lifestyle factors and level and type of medical insurance, as compared to the general population in the US. However, the working group notes that the Dupont workers in the reference group may be exposed to other hazardous agents but no information regarding this is provided in the publication. There were no statistically significant differences in mortality for any of the studied outcomes for the TiO_2 exposed workers compared to the US population. However, when the TiO_2 exposed workers were compared to the reference group of other Dupont workers, an increased mortality was observed for the following endpoints: all causes (SMR 1.23; 95% CI 1.15–1.32), all malignant neoplasms (SMR 1.17; 95% CI 1.02–1.33), and lung cancer (SMR 1.35; 95% CI 1.07–1.33). The associations were driven by an increased mortality found at the Edgemoor plant. Increased heart mortality was seen on the Edgemoor plant when this plant was compared to "other workers" as reference group, but not for all three plants combined. The risk estimates did not show clear dose-response relationship with increasing cumulative dose. Risk estimates for all causes and non-malignant respiratory disease increased marginally with increasing cumulative exposure using a 10 year lag, and risk estimates for all cancers and non-malignant respiratory disease increased marginally with increasing cumulative exposure using no lag. The observed difference in the SMRs using an employed population versus a general population as a reference group is typically associated with healthy worker effect. The studies by Ellis et al. (Ellis et al. 2010;Ellis et al. 2013) have some severe study limitations including lack of information on TiO_2 particle size, smoking history and a lack of a description of how the reference groups were selected (including the number of persons in the reference groups). Furthermore, there was no description of other possible occupational exposures of the Dupont workers in the reference group.

While none of the above mentioned studies had information on particle size, the adverse effects of TiO_2 in the nanosize have been specifically studied in two human biomonitoring studies:

In one study, biomarkers of inflammation, oxidative damage of nucleic acids, proteins and lipids were analyzed in the exhaled breath condensate (EBC) of a cohort of TiO_2 NM manufacturing workers (n=36). The controls were healthcare personnel and technical staff who were not employed at the factory and did not handle dusts (n=45). In the TiO_2 workshops, the median TiO_2 mass concentrations varied between 0.40 and 0.60 mg/m^3. In the facility, the median particle number concentration was approximately 2×10^4 particles/cm^3 of which approximately 80% of the particles were less than 100 nm. In the research workspace, the air concentration (0.16 mg/m^3) and the particle number ($1.32 * 10^4$ particles/cm^3) were lower. The results have been published in a series of articles and documented inflammation (Pelclova et al. 2016b), oxidative damage of nucleic acids and proteins (Pelclova et al. 2016a) and lipid oxidative damage (Pelclova et al. 2017) in the EBC of the cohort of TiO_2 NM manufacturing workers. TiO_2 concentrations in the EBC were statistically significantly increased in the production workers (~20 μg TiO_2/L, $p<0.001$)) compared to both research personnel (2.00 μg/L) and controls (1.12 μg/L). The levels of oxidative stress markers (8-OHdG, 8-OHG, 5-OHMeu, o-Tyr, 3-ClTyr, 3-chlorotyrosine and 3-NOTyr were higher in the production workers than the workers from the research wing of the plant and unexposed controls (Pelclova et al. 2016a).

Inflammation was evaluated by measuring leukotriens in EBC. All of the measured leukotrienes were statistically significantly increased compared to the control group (Pelclova et al. 2016b). Lipid oxidation measured as malondialdehyde, 4-hydroxy-trans-hexenal, 4-hydroxy-trans-noneal, 8-isoProstaglandin F2α and aldehydes C_6-C_{12} was increased in TiO_2 exposed workers compared to controls and a significant dose-response relationship was found between exposure to TiO_2 and markers of lipid oxidation in the EBC (Pelclova et al. 2017).

In another recent cross-sectional study, cardiopulmonary effects were analysed in exposed workers (n=83) and controls (n=85) in a TiO_2 NM manufacturing plant in China. The exposure is described in detail in (Xu et al. 2016) and is described in the above paragraph on exposure in the present report. In short, the highest dust concentration was measured in the packaging area where the total mass concentration of particles was 3.17 mg/m^3 of which 1.22 mg/m^3 was nanoparticles (39% of total mass). Only a minor part of the dust was TiO_2 (46.4 μg/m^3) and even less was TiO_2 NM (16.7 μg/m^3). A number of assessed markers of inflammation (IL-8, IL-6, IL-1β, TNF-α, and IL-10), oxidative stress (SOD and MDA), cardiovascular disease (VCAM-1, ICAM-1, low-density lipoproteins (LDL) and total cholesterol) and lung damage (surfactant protein D (SP-D) and pulmonary function) were associated with occupational exposure to TiO_2 NM. The acute phase proteins serum amyloid A (SAA) and high-sensitivity C reactive protein (hs-CRP) and the inflammatory markers IL-1β and IL-10 were also measured but for these markers no significant differences between the groups were observed. Among the measured biomarkers, SP-D was the only marker showing dose dependency: SP-D decreased with increasing working time (Zhao et al. 2018).

IARC and NIOSH (IARC 2010;NIOSH 2011), concluded that the included epidemiological studies did not show increased risks of lung cancer among workers occupationally exposed to TiO_2. However, in a recent study of workers exposed to TiO_2 at three different plants in the USA, statistically significantly elevated SMRs were found for all causes, all cancers, and lung cancers when non-TiO2 exposed workers at other Dupont factories were used as reference group while no increase was found when the US population was used as reference group (Ellis et al. 2013). Mortality of heart disease associated with TiO_2 exposure has been assessed by Chen et al. (Chen and Fayerweather 1988) and Ellis et al. (Ellis et al. 2010;Ellis et al. 2013). When using the US population or other workers as reference group neither study showed increased heart mortality among TiO_2 workers.

The present working group is of the opinion that the reference group of non-exposed Dupont workers is the most appropriate reference group as compared to the general US population, and therefore concludes that increased risk of all-cause mortality, malignant neoplasms, and lung cancer was found in the study by Ellis et al. (Ellis et al. 2013).

In relation to assessing the effects of TiO_2 NMs, a major limitation is that none of the studies on lung cancer and heart disease provided information on particle size. However, the literature search identified two biomonitoring studies with information of particle size. In these studies, biomarkers of effect were associated with occupational

exposure to TiO_2 NM. The biomarkers reflect a local biological response to TiO_2 NM in the pulmonary region of the exposed workers and a systemic response in the blood.

The ability to detect the effect of exposure to occupational carcinogens is also determined by the population-specific lung cancer incidence. In Denmark, the life time risk of getting lung cancer (0-74 years) is 4.9% for men and 4.5% for women, respectively, according to The National Board of Health. In the US, life time lung cancer risk is similar, 7% for men and 6% for women (American Cancer Society 2018). The relative lung risk caused by occupational exposure to a carcinogen, which causes lung cancer the different risk levels, 1%, 0.1% and 0.01% are given in table 1. As can be seen in the table, exposures that cause 1% excess lung cancer will give relative risks of 1.2. According to power calculation, detection of 1% excess cancer incidence with 5% lung cancer incidence in the reference group would require group sizes of 8 000 participants (with 80% chance of detecting the effect at 5% significance level). On the other hand, occupational exposures that cause 0.1% excess lung cancers (1 of 1 000, which is the acceptance level in the US), corresponds to a RR of 1.04, which requires group sizes of 750 000 persons if the background cancer incidence is 5%.

Table 1. Relative risk of lung cancer for carcinogens that cause 1%, 0.1% or 0.01% excess lung cancer risk in a population with the current Danish lung cancer incidence

	Men	**Women**
Life time risk (0-74 years) 2011-2015 in Denmark[1]	4.9%	4.5%
Excess lung cancer risk level	RR	RR
1:100	RR= (4.9+1)/ 4.9= 1.20	RR= (4.5+1)/4.5= 1.22
2:1000	RR= (49+2)/49= 1.041	RR= (45+2)/45=1.044
1:1 000	RR= (49+1)/49= 1.02	RR= (45+1)/45=1.02
1:10 000	RR= (490+1)/490= 1.002	RR= (450+1)/450= 1.002
1:100 000	RR= (4900+1)/4900= 1.000 2	RR= (4500+1)/4500= 1.000 2

Thus, the epidemiological studies on TiO_2 and lung cancer risk have limited statistical power to detect carcinogenic effects of TiO_2 exposure, unless the excess lung cancer risk associated with TiO_2 exposure was very high.

As none of the above mentioned epidemiological studies provided information on the size range of the TiO_2 particles, thus making it impossible to determine whether the exposures included TiO_2 NM, and no information on dose-response relationship, the present working group has decided to include and base the suggested OEL of experimental animal studies.

TOXICOKINETICS

Exposure to TiO_2 NM may occur by one of three exposure routes: inhalation, ingestion, or dermal. Of these, inhalation and to some degree dermal are the main exposure routes in the occupational setting. Dermal exposure through healthy skin is most likely not a risk following short term exposure. However, uptake may occur through damaged skin or if exposure is chronic (Christensen et al. 2011). The focus of this report is exposure by inhalation.

Inhalation of particles results in deposition in the respiratory tract (nasopharyngeal, tracheobronchial and alveolar regions) (Oberdorster et al. 2005). The deposition pattern of particles in the different parts of the respiratory tract is strongly dependent on size of the aerosolized particle agglomerate. Inhaled NMs deposit in the entire respiratory tract. However, a large fraction of the inhaled NMs deposit in the alveolar region. In contrast, most of the larger particles (> 1-2 µm) deposit in the upper airways (Oberdorster et al. 2005) (Shi et al. 2013;Koivisto et al. 2012). In a study, mice were exposed by inhalation 1h/day for 11 days to 42 mg/m^3 aerosolized powder of rutile TiO_2 with an average crystallite size of 21 nm. The pulmonary deposition fraction was estimated to be 8.6% based on the observed particle size distribution in the aerosol (Hougaard et al. 2010).

A 12 week inhalation study in rats showed that pulmonary clearance of 21 nm TiO_2 NM was slower ($t_{1/2}$ = 501 days) than 250 nm TiO_2 particles ($t_{1/2}$ = 174 days) (Ferin et al. 1992). . The main mechanism for particle clearance in the alveoli following TiO_2 NM inhalation was phagocytosis by macrophages(Shi et al. 2013;Koivisto et al. 2012). Smaller particles are less efficiently phagocytized than larger particles: A rat inhalation study with 20 nm TiO_2 particles demonstrated that nanoTiO_2 particles are not efficiently phagocytized by macrophages (Geiser et al. 2008). This results in prolonged residence time for particles in the lungs increasing the possibility for inflammatory reactions and translocation into lung tissue or the circulation. Ferin et al. showed that nanosized TiO_2 translocate from the lungs to the blood circulation to a greater extent than larger TiO_2 particles (Ferin et al. 1992). As reviewed by Geiser & Kreyling, human studies have shown that the translocated nanoparticle mass fraction is less than 1 % of the dose delivered to the lungs (Geiser and Kreyling 2010).

Only few studies have measured translocation of TiO_2 NM from the lung into the circulatory system to systemic tissue. Available data suggest that the rate of NM migration to the circulatory system is low. The rate of translocation is likely to depend on size, shape and surface modifications (Geiser and Kreyling 2010).

In a very comprehensive study, Kreyling and co-workers studied the biokinetics of radiolabeled 70 nm TiO_2 NM following intratracheal instillation (Kreyling et al. 2017b), intravenous injection (Kreyling et al. 2017a) and oral application (Kreyling et al. 2017c) in rats. For the intratracheal and the intravenous studies, biodistribution was assessed quantitatively 1 h, 4 h, 24 h, 7 d and 28 d after exposure. The biodistribution following oral application was assessed on the same time points except for the latest time point.

