**Risk Assessment of Titanium Dioxide Nanoparticles Using a Probabilistic Approach**

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**Abstract**:

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1. **Introduction**

Titanium dioxide (TiO2) is an important inorganic metal oxide used in industry today. Large scale industrial production of TiO2 began in the early 20th century and has steadily increased since then, with production capacity reaching 6.6 million metric-tons in 2012 [1]. Industrial production and use of TiO2 is generally in rutile or anatase form, although the rutile form has greater market-share [2]. Global TiO2 production is still largely driven by its past use asa pigment and in coatings [2] [3] [4], but due to its unique photocatalytic properties, nano-TiO2 has found its way into potential applications ranging from medical products to solar panels [5], [6]. Global production of nano-specific TiO2 is less certain as TiO2 in the nanomaterial size range has been produced for decades before the prefix nano became widespread and thus labeling these materials as such has not been straightforward [7]. However estimates in the U.S. alone have been placed as high as 38,000 metric-tons per year [8], [9].

The larger and coarser form of TiO2 is generally considered thermodynamically stable and inert [3]. For this reason, TiO2 was historically categorized as a nuisance dust in the occupational workplace. Nano-TiO2, on the other hand, are a less inert and more reactive material [3]. The nano-specific toxicological profile for TiO2 (i.e. nano-TiO2) has thus been put into question, especially for the occupational setting where highest human exposure is likely to occur [10].

The human health risk assessment (HHRA) paradigm for chemicals is generally considered applicable to engineered nanomaterials (EMN) [11] [12] [13]. It systematically applies scientific principles to estimate the probability that adverse human health effects emerge from exposure to chemicals. Although different organizations give different names to the RA phases, there is a general agreement that the framework is composed of problem formulation, hazard assessment, exposure assessment, risk characterization and uncertainty analysis [14]. Specifically, problem formulation is a systematic planning activity that sets the goals and the scope of the RA. The hazard assessment comprises hazard Identification and dose-response analysis. The hazard identification is carried out by gathering/generating and evaluating relevant physico-chemical and toxicological information from e.g. *in vitro* and *in vivo* studies to assess the intrinsic hazard of a substance. The dose-response analysis quantitatively characterizes the relationship between the dose of the substance and the incidence of adverse health effects in the exposed population. It typically involves the estimation of a point-of-departure (POD) such as the benchmark dose (BMD), and its extrapolation to a human effect threshold such as the derived-no-effect-level (DNEL) [15] by means of assessment factors[[1]](#footnote-1).

The exposure assessment begins by defining one or more exposure scenarios (ES), which describe(s) the conditions in which a substance (on its own, in a mixture or embedded in an article) is manufactured, handled or used across its life-cycle. This is followed by estimating the magnitude of exposure for one or more intake routes (i.e. inhalation, ingestion or dermal), either through direct measurements or through the use of models. In the risk characterization step, estimated exposure levels are typically compared to the DNEL, where risk can be defined when exposure is greater than the DNEL value. The uncertainty analysis estimates the level of ambiguity in each step of the RA process, pertaining to the quality and/or quantity of the input data and/or the applied models.

In 2011 the U.S. National Institute for Occupational Safety and Health (NIOSH) derived an recommended exposure limit (REL)[[2]](#footnote-2) for nano-TiO2 [10], acknowledging concerns over the higher hazard potential of ENMs [10]. A 2010 review of TiO2 toxicity by the U.S. Environmental Protection Agency found greater inflammatory responses, oxidative stress, toxicity and incidence of cancer for nano-TiO2 compared to its bulk counterpart[3]. NIOSH recommended an OEL[[3]](#footnote-3) of 0.3 mg/m3 based on dose-response modeling of lung tumor data involving two fine- (e.g. bulk) [16], [17] and one nano-TiO2 [18] chronic inhalation studies [10].

NIOSH also calculated, but did not recommend, a non-cancer REL of 0.004 mg/m3 for nano-TiO2. This value was based on four sub-chronic inhalation studies, which compared with short-term (e.g. acute) toxicity studies provide the types of relevant, repeated-dose, persistent exposures that are expected in the occupational setting. Three of these studies analyzed inflammation[[4]](#footnote-4) in the lung in response to fine-TiO2 exposure [19], [20], [21] and nano-TiO2 exposure [22]. NIOSH stated that their ultimate decision to recommend the REL-based lung tumor value instead of inflammation was because “the primary objective of preventing pulmonary inflammation is to prevent the development of lung tumors, and...lung tumors can be adequately controlled by exposures many-fold higher than inflammation-based exposure concentrations.” [10] This was further clarified by the fact that it is not known what level of sustained inflammation (e.g. neutrophil response) is required for tumor initiation [10]. This approach is valid and perfectly sound given the deterministic nature of the assessment.

NIOSH concluded their report with recommendations to evaluate nano-TiO2 exposure for specific job-titles and workplace activities, but there have been no documented attempts to compare the obtained OEL to exposure levels pertaining to such ES in order to estimate risks for workers. Currently, monitoring data on emission characteristics and/or source strengths are slowly emerging for some nano-TiO2 occupational ES, such as powder handling [23] and simulated sanding [24], [25]. However, most current measurement devices are unable to distinguish ENMs from background natural or incidental nano aerosols [26]. Although there are powerful instruments, able to measure the number, size, mass and surface area of ultrafine particles, they cannot discriminate among different types of EMNs [27].

In the absence of reliable measurements, risk assessors resort to models. This is why we applied NanoSafer (an advanced exposure model available for EMNs) with statistical dose-response modelling of *in vivo* toxicological data in order to perform probabilistic RA of nano-TiO2 for a number of occupational ES derived from the library of the EU FP7 MARINA and NANEX projects. The aim of this paper is to present the proposed methodology and the results of its application, including a quantitative sensitivity/uncertainty analysis to more effectively communicate the uncertainties (i.e. the probability thereof) in the RA results stemming from the input hazard and exposure data and/or from the application of models pertaining to the risks of nanomaterials.

1. **Methods**
   1. Problem Formulation

Problem Formulation is a systematic planning activity that identifies the major elements considered in a RA. In this step the goals and the