The intratracheal instillation study showed that after 1 h about 4% of the initial peripheral lung dose had translocated from the pulmonary region. The TiO_2 NM were mainly retained in the carcass (4% after 1 h and 0.3% after 28 d). In the liver and kidney the fractions of TiO_2 NM remained constant (0.03%) (Kreyling et al. 2017b). A comparison of the biodistribution after IV-injection (Kreyling et al. 2017a), gavage (Kreyling et al. 2017c) and intratracheal instillation (Kreyling et al. 2017b), showed that gavage and intratracheal instillation resulted in a similar patterns of biodistribution. However, the rate translocation to secondary organs was higher following pulmonary exposure (ca. 4.3% of the pulmonary deposited dose after 1 h) compared to oral exposure (0.6% of the administered dose passed the gastro-intestinal-barrier after one hour). The biodistribution following intravenous injection was very different from the biodistribution of following pulmonary and oral dosing.

ANIMAL STUDIES

Rodent versus human response

Inhalation studies in mice and rats are used to assess potential human hazard where human exposure studies and epidemiological studies are not available.
There is very limited data available on effects following inhalation of TiO_2 NMs in humans. Rats are the preferred animal model in particle toxicology and are more sensitive than mice to particle-induced lung cancer and fibrosis.

Intratracheal instillation versus inhalation

Inhalation studies are the gold standard of toxicity testing, as this exposure route is the closest surrogate to human exposure. However, the deposited pulmonary dose can be difficult to monitor after inhalation due to differences in sizes of the aerosolized particle agglomerates. This can result in differences in deposition (Schmid and Cassee 2017). In addition, exposure by inhalation requires a substantial amount of material and specialized inhalation facilities, and it poses an occupational health risk to the technicians handling the NMs.

Pulmonary deposition by intratracheal instillation is used in screening studies (Bourdon et al. 2012;Husain et al. 2013;Poulsen et al. 2015b;Saber et al. 2012b;Saber et al. 2012a) and has been proposed as an alternative to inhalation exposure. This exposure method ensures that the same dose is delivered to the lung for all NM exposures, demands less material and is more user-friendly. Intratracheal installation has previously been shown to give widespread distribution of particles throughout the lung (Mikkelsen et al. 2011).

A number of studies have compared the toxicological response following inhalation and instillation of nanomaterials. Two studies have compared the global transcriptional profiles as a means to investigate the pulmonary biological response after inhalation compared to instilled or aspirated NMs. Inhalation and intratracheal instillation of a surface modified TiO_2 NM resulted in similar transcriptional changes, with the acute phase response and inflammation as the most important pulmonary responses to inhaled and instilled TiO_2 (Halappanavar et al. 2011;Husain et al. 2013). Similarly, Kinaret et al (Kinaret et al. 2017) compared the global transcriptomic profiles of lung tissue from mice exposed to a straight and long multi-walled carbon nanotube (MWCNT) by inhalation or aspiration. The authors concluded that the perturbed pathways were very overlapping, suggesting that the transcriptiomic response to MWCNT exposure was very similar for inhaled and pulmonary dosed MWCNTs.

Other studies compared levels of pulmonary inflammation, measured as neutrophil influx, after exposure by inhalation or intratracheal instillation in rodents. Two studies using MWCNT reported that both methods resulted in pulmonary inflammation, with inhalation being more potent at inducing inflammation (Morimoto et al. 2012;Porter et al. 2013). Baisch et al. reported that instillation of a high dose of TiO_2 nanoparticles induced greater inflammation compared to low dose rate delivery through inhalation, even though the same pulmonary deposited dose were delivered. The authors concluded that

intratracheal instillation is useful for quantitative ranking of nanoparticle hazards, but not for quantitative risk assessment (Baisch et al. 2014).

Selection of studies and endpoints

In the present report inhalation studies will be prioritized. For the description of toxicological endpoints and mechanism of toxicity, studies using pulmonary deposition as intratracheal instillation will be included where no quality inhalation studies are available. Dose-response assessments, however, are solely conducted based on sub-chronic and chronic inhalation studies.

Endpoints were evaluated based on reported adverse effects of TiO_2 NM exposure in reports and in the scientific literature. The assessment by NIOSH used cancer as the endpoint (NIOSH 2011). However, other previous assessments have mainly focused on inflammation as critical effect (Christensen et al. 2011;Stockmann-Juvela et al. 2014;Nakanishi and Gamo 2011). This report will therefore include both endpoints.

Cancer and cardiovascular disease have been identified as two of the main mortality causing diseases in the world (World Health Organization 2018;Cancer Risks UK 2018). Both diseases are potentially initiated by inflammation, as described in *Mechanism of toxicity*. In conclusion, the critical endpoints were chosen based on literature review and mechanistic understanding.

Pulmonary inflammation

In a sub-chronic inhalation study by Ferin et al., rats were exposed to about 23 mg /m^3 of two different sized anatase TiO_2 particles (21 nm and 250 nm) for 6 h/day, 5 days/week for 12 week. Pulmonary inflammation was assessed 4, 8, 12, 41 and 64 weeks after start of exposure. The 21 nm TiO_2 NM induced more neutrophil influx than the 250 nm TiO_2 particles and the filtered air already after 4 weeks of exposure. The number of neutrophils were almost reduced to control level after 52 weeks post-exposure (Ferin et al. 1992).

In a sub-chronic inhalation study by Bermudez et al, female rats, mice and hamsters in groups of 25 were exposed to 0, 0.5, 2.0 or 10 mg/m^3 TiO_2 NM(P25, average primary particle size of 21 nm) for 6 hours/day, 5 days/week for 13 weeks (Bermudez et al. 2004). The mean mass-median aerodynamic diameter of TiO_2 NM and agglomerates was 1.37 µm in exposure chamber. Pulmonary endpoints (inflammation, cytotoxicity, lung cell proliferation and histopathology) were assessed 0, 4, 13, 26 and 52 weeks (49 weeks for TiO_2 NM exposed hamsters) after end of exposure.

To assess inflammation, the total number of broncho alveolar lavage (BAL) cells and the number of macrophages, neutrophils, eosinophils and lymphocytes in the BAL cells was determined. The neutrophil influx as percent of total BAL cells is shown in Table 2. Compared to controls, the percentage of neutrophils was not significantly increased in hamsters at any dose or time point after end of exposure. Immediately after end of exposure rats had increased percentage of neutrophils at 2 mg/m^3 and above while this

was not the case for exposure at 0.5 mg/m^3. From 4 weeks after exposure and later time points, both rats and mice had increased percentage of neutrophils at the highest dose (10 mg/m^3).

In rats exposed at the highest dose (10 mg/m^3), progressive epithelial and fibroproliferative changes were observed through 13 weeks post-exposure. Most of these changes were reported to be regressing over time (13-52 weeks post-exposure).

Inhalation and intratracheal instillation studies have shown that when rats and mice were exposed TiO_2, the ultrafine TiO_2 induced a much stronger pulmonary inflammatory response compared to the same mass of fine TiO_2 particles. The inflammatory response correlated with the surface area of the deposited particles irrespectively of size. This dose response relationship has been observed for a number of low-toxicity low – solubility particles and it is generally accepted that the inflammatory response of low toxicity-low solubility particles including TiO_2 is proportional to the surface area of the instilled particles rather than the mass (reviewed in Oberdörster et al (Oberdorster et al. 2005)).

We consider the Bermudez study (Bermudez et al. 2004) as a key study for this hazard assessment. It is the only of the identified studies that is a sub-chronic inhalation study with dose-response relationship of TiO_2 NM. In that study, a NOAEC of 0.5 mg/m^3 was identified for pulmonary influx of neutrophils in rats which was the most sensitive of the tested species. In addition to this study we have identified a range of inhalation studies with shorter exposure duration using TiO_2 NM particles (Ma-Hock et al. 2009;Noel et al. 2012;Rossi et al. 2010a;Rossi et al. 2010b;Baisch et al. 2014;Kwon et al. 2012;Lindberg et al. 2012). Overall they support that a NOAEC level for pulmonary influx of neutrophils is in the range of 0.5-2 mg/m^3.

Table 2. Pulmonary influx (%) of neutrophils in animals exposed by inhalation to TiO_2 NM (Bermudez et al. 2004).

Postexposure (weeks)	Concentration (mg/m^3)	Rats	Mice	Hamsters
0	0	0.4 ±0.42	0.00 ± 0.00	2.30 ± 1.15
	0,5	0.5 ± 0.35	0.00 ± 0.00	1.30 ± 6.84
	2	6.50 ± 4.23*	0.20 ± 0.27	1.00 ± 0.79
	10	64.80 ±5.35*	14.50 ± 5.73*	10.20 ± 14.65
4	0	0.3 ± 0.27	0.20 ± 0.27	2.30 ± 2.49
	0,5	0.2 ± 0.27	0.20 ± 0.27	5.20 ± 4.72
	2	0.90 ± 0.96	0.10 ± 0.22	4.10 ± 4.60
	10	43.30 ± 3.27*	12.40 ± 7.90*	3.70 ± 3.49
13	0	0.20 ± 0.27	0.10 ± 0.22	7.80 ± 8.24
	0,5	1.20 ± 1.15	0.40 ± 0.55	2.60 ± 1.71
	2	1.70 ± 1.60	0.30 ± 0.45	2.60 ± 0.89
	10	41.9 ±14.10*	13.90 ± 6.81*	2.70 ± 1.25
26	0	0.50 ±0.35	0.10 ± 0.22	4.70 ± 1.68
	0,5	0.30 ± 0.27	0.30 ± 0.27	3.10 ± 2.70
	2	1.90 ± 0.82	0.10 ± 0.22	3.60 ± 1.47
	10	20.8 ± 7.64*	17.00 ± 5.95*	2.30 ± 1.96
52	0	0.80 ± 0.84	0.10 ± 0.22	4.50 ± 2.50
	0,5	0.60 ± 0.42	0.00 ± 0.00	5.50 ± 6.28
	2	0.70 ± 0.57	0.40 ± 0.65	5.20 ± 3.19
	10	12.00 ± 4.51*	12.30 ± 4.80*	4.50 ± 1.70

***Significantly different from control, $p < 0.05$; NOAECs and the lowest observed adverse effect concentrations (LOAECs) for each species at different time-points after end of exposure are indicated with green and red text, respectively. Table is generated based on Tables S1-S3 in Bermudez et al (Bermudez et al. 2004).**

Genotoxicity and cancer

Genotoxicity and cancer are well studied, possible adverse effects of exposure to TiO_2 NM. Genotoxicity often occurs relative rapidly after exposure, whereas cancer is a more complex pathological endpoint that requires longer time to develop. In this report, we therefore chose to differentiate between genotoxicity in shorter-term studies and cancer in long-term studies.

Cancer

Three chronic cancer TiO_2 inhalation studies were identified. One of these studies used TiO_2 NM (P25, 80%anatase/20% rutile) which was tested in female Wistar rats (Heinrich et al. 1995;Heinrich et al. 1995), while the other two studies used both fine, rutile TiO_2 but tested in male/female Wistar rats (Muhle et al. 1991) and in male/female Sprague-

Dawley rats (Lee et al. 1985), respectively. Details on the study set-ups are summarized in Table 3. An increased cancer incidence was detected in rats exposed to 10 mg/m^3 of TiO_2 NM (Heinrich et al. 1995), while exposure to fine TiO_2 only increased cancer incidence in rats exposed to the highest tested concentration (250 mg/m^3) (Lee et al. 1985). In rats exposed to 10 mg/m^3 of TiO_2 NM, slight to moderate interstitial fibrosis in the lungs was observed in all animals after 2 years of exposure (Heinrich et al. 1995). The present working group notes that dose response relationship for TiO2 NM-induced cancer could not be established since only one dose level was tested. However, NIOSH has evaluated the rat cancer data from inhalation studies of TiO_2 in different sizes (ultrafine and fine) and concluded that they fit on the same dose-response curve when dose is expressed as total particle surface area in the lungs (NIOSH 2011). The present working group considers this sufficient evidence of dose response relationship.

In summary, in the only identified chronic inhalation study of rats exposed to TiO_2 NM, cancer was induced at 10 mg/m^3 (LOAEC = 10 mg/m^3). TiO_2 has been classified as possibly carcinogenic by IARC based on sufficient evidence of carcinogenicity in experimental animals (IARC 2010). When NIOSH evaluated rat cancer data from inhalation studies of TiO_2 in different sizes (ultrafine and fine) they concluded that all the data points fit on the same dose-response curve when dose was expressed as total particle surface area in the lungs (NIOSH 2011).

Table 3. Overview of chronic rat inhalation studies

Reference	Type of TiO_2	Exposure	Lung tumor increase compared to controls
Muhle et al. (Muhle et al. 1991)	Fine, rutile	2 year, 0, or 5 mg/m^3	5 mg/m^3: No increase
Lee et al. (Lee et al. 1985) (reclassification of tumors in Warheit and Frame (Warheit and Frame 2006))	Fine, rutile	Whole body inhalation for 6 hour/day, 5 days/week for up to 2 years, to 0, 10, 50, or 250 mg/m^3 No follow up time after end of exposure.	10 mg/m^3: No increase 50 mg/m^3: No increase 250 mg/m^3: Increased For the 250 mg/m^3: Bronchioalveolar carcinomas in 12/77 male rats and 13/74 female rats Squamous cell carcinomas in 1/77 males and 13/74 females) Controls: Bronchioalveolar carcinomas in 2/79 male rats and 0/77 female rats No squamous cell carcinomas.
Heinrich et al. (Heinrich et al. 1995)	Ultrafine P25 (15-40 nm primary particle size, 0.8 µm MMAD, 48 m^2/g specific surface area, 80% anatase/20% rutile)	18 hour/day, 5 days/week for up to 2 years to 0, or 10 mg/m^3 followed by 6 months without TiO_2 exposure	10 mg/m^3: Increased At 30 months: 13/100: Adenocarcinoma 3/100: Squamous cell carcinoma 4/100: Adenocarcinoma 20/100: keratinzing cystic squamous-cell tumors 32/100: Total number with tumors Controls: Adenocarcinomas: 1/217 No other lung tumors were observed

Genotoxicity

The genotoxic potential has been tested in many *in vivo* studies by analysis of different endpoints including DNA strand breaks, DNA adducts and micronuclei. Some studies indicate that TiO_2 NMs are genotoxic, while other studies do not (reviewed by (Shi et al. 2013)). The different physico-chemical properties of the tested TiO_2 particles (specific surface area, coating, anatase/rutile, form) may explain why some studies are negative and others positive. The present working group concludes that no firm conclusions can be reached regarding genotoxicity.

Cardiovascular effects

Only few studies have investigated the cardiovascular effects of pulmonary TiO_2 NM exposure. Some studies have assessed promising biomarkers for cardiovascular disease.

Plaque progression and vascular dysfuction

The lipid profile of mice significantly differs from that of humans. Mice do not develop atherosclerosis, because rapid clearance of hepatic LDL results in low and rather stabile total serum cholesterol levels, even after increased cholesterol intake and synthesis. Atherosclerotic changes are therefore mainly investigated in ApoE -/- mice, which are deficient in apolipoprotein E (apoE), a glycoprotein associated with all lipoproteins except LDL. ApoE -/- mice develop spontaneous atherosclerosis as early as 3–4 months of age when fed normal chow (Nakashima et al. 1994). This makes them suitable for investigating cardiovascular effects.

A few studies have reported TiO_2 NM-induced accelerated plaque progression:

Modest effect on plaque progression was detected in ApoE-/- mice intratracheally instilled with 0.5 mg/kg bodyweight TiO_2 NM (21 nm) once a week for 4 weeks. No effect on vasodilatory function was detected in ApoE-/- mice intratracheally instilled with 0.5 mg/kg bodyweight of three types of TiO_2 (rutile 288 nm TiO_2, anatase/rutile 12 nm TiO_2, and rutile 21 nm TiO_2) at 26 and 2 hours before measurement (Mikkelsen et al. 2011).

ApoE-/- mice were exposed by tracheal instillation of 0, 10, 50 and 100 μg 5-10 nm TiO_2 NM once a week for 6 weeks. Compared to vehicle controls, the high dose group had increased levels of CRP, nitrogen oxide (NO), endothelial nitric oxide synthase (eNOS), total and high density lipoprotein (HDL) cholesterol in serum. In addition, the medium and high dose group had increased plaque area and increased ratio of the lipid-rich core area to plaque area, respectively. At the highest dose, TiO_2 NM exposure induced systemic inflammation (measured as increased level of Hs-CRP), endothelial dysfunction (measured as reduced serum level of nitric oxide and eNOS) and changed lipid metabolism (measured as increased total cholesterol and decreased HDL in the serum) (Chen et al. 2013).

Microvascular dysfunction was observed in rats exposed by inhalation to TiO_2 NM. The microvascular dysfunction was associated with increased oxidative stress and decreased NO production (Nurkiewicz et al. 2009).

Thrombus formation

Systemic administration of a single dose (1 mg/kg) of anatase TiO_2 NM (38 nm, 320 m^2/g) but not rutile TiO_2 NM (67 nm, 60 m^2/g) accelerated thrombus formation in the microcirculation in mice (Haberl et al. 2015).

Activation of complement factor 3 (C3) may promote the atherosclerotic process because C3 activation products (C3a and C3b) are involved in the atherothrombotic process and they are associated with lipid components in the vessel wall. Activation of C3 in blood

was detected in C57BL/6 mice exposed by intratracheal instillation to 18 or 162 μg of TiO_2 NM (rutile, 21 nm) compared with vehicle controls (Husain et al. 2015).

Acute phase response

The acute phase response is induced in humans in response to infection, infarction and trauma, and it is defined by increases in acute phase response proteins with the most predominant being CRP, SAA, and fibrinogen. During an acute phase response these proteins can increase thousand fold (Gabay and Kushner 1999). Elevated plasma levels of CRP and SAA have been reported as a risk factor for cardiovascular disease in humans (Johnson et al. 2004;Lowe 2001;Mezaki et al. 2003;Ridker et al. 2000). In mice, the SAA isoforms are the main acute phase response proteins, while CRP is only moderately induced by inflammatory stimuli (Whitehead et al. 1990;Pepys and Hirschfield 2003). SAA (SAA1-4) is a highly conserved family of apolipoproteins associated with HDL.

Several studies have reported changes in *Saa* expression levels after pulmonary exposure to TiO_2 NM. Inhalation of TiO_2 NMs as well as intratracheal instillation of a single dose of TiO_2 NM (21 nm) in female C57BL/6 mice strongly increased *Saa1*, *Saa2* and *Saa3* mRNA levels in lung tissue in a dose-dependent manner (Saber et al. 2013;Halappanavar et al. 2011;Husain et al. 2013). Time-mated mice were exposed by inhalation 1h/day to 42 mg/m^3 TiO_2 NM on gestation days 8–18. *Saa3* mRNA expression levels were increased 5 days and 4 weeks after the end of exposure (Saber et al. 2013).

Reproductive toxicity

Time mated female rats were exposed to TiO_2 NM (P25, 21 nm in diameter) by inhalation for 8 non-consecutive days (4-6 h/day for 7.8 days). The mass concentration was 10 mg/m^3. The calculated cumulative, deposited dose was 217 ± 1.0 μg. Chromatin immunoprecipitation and DNA sequencing were performed in the offspring fetal hearts at gestation day 20; and it was reported that the experiments provide initial evidence that significant epigenetic and transcriptomic changes occur in the cardiac tissue of maternally TiO_2 NM exposed progeny (Stapleton et al. 2018a). Two other studies used a similar dosing regimen. One found that gestational exposure to the 21 nm TiO_2 particles (median aerodynamic diameter 130-150 nm) disrupted progeny cardiac function and bioenergetics (Hathaway et al. 2017). In the other study, the median aerodynamic diameter of the inhaled 21 TiO_2 NM particles was 171 nm and the calculated daily maternal deposition was 13.9 ± 0.5 μg. At 5 months of age a standard battery of several locomotion, learning, and anxiety tests was applied for testing of male offspring from four control and four exposed dams (n=11). TiO_2 NM was associated with significant working memory impairments in the radial arm maze and deficits in the visual platform test, possibly reflecting deficits in initial motivation in male F1 adults (Engler-Chiurazzi et al. 2016). In a final study from this research group, virgin and late stage pregnant [GD 19] female rats were exposed to TiO_2 NM (21 nm in diameter) by inhalation for 5 h. The mass concentration was 10 mg/m^3 (median aerodynamic diameter particle diameter 173.2±6.4 nm in the exposure atmosphere), leading to a calculated deposited dose of 42.2 ± 1.9 μg TiO_2. Assessment in live animals showed that inhalation of TiO_2 NM disturbed vascular reactivity differentially in different stages of estrous in non-pregnant females. In addition, increased inflammatory activity was observed in these animals. The level of

inflammatory markers in blood was altered during estrus and late gestation. The authors suggest that female fertility may be impaired by TiO_2 NM inhalation (Stapleton et al. 2018b).

Another research group exposed time-mated female mice to TiO_2 NM (UV Titan, 21 nm in diameter) by inhalation for 1h/day on gestation days 8 to 18. The mass concentration was 42 mg/m^3 and the major particle size-mode was ~100 nm. Several outcomes were studied in the time-mated females and their offspring. TiO_2 NM exposure was associated with lung inflammation in the time-mated females 5 and 27 days post-exposure. In the adult offspring, TiO_2 NM exposure was associated with moderate neurobehavioral alterations. Cognitive function was unaffected but the offspring tended to avoid the central zone in the open field assay. In addition, exposed female offspring displayed enhanced prepulse inhibition in the acoustic startle test (Hougaard et al. 2010). Levels of DNA strand breaks were evaluated using the comet assay. No effects were observed on this endpoint in BAL cells or liver of time-mated females 5 and 27 days post exposure, nor in the livers of their offspring at postnatal day 2 and 22 (Jackson et al. 2013). At maturity, female F1 offspring were mated with unexposed males. Expanded-simple-tandem-repeat-loci germline mutation rates were determined in the F2-generation and found not to differ between TiO_2 and control F2 offspring (Boisen et al. 2012). Also testicles were collected from the mature F1 and F2 males. Daily sperm production was not statistically significantly affected in the F1- or F2-generation males originating in dam with TiO_2 exposure compared to sham exposed dams (Kyjovska et al. 2013). Overall, the above described developmental toxicity effects were observed after 8 days of exposure for 4-6 h per day at 10 mg TiO_2 NM/m^3, or following exposure to 42 mg TiO_2 NM/m^3 for 1 h per day for 11 days. The corresponding daily doses were 5-7.5 mg/m3 per 8-h workday. When taking into account the LOAEC/NOAEC observed on increased BAL neutrophils in BAL in other studies at 2/0.5 mg/m^3 (E.g. (Bermudez et al. 2004)), developmental toxicity is not deemed to be the critical effect in the current investigation. This, however, may change if experiments with lower mass concentrations of TiO_2 are undertaken in the future.

MECHANISMS OF TOXICITY

Pulmonary inflammation, genotoxicity and cancer

Pulmonary exposure to TiO_2 NM has consistently shown dose-dependent pulmonary inflammation (NIOSH 2011)and deposited surface area has been identified as an important predictor of pulmonary inflammation (NIOSH 2011). The present working group notes that there is limited available data on the biological effects of TiO_2 NM with different physico-chemical properties, but concludes that the majority of available data support that the surface area (and therefore the size) of TiO_2 is a critical driver of particle-induced inflammation in the lungs. The present working group concludes that inhalation of TiO_2 NM induces dose dependent pulmonary inflammation and that neutrophil influx is predicted by the total surface area of deposited particles. The working group considers inflammation as a threshold effect.

Shi et al. reviewed TiO_2 NM-induced genotoxicity *in vivo* and *in vitro* and concluded that:" The possible mechanisms for TiO_2 NM-induced genotoxicity involve DNA damage directly or indirectly via oxidative stress and/or inflammatory responses"(Shi et al. 2013).

IARC has classified TiO_2 as possibly carcinogenic to humans (group 2B) based on sufficient evidence of carcinogenicity in experimental animals and insufficient evidence in humans. IARC does not differentiate between nano- and fine particles in their classification (IARC 2010).

NIOSH concludes that the inflammatory response and the induction of lung tumors by TiO_2 and other low-toxicity low-solubility particles correlates well with the total surface area of pulmonary deposited particles (NIOSH 2011). NIOSH furthermore concludes that "TiO_2 is not a direct-acting carcinogen, but acts through a secondary mechanism that is not specific to TiO_2 but primarily related to particle size and surface area (NIOSH 2011).

EU's Scientific Committee on Consumer Safety (SCCS) concluded similarly in a recent report that "..an inflammatory process and indirect genotoxic effect by ROS production seems to be the major mechanism to explain the effects induced by TiO_2". However, SCCS also stated that "a genotoxic effect by direct interaction with DNA cannot be excluded since TiO_2 was found in the cell nucleus in various in vitro and in vivo studies" (Scientific Committee on Consumer Safety (SCCS) 2017).

The present working group found that the mechanism of action of the genotoxic and carcinogenic effects have not been fully clarified (Shi et al. 2013). Secondary genotoxicity due to particle-induced inflammation is an important and well documented mechanism of action for the development of lung cancer. However, the available data did not allow ruling out that TiO_2 NM could also induce cancer through a direct genotoxic mechanism. Therefore, the present working group considers carcinogenicity as a non-threshold effect.

Consequently, the present working group decided to perform the risk assessment based on both a threshold and a non-threshold mechanism of action.

Cardiovascular effects

NM exposure can lead to cardiovascular effects either: 1. Directly, by translocation of NMs from the lung to the vascular system. 2. Indirectly, as a consequence of pulmonary inflammation and acute phase response. 3. Alterations in autonomic nervous system activity to elicit peripheral effects.

Atherosclerosis is a central cardiovascular effect, which is manifested as increased plaque deposition or build-up in the arteries. It is initiated by a biological, chemical or physical insult to the artery walls. Translocated NMs could induce this insult by interacting directly with the endothelium. This leads to the expression of cell adhesion molecules (selectins, VCAM-1 and ICAM-1) on the endothelial lining of the arteries, which facilitates the activation, recruitment and migration of monocytes through the endothelial monolayer (Hansson and Libby 2006;Cybulsky et al. 2001). Inside the intima layer, the monocytes differentiate into macrophages and internalize fatty deposits (mainly oxidized low density lipoprotein), transforming them into foam cells, which is a major component of the atherosclerotic fatty streaks. The fatty streaks reduce the elasticity of the artery walls and the foam cells promote a pro-inflammatory environment by secretion of cytokines and ROS. In addition, foam cells also induce the recruitment of smooth muscle cells to the intima. Added together, these changes lead to the formation of plaques on the artery walls. A fibrous cap of collagen and vascular smooth muscle cells protects the necrotic core and stabilizes the plaque (Libby 2002;Virmani et al. 2005). Although initially asymptomatic, narrowing of the blood vessels can lead to other cardiovascular diseases, such as coronary artery disease or stroke. In addition, blood clots can be formed if the plaque ruptures. These may travel with the bloodstream and obstruct the blood flow of smaller vessels.

Pulmonary exposure to NMs may also promote accelerated atherosclerosis indirectly through an induced pulmonary acute phase response. Introduction of NMs to the lung promotes neutrophil influx and release of pro-inflammatory cytokines, which leads to increased production of SAA proteins. The SAAs are hydrophobic proteins that upon secretion in their target organs are able to translocate to the blood. A statistically significant correlation between Saa3 mRNA levels in the lung and SAA3 protein levels in the blood have previously been reported (Poulsen et al. 2015a), indicating that SAA3 produced in the target organ translocate to systemic circulation. SAA circulating in the blood becomes incorporated with HDL, thereby replacing Apolipoprotein A1 (Apo-A1) as the major HDL-associated protein and forming HDL-SAA. The formation of HDL-SAA has a double effect on plaque progression: 1. HDL is a major component of reverse cholesterol transport, a multi-stepped process resulting in the movement of cholesterol through the blood from peripheral tissues (including the artery walls) to the liver. The formation of SAA-HDL impairs the HDL-mediated reverse cholesterol transport, resulting in reduced cholesterol transport and an increased systemic total cholesterol pool (Lindhorst et al. 1997;Steinmetz et al. 1989). 2. SAA and SAA-HDL have been shown to directly stimulate the transformation of macrophages into foam cells and to

stimulate uptake of oxidized LDL in the macrophages (Lee et al. 2013). In addition, SAA-HDL has a lower capacity to promote cellular cholesterol efflux from macrophages than native HDL (Artl et al. 2000). Pulmonary neutrophil influx has been shown to correlate with pulmonary Saa3 mRNA levels, SAA3 levels in blood and with deposited surface area of instilled particles (Saber et al. 2014), which links deposited particle surface area with biomarkers of risk of developing cardiovascular disease.

In conclusion, the present working group is of the opinion that pulmonary exposure to particles including TiO_2 NMs can lead to accelerated plaque progression directly, through translocation, or indirectly, through an induced acute phase response. No single physicochemical property has been identified as the driver of cardiovascular effects, but TiO_2 NM surface area a likely important due to the close association with pulmonary inflammation. As for inflammation, we consider cardiovascular effects as a threshold effect. This is based on identified dose-response relationships between particle exposure dose and induced acute phase response (Poulsen et al. 2015a;Saber et al. 2013), and the close interplay between inflammation, acute phase response and plaque progression.

Dose-response relationships

Inflammation

Strong dose-response relationships have been observed following inhalation (Bermudez et al. 2004) and intratracheal instillation of TiO_2 NM (Saber et al. 2012a) when dose is expressed as mass. Inhalation and intratracheal instillation studies have shown that when rats and mice were exposed TiO_2, the TiO_2 NM induced a much stronger pulmonary inflammatory response compared to the same mass of fine TiO_2 particles. The inflammatory response correlated with the surface area of the deposited particles irrespectively of size. This dose response relationship has been observed for a number of low-toxicity, low-solubility particles and it is generally accepted that the inflammatory response of low toxicity-low solubility particles including TiO_2 is proportional to the surface area of the deposited particles rather than the mass (reviewed by Oberdörster et al. (Oberdorster et al. 2005)).

Acute phase response

Strong dose-response relationship has been observed for pulmonary *Saa* mRNA expression levels in mice intratracheally instilled with TiO_2 NM (Saber et al. 2013). *Saa* mRNA expression levels correlates with neutrophil influx and total deposited surface area (Saber et al. 2013).

Cancer

As for other low-toxicity low–solubility particles, on a mass basis, the rat tumor response following pulmonary exposure to ultrafine TiO_2 (Heinrich et al. 1995) is much greater than for fine TiO_2 (Lee et al. 1985). However, the tumor response in rats exposed to fine and ultrafine TiO_2 fit on the same dose-response curve when dose is expressed as particle surface area (NIOSH 2011). This indicates that for the same mass dose of TiO_2 the tumour response is higher for ultrafine than for fine particles. Based on this, dose-dependency is assumed for TiO_2 NM-induced lung cancer.

Particle characteristics

TiO_2 NMs may vary regarding size (and therefore also surface area), crystal form, coating etc. These are all characteristics that could influence the toxicity. As described above in the paragraph on dose-response relationship, the surface area of TiO_2 NM is the best dose predictor for both the inflammatory response and for lung tumors.

TiO_2 exists in different naturally occurring polymorphs including rutile and anatase. NIOSH concluded that the dose-response relationships for pulmonary inflammation and lung tumors were not affected by different crystal structures: "The difference in TiO_2 crystal structure in these sub-chronic and chronic studies did not influence the dose-response relationships for pulmonary inflammation and lung tumors [Bermudez et al. 2002, 2004; Lee et al. 1985; Heinrich et al. 1995]. That is, the particle surface area dose and response relationships were consistent for the ultrafine (80% anatase, 20% rutile) and fine (99% rutile) TiO_2 despite the differences in crystal structure."(NIOSH 2011).

The present working group notes that there is limited available data on the biological effects of different physico-chemical properties, but the present working group concludes that the majority of available data support that the surface area (and therefore also the size) of TiO_2 is a critical driver of particle-induced inflammation and the acute phase response in the lungs.

PREVIOUS RISK ASSESSMENTS OF TiO_2

During the last couple of years, researchers, producers and organizations have proposed recommended exposure limits (RELs), indicative or derived no-effect-level (INEL/DNEL) and occupational exposure levels for TiO_2 NM. These have been set based on pulmonary inflammation or lung cancer. The previous recommendations of exposure limits are presented below and an overview can be found in table 4.

IARC

In 2006, the International Agency for Research on Cancer (IARC) classified TiO_2 as possibly carcinogenic to humans (group 2B). This classification was based on sufficient evidence of carcinogenicity in experimental animals exposed by inhalation and insufficient evidence in humans. IARC does not differentiate between nano- and fine particles in their classification (IARC 2010). In Denmark, substances classified as group 1, 2A and 2B by IARC are considered carcinogenic.

ENRHES

One of the first suggestions of a limit value for TiO_2 NM was made within the EU project ENRHES (Christensen et al. 2011). Due to limited data on TiO_2 NM, the authors suggest an *indicative* no effect level instead of a *derived* no effect level. The derivation of an INEL was made under the assumption of a threshold driven mechanism of TiO_2 NM toxicity: TiO_2 NM induced oxidative stress/inflammation which may result in other effects such as e.g. cancer.

The INEL 17 $\mu g/m^3$ was derived based on the sub-chronic inhalation study of mice, rats and hamsters by Bermudez et al. (Bermudez et al. 2004). Because rats were the most sensitive of the tested species, the data from the rats are used for the risk assessment. A NOAEC ($NOAEC_{Bermudez}$) of 0.5 mg/m^3 was identified for pulmonary influx of neutrophils immediately after end of exposure in rats exposed 6 hour/day, 5 days/week for 13 weeks to P25 TiO_2 NM (21nm, 80% anatase/20% rutile).

The calculations of INEL follow the approach given by ECHA (ECHA 2012):

First, the $NOAEC_{Bermudez}$ is modified to correct for an 8 hour working day (in Bermudez et al. (Bermudez et al. 2004) the rats were exposed 6 hour a day) and to correct for a higher breathing rate in workers (10 m^3/day) compared to 6.7 m^3/day at rest:

$$NOAEC_{Corrected} = NOAEC_{Bermudez} * 6 \text{ hour}/8 \text{ hour} * 6.7\ m^3/10\ m^3$$
$$= 0.25\ mg/m^3$$

Secondly, the corrected NOAEC is adjusted by a number of assessment factors (most of these are default values suggested by ECHA (ECHA 2012). The following assessment factors are used. To adjust for interspecies extrapolation, an assessment factor of 1.5 was used (default factor is 2.5) because the observed toxic effects do not involve metabolism and therefore there is no need for allometric scaling):

Interspecies extrapolation (default factor is 2.5):	1.5
Intraspecies interpolation (default factor for workers):	5
Extrapolation from sub-chronic to chronic (default factor):	2

The overall assessment factor,

$AF_{Total} = 1.5 * 5 * 2 = 15$

This results in an INEL for chronic inhalation for pulmonary inflammation of:

$INEL = NOAEC_{Corrected}/AF_{Total} = 0.25\ mg/m^3 / 15 = 0.017\ mg/m^3 = 17\ \mu g/m^3$

NEDO

The NEDO project also used the sub-chronic inhalation study by Bermudez et al. (Bermudez et al. 2004) as basis for calculation (Nakanishi and Gamo 2011). However, in contrast to Christensen et al. (Christensen et al. 2011), Nakanishi and Gamo chose to use a NOAEC ($NOAEC_{Bermudez}$) of 2.0 mg/m^3 for pulmonary influx of neutrophils in rats. From 4 weeks after end of exposure and the following time points the NOAEC is 2.0 mg/m^3, while the NOAEC used by Christensen et al (Christensen et al. 2011) is the NOAEC immediately after end of exposure (please see table 2 for details, paragraph on subacute studies).
The corrected NOAEC for human exposure is calculated to be 1.8 mg/m^3.
The assessment factor is: AF=3
This results in the following recommendation by Nakanishi and Gamo, 2011:
$OEL = NOAEC_{corrected}/AF = 1.8\ mg/m^3/3 = 0.61\ mg/m^3$

NIOSH

NIOSH suggested the following recommended airborne exposure limits (as time-weighted average (TWA) concentrations for up to 10 hr/day during a 40-hour week): 0.3 mg/m^3 for ultrafine (including engineered nanoscale) TiO_2 and 2.4 mg/m^3 for fine TiO_2. "These recommendations represent levels that over a working lifetime are estimated to reduce risks of lung cancer to below 1 in 1,000. The recommendations are based on using chronic inhalation studies in rats to predict lung tumor risks in humans."(NIOSH 2011).

NIOSH also derived exposure concentrations that are designed to prevent pulmonary inflammation. These are 0.004 mg/m^3 for ultrafine TiO_2 and 0.04 mg/m^3 for fine TiO_2. These were derived based on a benchmark dose analysis for pulmonary inflammation in rats followed by an extrapolation of the rat benchmark doses to humans. The starting points for the calculations were 0.9 mg/m^3 and 0.11 mg/m^3 for the fine and ultrafine TiO_2, respectively. Compared to the RELs accepting a risk of cancer below 1 out of 1,000 there would be a zero excess risk of cancer development due to secondary toxicity at exposure limits preventing pulmonary inflammation.

NIOSH showed that total deposited particle surface area of TiO_2 particles of different sizes (fine and ultrafine) and different crystal structure (80% anatase/20% rutile and 99%

rutile) can explain the observed variation in TiO_2 particle-induced pulmonary inflammation and lung cancer in rat inhalation studies: "…when rats were exposed to TiO_2 in sub-chronic inhalation studies, no difference in pulmonary inflammation response to fine and ultrafine TiO_2 particles of different crystal structure (i.e., 99% rutile vs. 80% anatase/20% rutile) was observed once dose was adjusted for particle surface area [Bermudez et al. 2002, 2004]; nor was there a difference in the lung tumor response in the chronic inhalation studies in rats at a given surface area dose of these fine and ultrafine particles (i.e., 99% rutile vs. 80% anatase/20% rutile) [Lee et al. 1985; Heinrich et al. 1995]. Therefore, NIOSH concludes that the scientific evidence supports surface area as the critical metric for occupational inhalation exposure to TiO_2."(NIOSH 2011).

Scaffold project

Recently, a recommendation of a limit value for TiO_2 NM was made within the frames of the EU project Scaffold and was published by Stockmann-Juvala et al.(Stockmann-Juvela et al. 2014).The Scaffold project identified pulmonary inflammation as the critical health effect for TiO_2. Similar to Christensen et al. (Christensen et al. 2011) and the NEDO project (Nakanishi and Gamo 2011), the project uses the sub-chronic inhalation study by Bermudez et al. (Bermudez et al. 2004) as basis for the calculation. Similar to Christensen et al. 2011, Stockmann-Juvala et al., 2014 uses a NOAEC ($NOAEC_{Bermudez}$) of 0.5 mg/m³ for pulmonary influx of neutrophils in rats immediately after end of exposure is chosen as starting point for the calculations.

First, the $NOAEC_{Bermudez}$ is modified to correct for an 8 hour working day (in Bermudez et al. (Bermudez et al. 2004) the rats were exposed 6 hour a day) and to correct for a higher breathing rate in workers (10 m³/day) compared to 6.7 m³/day at rest:

$NOAEC_{Corrected}$ = $NOAEC_{Bermudez}$ *6 hour/8 hour * 6.7 m³/10 m³
= 0.25 mg/m³

Secondly, the corrected NOAEC is adjusted by an assessment factor to take differences between sensitivity between individuals into account:

AF = 2.5

This results in an OEL for nanoTiO_2:

OEL = $NOAEC_{Corrected}$/AF = 0.25 mg/m³ / 2.5 = 0.1 mg/m³ = 100 μg/m³

No data were identified for the determination of an OEL for dermal exposure.

ECHA's Committee for Risk Assessment (RAC)

ECHA's Committee for Risk Assessment (RAC) has concluded that the available scientific evidence meets the criteria in the CLP Regulation to classify TiO_2 as a substance suspected of causing cancer through the inhalation route (RAC, 2017).

Summary of the evaluations

As shown in table 4, three of the recommendations use the same approach on the results from the same sub-chronic rat inhalation study (Bermudez et al. 2004). However, due to different choice of starting point and/or different assessment factors the derived recommendations are in the range from 17 μg/m^3 – 610 μg/m^3 (Christensen et al. 2011;Stockmann-Juvela et al. 2014;Nakanishi and Gamo 2011). Based on a benchmark dose approach NIOSH suggested the following recommended airborne exposure limits (as time-weighted average (TWA) concentrations for up to 10 hr/day during a 40-hour week): 0.3 mg/m^3 for ultrafine (including engineered nanoscale) TiO_2 and 2.4 mg/m^3 for fine TiO_2. "These recommendations represent levels that over a working lifetime are estimated to reduce risks of lung cancer to below 1 in 1,000. The recommendations are based on using chronic inhalation studies in rats to predict lung tumor risks in humans."(NIOSH 2011)

Table 4. Overview of suggested OELs for TiO_2 NM by different organizations/researchers

			Methodology for OEL development and reference/project			
		ENRHES (Christensen et al. 2011)	**NEDO (Nakanishi and Gamo 2011)**	**NIOSH (NIOSH 2011)**		**Scaffold (Stockmann-Juvela et al. 2014)**
Critical effect		Pulmonary inflammation	Pulmonary inflammation	Lung cancer[a]	Pulmonary inflammation[a]	Pulmonary inflammation
Key study		(Bermudez et al. 2004)	(Bermudez et al. 2004)	(Lee et al. 1985;Muhle et al. 1991;Heinrich et al. 1995)	(Bermudez et al. 2002;Bermudez et al. 2004;Cullen et al. 2002;Tran et al. 1999)	(Bermudez et al. 2004)
Risk determinant		NOAEC	NOAEC	BMDL associated with 1/1000 excess risk of cancer	BMD Particle surface area per gram of lung tissue associated with 4% inflammatory response of neutrophils	NOAEC
Risk level in rodents		0.5 mg/m^3	2 mg/m^3			0.5 mg/m^3
Corrected starting point		0.25 mg/m^3[a]		0.29 mg/m^3	0.11 mg/m^3	0.25 mg/m^3
Uncertainty factors						
	Interspecies	1.5	3		2.5	1
	Intraspecies	5	1		10	2.5
	Sub-chronic to chronic	2	1		-	1
Overall uncertainty factor		15	3		25	2.5
Suggested OEL		0.017 mg/m^3 (17 μg/m^3)	0.61 mg/m^3 (610 μg/m^3)	0.3 mg/m^3 (300 μg/m^3)	0.004 mg/m^3 (4 μg/m^3)	0.1 mg/m^3 (100 μg/m^3)

[a]NIOSH calculated exposure limits based on both pulmonary inflammation and lung cancer. However, NIOSH's final recommendation is based on lung cancer rather than pulmonary inflammations. For transparency, both results are shown in the table.

SCIENTIFIC BASIS FOR SETTING AN OCCUPATIONAL EXPOSURE LIMIT

Different methods exist for calculating OELs. The choice of method depends on the mode of action of the substance, and can fundamentally be split up in two approaches: Threshold effects or non-threshold effects. The threshold effect approach relies on the assumption that the organism can withstand a certain dose before adverse effects occur, whereas for non-threshold effects it is assumed that any exposure to the substance can result in adverse effects. In this report, we will calculate proposed OELs based both on threshold effects and non-threshold effects.

Endpoint: Inflammation

Pulmonary inflammation is a defense mechanism when particles or other types of foreign material enter the lungs. Particle-induced pulmonary inflammation is considered to be one of the key steps leading to lung cancer by a secondary genotoxic mechanism (NIOSH 2011). Furthermore there is a close interplay between inflammation, the acute phase response and cardiovascular plaque progression (Poulsen et al. 2015a;Saber et al. 2013). Thus, the derivation of a DNEL based on inflammation has been made under the assumption of a threshold-driven mechanism of TiO_2 NM toxicity: TiO_2 NM induced oxidative stress/inflammation which may result in other effects such as e.g. cancer and cardiovascular disease.

Our approach for an OEL for TiO_2 NM follows the traditional approach for setting health-based OELs: 1) identification of critical effect, 2) identification of the NOAEC, 3) calculation of OEL using assessment factors adjusting for inter and intra species differences).

In the current report we use the DNEL as recommended by ECHA as the OEL for toxicological effects having thresholds (ECHA 2012).

The DNEL of 10 $\mu g/m^3$ is derived based on the sub-chronic inhalation study of mice, rats and hamsters by Bermudez et al. (Bermudez et al. 2004). Rats were the most sensitive of the tested species, and the data from the rats are used for the DNEL derivation. A NOAEC ($NOAEC_{Bermudez}$) of 0.5 mg/m^3 was identified for pulmonary influx of neutrophils immediately after end of exposure in rats exposed 6 hour/day, 5 days/week for 13 weeks to P25 TiO_2 NM (21nm, 80% anatase/20% rutile). Histopathological changes in the lungs were dose and time dependent.
The study by Bermudez et al (Bermudez et al. 2004) is the only sub-chronic dose-response inhalation study with TiO_2 NM identified. In addition to this study we have identified a range of inhalation studies of shorter exposure time using TiO_2 particles (Ma-Hock et al. 2009;Noel et al. 2012;Rossi et al. 2010a;Rossi et al. 2010b;Baisch et al. 2014;Lindberg et al. 2012). Overall they support that a NOAEC level is in the range of 0.5-2 mg/m^3.

The calculations of the DNEL follow the approach as set out in the REACH guidance (ECHA 2012):

First, the $NOAEC_{Bermudez}$ is modified to correct for an 8 hour working day (in Bermudez et al. (Bermudez et al. 2004)) the rats were exposed 6 hour a day) and to correct for a higher breathing rate in workers at light work (10 m^3/day) compared to 6.7 m^3/day at rest:

$NOAEC_{Corrected}$ = $NOAEC_{Bermudez}$ *6 hour/8 hour * 6.7 m^3/10 m^3
= 0.25 mg/m^3

Secondly, the corrected NOAEC is adjusted by a number of assessment factors (most of these are default values suggested by ECHA.
Inflammation is considered an acute response. Due to the accumulation of particles over time, we have chosen to use the default assessment factor 2 to extrapolate from sub-chronic to chronic exposure. The following default assessment factors are used:

Interspecies extrapolation:	2.5
Intraspecies interpolation (default factor for workers):	5
Extrapolation from sub-chronic to chronic:	2

The overall assessment factor (AF_{Total}),

AF_{Total} = 2.5 * 5 * 2 = 25

This results in a DNEL for chronic inhalation for pulmonary inflammation of:

DNEL = $NOAEC_{Corrected}/AF_{Total}$ = 0.25 mg/m^3 / 25 = 0.01 mg/m^3 = 10 $\mu g/m^3$

Endpoint: Cancer

The present working group has chosen not to use the epidemiological study by Ellis et al. (Ellis et al. 2013) as basis for an OEL suggestion for several reasons, including the lack of information on particle size, lack of dose-response relationship between lung cancer incidence and cumulative TiO_2 dose, lack of description of the reference groups including information on exposure, lack of information about the lung cancer incidence in the reference groups.

Instead, the derivation of an OEL based on cancer has been made under the assumption of a non-threshold driven mechanism of TiO_2 NM toxicity.

The OEL is derived based on the chronic inhalation study of mice and rats by Heinrich et al. (Heinrich et al. 1995). Lung tumor rate in mice exposed to TiO_2 was not statistically different from the lung tumor rate in mice exposed to filtered air. Therefore, as the most sensitive of the tested species, data from the rats are used for the risk assessment.

The lowest effect level for lung cancer was observed in rats, where increased lung cancer incidence was found at 10 mg/m^3. Lung cancer incidence in TiO_2 exposed rats was 32% (32/100), while the cancer incidence in control rats was 0.5% (1/217).

Increased lung cancer incidence was observed in rats at 10 mg/m^3. Both malignant and non-malignant tumors were included in accordance with the REACH guideline stating that: "malignant tumours as well as benign tumours that are suspected of possibly progressing to malignant tumours are taken into account in obtaining the dose-descriptor values" (ECHA, 2012).

Table 5. Cancer incidence and TiO_2 lung burden at different TiO_2 mass air concentrations in a chronic inhalation study (Heinrich et al. 1995).

	0 mg/m^3	**10 mg/m^3**
Total cancer incidence	1/217	32/100
TiO_2 lung burden (mg/lung)		39

Observed excess cancer incidence at 10 mg/m^3:
(32/100- 1/217)/(1-1/217)= 0.32 =32 %

Method I

The present working group has chosen to use the approach used by Kasai et al (Kasai et al. 2016) and Erdely et al (Erdely et al. 2013), who use the measured lung burden in rats exposed by inhalation and the alveolar surface area of rats and humans to estimate the human equivalent lung burden:

At 10 mg/m^3, the amount of pulmonary deposited TiO_2 after 2 years of inhalation was determined to be 39 mg/rat lung (Heinrich et al. 1995).

Human lung burden equals:
Rat lung burden (39 mg) × Human alveolar surface area (102 m^2) / rat alveolar surface area (0.4 m^2) = 9945 mg per human lung.

We assume using standard values that human ventilation is 20 L/min during light work (1.2 m^3/h), work related exposure for 8 h per day, 5 days per week, 45 working weeks per year, over a working lifetime of 45 years. The deposition rate was not reported to in the Heinrich study. For the calculation, we have used a deposition of 8.6% based on an inhalation study by (Hougaard et al. 2010). In that study, mice were exposed by inhalation 1h/day for 11 days to 42 mg/m^3 aerosolized powder of rutile TiO_2 with an average crystallite size of 21 nm. The pulmonary deposition fraction was estimated to be 8.6% based on the observed particle size distribution in the aerosol.

A lung burden of 9945 mg in humans would require that workers are exposed:

Air concentration = 9945 mg/(8h/day x 5 day/week x 45 weeks/year x 45 years x 1.2 m^3/h x 0.086) = 1.2 mg/m^3.

Thus, at an air concentration of 1.2 mg/m^3 during a 45 year work life, an excess lung cancer incidence of 32% is expected.

Assuming a linear dose-response relationship, then 1% excess lung cancer is expected at (1.2 mg/m^3)/32 = 0.04 mg/m^3 (40 μg/m^3).

The TiO_2 NM air concentrations resulting in different excess lung cancer incidences are given in the table below.

Table 6. Calculated excess lung cancer incidences at different TiO_2 NM mass concentrations based on method I.

Excess lung cancer incidence	TiO_2 NM Air concentration (μg/m^3)
1:1 000	4
1: 10 000	0.4
1: 100 000	0.04

Method II

ECHA (ECHA 2012; SCHER/SCCP/SCENIHR 2009), calculated based on the two year TiO_2 NM inhalation study in rats by (Heinrich et al. 1995) (Table 5):

Excess cancer risk:

Observed excess cancer incidence at 10 mg/m^3:

(32/100- 1/217)/(1-1/217)= 0.32 =32 %

Correction of dose metric for humans during occupational exposure (8h/d):

10 mg/m^3 x (18 h/day)/(8 h/day) x (6.7 m^2/10 m^2) = 15 mg/m^3

Calculation of unit risk for cancer:

Risk level = exposure level x unit risk
0.32 = 15 000 μg/m^3 x unit risk
Unit risk = 2.1 x 10^{-5} per μg/m^3

At a dose of 1 μg/m^3, 2.1 x 10^{-5} excess cancers are expected.

Calculation of dose levels corresponding to risk level of 10^{-5} (and other risk levels)

10^{-5} risk level = exposure level x unit risk (2.1 x 10^{-5} per μg/m^3)
Exposure level (10^{-5}) = 0.47 μg/m^3

Thus, at 0.47 μg/m^3, 1:100 000 excess lung cancer cases can be expected.

Table 7. Calculated excess lung cancer incidence at different TiO_2 NM mass concentrations based on method II.

Excess lung cancer incidence	TiO_2 NM Air concentration ($\mu g/m^3$)
1:1,000	47
1: 10,000	4.7
1: 100,000	0.47

CONCLUSION

The present working group evaluated the relevant literature on TiO_2 NM from both epidemiological and animal inhalation studies. None of the identified epidemiological studies provided information on the particle size range of the TiO_2, thus making it impossible to determine whether the exposures included TiO_2 NM. Therefore it was decided to base the suggested health-based OEL on data from experimental animal studies.

Pulmonary inflammation and carcinogenicity was observed in sub-chronic and chronic inhalation studies in rats. The present working group regards inflammation and carcinogenicity as the critical adverse effects and the subsequent risk assessments are conducted based on studies reporting these effects. TiO_2 NM induced cardiovascular effects were also identified in animal studies. But as none of these studies were sub-chronic or chronic inhalation studies, they were not suitable for risk assessment. However, the present working group regards the acute phase response as a biomarker of cardiovascular effects. Due to the close association between pulmonary inflammation and the acute phase response, the present working group regards inflammation as a proxy for cardiovascular effects.

The present working group found strong dose response relationships for neutrophil influx as a marker of pulmonary inflammation (Bermudez et al. 2004). Neutrophil influx was predicted by deposited surface area. The working group considers inflammation as a threshold effect.

The present working group found that the mechanism of action of the carcinogenic effect has not been fully clarified. Secondary genotoxicity due to particle-induced inflammation is an important and well documented mechanism of action for the development of lung cancer. However, the available data did not allow ruling out that TiO_2 NM could also induce cancer through a direct genotoxic mechanism. Therefore, the present working group considers carcinogenicity as a non-threshold effect. Consequently, the present working group decided to perform the risk assessment based on both a threshold and a non-threshold mechanism of action.

The working group considered that data from two rat inhalation studies were the best basis for risk assessment. The following studies were selected to be used for calculation of DNEL and excess cancer risk, respectively: A 13 week sub-chronic inhalation study in rats (0, 0.5, 2.0 and 10 mg/m^3) (Bermudez et al. 2004) and a 2 year chronic cancer inhalation study in rats (0 and 10 mg/m^3) (Heinrich et al. 1995). Table 8 shows a DNEL for pulmonary inflammation derived based on the sub-chronic inhalation study of rats as the most sensitive of three tested species, and exess lung cancer risk at 1 in 1 000, 1 in 10 000 and 1 in 100 000 derived using two different approaches.

Table 8. Overview of DNEL based on a threshold based mechanism of action and exposure levels resulting in extra cancer risk levels at 1:1000, 1:10 000 and 1: 100 000 based on a non-threshold based mechanism of action using two different approaches.

		Suggestion of an OEL for TiO_2 NM		
		Inflammation	**Lung Cancer (method I)**	**Lung cancer (method II)**
Threshold based	DNEL	10 μg/m³		
Non-threshold based	Extra cancer risk			
	1:1000		4 μg/m³	47
	1:10 000		0.4 μg/m³	4.7
	1:100 000		0.04 μg/m³	0.47 μg/m³

Both of studies used for the risk assessment used P25 TiO_2 NM (15-40 nm diameter, 80% anatase/20% rutile). TiO_2 NMs differ regarding size and surface area but also coating, shape, crystal structure etc. The present working group notes that there is limited available data on the biological effects of different physico-chemical properties, but the present working group concludes that the majority of available data support that the surface area (and therefore the size) of TiO_2 is a critical driver of particle-induced inflammation and the acute phase response in the lungs.

The present working group also notes that NIOSH showed that particle surface area of TiO_2 particles of different sizes (fine and ultrafine) and different crystal structure (80% anatase/20% rutile and 99% rutile) can explain the observed variation in TiO_2 particle-induced pulmonary inflammation and lung cancer in rat inhalation studies. This stresses the importance of the surface area as a predictor for the inflammatory and carcinogenic response.

The present working group regards cancer as the most critical effect.
The DNEL approach relies heavily on the assumption of a threshold effect on inflammation and carcinogenicity. The present working group is of the opinion that there is still uncertainly whether this is the case for TiO_2 NM –induced carcinogenicity.

Two different approaches were used for calculating excess lung cancer risk based on the same chronic inhalation study. In the first approach, lung burden in rats after two years of exposure was used to estimate the exposure levels for occupational exposure. In the second approach, air concentrations were used directly. Independently of the applied method for risk assessment, the resulting exposure levels were all very low. These levels are all more than 100-fold lower than the current Danish OEL for titanium of 6 mg/m³ (measured as Ti, corresponding to 10 mg/m³ for TiO_2).

The present working group recommends the approach using the excess lung cancer risk estimates based on lung burden, since this approach takes the actual retained pulmonary dose into account. Thus, the expected excess lung cancer risk based on lung burden approach is 1:1 000 at 4 μg/m³, 1:10 000 at 0.4 μg/m³ and 1:100 000 at 0.04 μg/m³ TiO_2 NM.

REFERENCES

American Cancer Society. Lifetime risk of developing or dying from cancer. American Cancer Society, 2018. https://www.cancer.org/cancer/cancer-basics/lifetime-probability-of-developing-or-dying-from-cancer.html

Artl A, Marsche G, Lestavel S, Sattler W, Malle E. Role of serum amyloid A during metabolism of acute-phase HDL by macrophages. Arterioscler Thromb Vasc Biol 2000;20:763-772.

Baisch BL, Corson NM, Wade-Mercer P, Gelein R, Kennell AJ, Oberdörster G, Elder A. Equivalent titanium dioxide nanoparticle deposition by intratracheal instillation and whole body inhalation: The effect of dose rate on acute respiratory tract inflammation. Part Fibre Toxicol 2014;11:5.

Bermudez E, Mangum JB, Asgharian B, Wong BA, Reverdy EE, Janszen DB, Hext PM, Warheit DB, Everitt JI. Long-term pulmonary responses of three laboratory rodent species to subchronic inhalation of pigmentary titanium dioxide particles. Toxicol Sci 2002;70:86-97.

Bermudez E, Mangum JB, Wong BA, Asgharian B, Hext PM, Warheit DB, Everitt JI. Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles. Toxicol Sci 2004;77:347-357.

Boffetta P, Gaborieau V, Nadon L, Parent ME, Weiderpass E, Siemiatycki J. Exposure to titanium dioxide and risk of lung cancer in a population-based study from Montreal. Scand J Work Environ Health 2001;27:227-232.

Boffetta P, Soutar A, Cherrie JW, Granath F, Andersen A, Anttila A, Blettner M, Gaborieau V, Klug SJ, Langard S, Luce D, Merletti F, Miller B, Mirabelli D, Pukkala E, Adami HO, Weiderpass E. Mortality among workers employed in the titanium dioxide production industry in Europe. Cancer Causes Control 2004;15:697-706.

Boisen AM, Shipley T, Jackson P, Hougaard KS, Wallin H, Yauk CL, Vogel U. NanoTIO(2) (UV-Titan) does not induce ESTR mutations in the germline of prenatally exposed female mice. Part Fibre Toxicol 2012;9:19.

Bourdon JA, Saber AT, Jacobsen NR, Jensen KA, Madsen AM, Lamson JS, Wallin H, Møller P, Loft S, Yauk CL, Vogel UB. Carbon black nanoparticle instillation induces sustained inflammation and genotoxicity in mouse lung and liver. Part Fibre Toxicol 2012;9:5.

Cancer Risks UK. Worldwide cancer mortality statistics. Cancer Risks UK. 2018. https://www.cancerresearchuk.org/health-professional/cancer-statistics/worldwide-cancer/mortality

Chen JL, Fayerweather WE. Epidemiologic study of workers exposed to titanium-dioxide. J Occup Environ Med 1988;30:937-942.

Chen T, Hu J, Chen C, Pu J, Cui X, Jia G. Cardiovascular effects of pulmonary exposure to titanium dioxide nanoparticles in ApoE knockout mice. J Nanosci Nanotechnol 2013;13:3214-3222.

Christensen FM, Johnston HJ, Stone V, Aitken RJ, Hankin S, Peters S, Aschberger K. Nano-TiO_2--feasibility and challenges for human health risk assessment based on open literature. Nanotoxicology 2011; 5:110-124.

Cullen RT, Jones AD, Miller BG, Tran CL, Davis JMG, Donaldson K, Wilson M, Stone V, Morgan A. Toxicity of volcanic ash from Montserrat. Research Report TM/02/01. Edingburgh: Institute of Occupational Medicine, 2002.

Cybulsky MI, Iiyama K, Li H, Zhu S, Chen M, Iiyama M, Davis V, Gutierrez-Ramos JC, Connelly PW, Milstone DS. A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. J Clin Invest 2001;107:1255-1262.

Debia M, Bakhiyi B, Ostiguy C, Verbeek JH, Brouwer DH, Murashov V. A systematic review of reported exposure to engineered nanomaterials. Ann Occup Hyg 2016;60:916-935.

ECHA. Guidance on information requirements and chemical safety assessment. Appendix R8-15 Recommendations for nanomaterials applicable to Chapter R.8 Characterisation of dose [concentration] - response for human health. ECHA-12-G-09-EN. European Chemicals Agency, 2012.

ECHA. Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health. ECHA-2010-G-19-EN. European Chemicals Agency, 2012.

Ellis ED, Watkins J, Tankersley W, Phillips J, Girardi D. Mortality among titanium dioxide workers at three DuPont plants. J Occup Environ Med 2010;52:303-309.

Ellis ED, Watkins JP, Tankersley WG, Phillips JA, Girardi DJ. Occupational exposure and mortality among workers at three titanium dioxide plants. Am J Ind Med 2013;56:282-291.

Engler-Chiurazzi EB, Stapleton PA, Stalnaker JJ, Ren X, Hu H, Nurkiewicz TR, McBride CR, Yi J, Engels K, Simpkins JW. Impacts of prenatal nanomaterial exposure on male adult Sprague-Dawley rat behavior and cognition. J Toxicol Environ Health A 2016;79:447-452.

Erdely A, Dahm M, Chen BT, Zeidler-Erdely PC, Fernback JE, Birch ME, Evans DE, Kashon ML, Deddens JA, Hulderman T, Bilgesu SA, Battelli L, Schwegler-Berry D, Leonard HD, McKinney W, Frazer DG, Antonini JM, Porter DW, Castranova V, Schubauer-Berigan MK. Carbon nanotube dosimetry: from workplace exposure assessment to inhalation toxicology. Part Fibre Toxicol 2013;10:53.

European Commission. Definition of a nanomaterial. Last update: 22-2-2017. European Commission, 2018.
http://ec.europa.eu/environment/chemicals/nanotech/faq/definition_en.htm

Ferin J, Oberdörster G, Penney DP. Pulmonary Retention of Ultrafine and Fine Particles in Rats. Am J Respir Cell Mol Biol 1992;6:535-542.

Fryzek JP, Chadda B, Marano D, White K, Schweitzer S, McLaughlin JK, Blot WJ. A cohort mortality study among titanium dioxide manufacturing workers in the United States. J Occup Environ Med 2003;45:400-409.

Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448-454.

Geiser M, Casaulta M, Kupferschmid B, Schulz H, Semmler-Behnke M, Kreyling W. The role of macrophages in the clearance of inhaled ultrafine titanium dioxide particles. Am J Respir Cell Mol Biol 2008;38:371-376.

Geiser M, Kreyling WG. Deposition and biokinetics of inhaled nanoparticles. Part Fibre Toxicol 2010;7:2.

Haberl N, Hirn S, Holzer M, Zuchtriegel G, Rehberg M, Krombach F. Effects of acute systemic administration of TiO2, ZnO, SiO2, and Ag nanoparticles on hemodynamics, hemostasis and leukocyte recruitment. Nanotoxicology 2015;9:963-971.

Halappanavar S, Jackson P, Williams A, Jensen KA, Hougaard KS, Vogel U, Yauk CL, Wallin H. Pulmonary response to surface-coated nanotitanium dioxide particles includes induction of acute phase response genes, inflammatory cascades, and changes in microRNAs: A toxicogenomic study. Environ Mol Mutagen 2011;52:425-439.

Hansson GK, Libby P. The immune response in atherosclerosis: A double-edged sword. Nat Rev Immunol 2006;6:508-519.

Hathaway QA, Nichols CE, Shepherd DL, Stapleton PA, McLaughlin SL, Stricker JC, Rellick SL, Pinti MV, Abukabda AB, McBride CR, Yi J, Stine SM, Nurkiewicz TR, Hollander JM. Maternal-engineered nanomaterial exposure disrupts progeny cardiac function and bioenergetics. Am J Physiol Heart Circ Physiol 2017;312:H446-H458.

Heinrich U, Fuhst R, Rittinghausen S, Creutzenberg O, Bellmann B, Koch W, Levsen K. Chronic inhalation exposure of wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide. Inhal Toxicol 1995;7:533-556.

Hougaard KS, Jackson P, Jensen KA, Sloth JJ, Löschner K, Larsen EH, Birkedal RK, Vibenholt A, Boisen AM, Wallin H, Vogel U. Effects of prenatal exposure to surface-coated nanosized titanium dioxide (UV-Titan). A study in mice. Part Fibre Toxicol 2010;7:16.

Husain M, Saber AT, Guo C, Jacobsen NR, Jensen KA, Yauk CL, Williams A, Vogel U, Wallin H, Halappanavar S. Pulmonary instillation of low doses of titanium dioxide nanoparticles in mice leads to particle retention and gene expression changes in the absence of inflammation. Toxicol Appl Pharmacol 2013;269:250-262.

Husain M, Wu D, Saber AT, Decan N, Jacobsen NR, Williams A, Yauk CL, Wallin H, Vogel U, Halappanavar S. Intratracheally instilled titanium dioxide nanoparticles translocate to heart and liver and activate complement cascade in the heart of C57BL/6 mice. Nanotoxicology 2015;9:1013-1022.

IARC. Carbon Black, Titanium Dioxide, and Talc. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 93. Lyon: WHO, The International Agency for Research on Cancer, 2010.

Jackson P, Halappanavar S, Hougaard KS, Williams A, Madsen AM, Lamson JS, Andersen O, Yauk C, Wallin H, Vogel U. Maternal inhalation of surface-coated nanosized titanium dioxide (UV-Titan) in C57BL/6 mice: Effects in prenatally exposed offspring on hepatic DNA damage and gene expression. Nanotoxicology 2013;7:85-96.

Johnson BD, Kip KE, Marroquin OC, Ridker PM, Kelsey SF, Shaw LJ, Pepine CJ, Sharaf B, Bairey Merz CN, Sopko G, Olson MB, Reis SE. Serum amyloid A as a predictor of coronary artery disease and cardiovascular outcome in women: The National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). Circulation 2004;109:726-732.

Kasai T, Umeda Y, Ohnishi M, Mine T, Kondo H, Takeuchi T, Matsumoto M, Fukushima S. Lung carcinogenicity of inhaled multiwalled carbon nanotube in rats. Part Fibre Toxicol 2016;13:53.

Kinaret P, Ilves M, Fortino V, Rydman E, Karisola P, Lahde A, Koivisto J, Jokiniemi J, Wolff H, Savolainen K, Greco D, Alenius H. Inhalation and oropharyngeal aspiration exposure to rod-like carbon nanotubes induce similar airway inflammation and biological responses in mouse lungs. ACS Nano 2017;11:291-303.

Koivisto AJ, Lyyranen J, Auvinen A, Vanhala E, Hameri K, Tuomi T, Jokiniemi J. Industrial worker exposure to airborne particles during the packing of pigment and nanoscale titanium dioxide. Inhal Toxicol 2012;24:839-849.

Kreyling WG, Holzwarth U, Haberl N, Kozempel J, Hirn S, Wenk A, Schleh C, Schaffler M, Lipka J, Semmler-Behnke M, Gibson N. 2017a. Quantitative biokinetics of titanium dioxide nanoparticles after intravenous injection in rats: Part 1. Nanotoxicology 2017a;11:434-442.

Kreyling WG, Holzwarth U, Haberl N, Kozempel J, Wenk A, Hirn S, Schleh C, Schaffler M, Lipka J, Semmler-Behnke M, Gibson N. Quantitative biokinetics of titanium dioxide nanoparticles after intratracheal instillation in rats: Part 3. Nanotoxicology 2017b;11:454-464.

Kreyling WG, Holzwarth U, Schleh C, Kozempel J, Wenk A, Haberl N, Hirn S, Schaffler M, Lipka J, Semmler-Behnke M, Gibson N. Quantitative biokinetics of titanium dioxide nanoparticles after oral application in rats: Part 2. Nanotoxicology 2017c;11:443-453.

Kwon S, Yang YS, Yang HS, Lee J, Kang MS, Lee BS, Lee K, Song CW. Nasal and pulmonary toxicity of titanium dioxide nanoparticles in rats. Toxicol Res 2012;28:217-224.

Kyjovska ZO, Boisen AM, Jackson P, Wallin H, Vogel U, Hougaard KS. Daily sperm production: Application in studies of prenatal exposure to nanoparticles in mice. Reprod Toxicol 2013;36:88-97.

Lee HY, Kim SD, Baek SH, Choi JH, Cho KH, Zabel BA, Bae YS. Serum amyloid A stimulates macrophage foam cell formation via lectin-like oxidized low-density lipoprotein receptor 1 upregulation. Biochem Biophys Res Commun 2013;433:18-23.

Lee KP, Trochimowicz HJ, Reinhardt CF. Pulmonary response of rats exposed to titanium dioxide (TiO2) by inhalation for two years. Toxicol Appl Pharmacol 1985;79:179-192.

Libby P. Inflammation in atherosclerosis. Nature 2002;420:868-874.

Lindberg HK, Falck GC, Catalán J, Koivisto AJ, Suhonen S, Järventaus H, Rossi EM, Nykäsenoja H, Peltonen Y, Moreno C, Alenius H, Tuomi T, Savolainen KM, Norppa H. Genotoxicity of inhaled nanosized TiO(2) in mice. Mutat Res 2012;745:58-64.

Lindhorst E, Young D, Bagshaw W, Hyland M, Kisilevsky R. Acute inflammation, acute phase serum amyloid A and cholesterol metabolism in the mouse. Biochim Biophys Acta 1997;1339:143-154.

Lowe GD. The relationship between infection, inflammation, and cardiovascular disease: an overview. Ann Periodontol 2001;6:1-8.

Ma-Hock L, Burkhardt S, Strauss V, Gamer AO, Wiench K, van Ravenzwaay B, Landsiedel R. Development of a short-term inhalation test in the rat using nano-titanium dioxide as a model substance. Inhal Toxicol 2009;21:102-118.

Mezaki T, Matsubara T, Hori T, Higuchi K, Nakamura A, Nakagawa I, Imai S, Ozaki K, Tsuchida K, Nasuno A, Tanaka T, Kubota K, Nakano M, Miida T, Aizawa Y. Plasma levels of soluble thrombomodulin, C-reactive protein, and serum amyloid A protein in the atherosclerotic coronary circulation. Jpn Heart J 2003;44:601-612.

Mikkelsen L, Sheykhzade M, Jensen KA, Saber AT, Jacobsen NR, Vogel U, Wallin H, Loft S, Møller P. Modest effect on plaque progression and vasodilatory function in atherosclerosis-prone mice exposed to nanosized TiO(2). Part Fibre Toxicol 2011;8:32.

Morimoto Y, Hirohashi M, Ogami A, Oyabu T, Myojo T, Todoroki M, Yamamoto M, Hashiba M, Mizuguchi Y, Lee BW, Kuroda E, Shimada M, Wang WN, Yamamoto K, Fujita K, Endoh S, Uchida K, Kobayashi N, Mizuno K, Inada M, Tao H, Nakazato T, Nakanishi J, Tanaka I. Pulmonary toxicity of well-dispersed multi-wall carbon nanotubes following inhalation and intratracheal instillation. Nanotoxicology 2012;6:587-599.

Muhle H, Bellmann B, Creutzenberg O, Dasenbrock C, Ernst H, Kilpper R, MacKenzie JC, Morrow P, Mohr U, Takenaka S. Pulmonary response to toner upon chronic inhalation exposure in rats. Fundam Appl Toxicol 1991;17:280-299.

Nakanishi J, Gamo M. Risk Assessment of Manufactured Nanomaterials Titanium dioxide (TiO2). Final report issued on July 22, 2011. NEDO project P06041 "Research and development of nanoparticle characterization methods". NEDO, 2011.

Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Arterioscler Thromb 1994;14:133-140.

NIOSH. Occcupational exposure to Titanium dioxide. Current Intelligence Bulletin 63. Cincinnati, OH: Department of Health and Human Services; Centers for Disease Control and Prevention; National Institute for Occupational Safety and Health, 2011.

Noel A, Maghni K, Cloutier Y, Dion C, Wilkinson KJ, Halle S, Tardif R, Truchon G. Effects of inhaled nano-TiO2 aerosols showing two distinct agglomeration states on rat lungs. Toxicol Lett 2012;214:109-119.

Nurkiewicz TR, Porter DW, Hubbs AF, Stone S, Chen BT, Frazer DG, Boegehold MA, Castranova V. Pulmonary nanoparticle exposure disrupts systemic microvascular nitric oxide signaling. Toxicol Sci 2009;110:191-203.

Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 2005;113:823-839.

OECD Environment HaSP. Titanium dioxide: Summary of the dossier. Series on the Safety of Manufactured Nanomaterials: 73. Paris: OECD, 2016.

Pelclova D, Zdimal V, Fenclova Z, Vlckova S, Turci F, Corazzari I, Kacer P, Schwarz J, Zikova N, Makes O, Syslova K, Komarc M, Belacek J, Navratil T, Machajova M, Zakharov S. Markers of oxidative damage of nucleic acids and proteins among workers exposed to TiO2 (nano) particles. Occup Environ Med 2016a;73:110-118.

Pelclova D, Zdimal V, Kacer P, Fenclova Z, Vlckova S, Komarc M, Navratil T, Schwarz J, Zikova N, Makes O, Syslova K, Belacek J, Zakharov S. Leukotrienes in exhaled breath condensate and fractional exhaled nitric oxide in workers exposed to TiO2 nanoparticles. J Breath Res 2016b;10:036004.

Pelclova D, Zdimal V, Kacer P, Zikova N, Komarc M, Fenclova Z, Vlckova S, Schwarz J, Makes O, Syslova K, Navratil T, Turci F, Corazzari I, Zakharov S, Bello D. Markers of lipid oxidative damage in the exhaled breath condensate of nano TiO2 production workers. Nanotoxicology 2017;11:52-63.

Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003;111:1805-1812.

Porter DW, Hubbs AF, Chen BT, McKinney W, Mercer RR, Wolfarth MG, Battelli L, Wu N, Sriram K, Leonard S, Andrew M, Willard P, Tsuruoka S, Endo M, Tsukada T, Munekane F, Frazer DG, Castranova V. Acute pulmonary dose-responses to inhaled multi-walled carbon nanotubes. Nanotoxicology 2013;7:1179-1194.

Poulsen SS, Saber AT, Mortensen A, Szarek J, Wu D, Williams A, Andersen O, Jacobsen NR, Yauk CL, Wallin H, Halappanavar S, Vogel U. Changes in cholesterol homeostasis and acute phase response link pulmonary exposure to multi-walled carbon nanotubes to risk of cardiovascular disease. Toxicol Appl Pharmacol 2015a;283:210-222.

Poulsen SS, Saber AT, Williams A, Andersen O, Købler C, Atluri R, Pozzebon ME, Mucelli SP, Simion M, Rickerby D, Mortensen A, Jackson P, Kyjovska ZO, Mílhave K, Jacobsen NR, Jensen KA, Yauk CL, Wallin H, Halappanavar S, Vogel U. MWCNTs of different physicochemical properties cause similar inflammatory responses, but differences in transcriptional and histological markers of fibrosis in mouse lungs. Toxicol Appl Pharmacol 2015b;284.:16-32.

RAC. Titanium dioxide proposed to be classified as suspected of causing cancer when inhaled. ECHA's Committee for Risk Assessment (RAC). RAC, 2017.

Ramanakumar AV, Parent ME, Latreille B, Siemiatycki J. Risk of lung cancer following exposure to carbon black, titanium dioxide and talc: Results from two case-control studies in Montreal. Int J Cancer 2008;122:183-189.

Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836-843.

Rossi EM, Pylkkanen L, Koivisto AJ, Nykasenoja H, Wolff H, Savolainen K, Alenius H. Inhalation exposure to nanosized and fine TiO2 particles inhibits features of allergic asthma in a murine model. Part Fibre Toxicol 2010a;7:35.

Rossi EM, Pylkkänen L, Koivisto AJ, Vippola M, Jensen KA, Miettinen M, Sirola K, Nykäsenoja H, Karisola P, Stjernvall T, Vanhala E, Kiilunen M, Pasanen P, Mäkinen M, Hämeri K, Joutsensaari J, Tuomi T, Jokiniemi J, Wolff H, Savolainen K, Matikainen S, Alenius H. Airway exposure to silica-coated TiO2 nanoparticles induces pulmonary neutrophilia in mice. Toxicol Sci 2010b;113:422-433.

Saber AT, Jacobsen NR, Jackson P, Poulsen SS, Kyjovska ZO, Halappanavar S, Yauk CL, Wallin H, Vogel U. Particle-induced pulmonary acute phase response may be the causal link between particle inhalation and cardiovascular disease.
Wiley Interdiscip Rev Nanomed Nanobiotechnol 2014;6:517-531.

Saber AT, Jacobsen NR, Mortensen A, Szarek J, Jackson P, Madsen AM, Jensen KA, Koponen IK, Brunborg G, Gutzkow KB, Vogel U, Wallin H. Nanotitanium dioxide toxicity in mouse lung is reduced in sanding dust from paint. Part Fibre Toxicol 2012a;9:4.

Saber AT, Jensen KA, Jacobsen NR, Birkedal R, Mikkelsen L, Møller P, Loft S, Wallin H, Vogel U. Inflammatory and genotoxic effects of nanoparticles designed for inclusion in paints and lacquers. Nanotoxicology 2012b;6:453-471.

Saber AT, Lamson JS, Jacobsen NR, Ravn-Haren G, Hougaard KS, Nyendi AN, Wahlberg P, Madsen AM, Jackson P, Wallin H, Vogel U. Particle-induced pulmonary acute phase response correlates with neutrophil influx linking inhaled particles and cardiovascular risk. PLoS One 2013;8:e69020.

SCHER/SCCP/SCENIHR. Risk assessment methodologies and approaches for genotoxic and carcinogenic substances. European Commission Health & Consumer Protection, 2009. http://ec.europa.eu/health/ph_risk/committees/04_scher/docs/scher_o_113.pdf

Schmid O, Cassee FR. On the pivotal role of dose for particle toxicology and risk assessment: Exposure is a poor surrogate for delivered dose. Part Fibre Toxicol 2017;14:52.

Scientific Committee on Consumer Safety (SCCS). Opinion on Titanium Dioxide (nano form) as UV-filter in sprays. SCCS/1583/17. Luxembourg: European Commission, 2017.

Shi H, Magaye R, Castranova V, Zhao J. Titanium dioxide nanoparticles: a review of current toxicological data. Part Fibre Toxicol 2013;10:15.

Stapleton PA, Hathaway QA, Nichols CE, Abukabda AB, Pinti MV, Shepherd DL, McBride CR, Yi J, Castranova VC, Hollander JM, Nurkiewicz TR. Maternal engineered nanomaterial inhalation during gestation alters the fetal transcriptome.
Part Fibre Toxicol 2018a;15:3.

Stapleton PA, McBride CR, Yi J, Abukabda AB, Nurkiewicz TR. Estrous cycle-dependent modulation of in vivo microvascular dysfunction after nanomaterial inhalation.
Reprod Toxicol 2018b;78:20-28.

Steinmetz A, Hocke G, Saile R, Puchois P, Fruchart JC. Influence of serum amyloid A on cholesterol esterification in human plasma. Biochim Biophys Acta 1989;1006:173-178.

Stockmann-Juvela T, Taxell P, Santonen T. Formulating occupational exposure limits values (OELs) (inhalation & dermal). Scaffold SPD7. [European Community's Seventh Framework Programme (FP7/20072013)]. Scaffold Public Documents, 2014.

Stone V, Miller MR, Clift MJD, Elder A, Mills NL, Møller P, Schins RPF, Vogel U, Kreyling WG, Alstrup JK, Kuhlbusch TAJ, Schwarze PE, Hoet P, Pietroiusti A, De Vizcaya-Ruiz A, Baeza-Squiban A, Teixeira JP, Tran CL, Cassee FR. Nanomaterials versus ambient ultrafine particles: An opportunity to exchange toxicology knowledge. Environ Health Perspect 2017;125:106002.

Tran CL, Cullen RT, Buchanan D, Jones AD, Miller BG, Searl A, Davis JMG, Donaldson K. Investigation and prediction of pulmonary responses to dust Part II. In: Investigations into the pulmonary effects of low toxicity dusts. Contract Research Report: 216. Suffolk, UK: HSE Books, 1999.

Virmani R, Kolodgie FD, Burke AP, Finn AV, Gold HK, Tulenko TN, Wrenn SP, Narula J. Atherosclerotic plaque progression and vulnerability to rupture: Angiogenesis as a source of intraplaque hemorrhage. Arterioscler Thromb Vasc Biol 2005;25:2054-2061.

Warheit DB, Frame SR. Characterization and reclassification of titanium dioxide-related pulmonary lesions. J Occup Environ Med 2006;48:1308-1313.

Whitehead AS, Zahedi K, Rits M, Mortensen RF, Lelias JM. Mouse C-reactive protein. Generation of cDNA clones, structural analysis, and induction of mRNA during inflammation. Biochem J 1990;266:283-290.

World Health Organization. Cardiovascular Diseases (CVDs). World Health Organization, 2018.
http://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

Xu H, Zhao L, Chen Z, Zhou J, Tang S, Kong F, Li X, Yan L, Zhang J, Jia G. Exposure assessment of workplace manufacturing titanium dioxide particles. J Nanopart Res 2016;18.

Zhao L, Zhu Y, Chen Z, Xu H, Zhou J, Tang S, Xu Z, Kong F, Li X, Zhang Y, Li X, Zhang J, Jia G. Cardiopulmonary effects induced by occupational exposure to titanium dioxide nanoparticles. Nanotoxicology 2018;12:169-184.

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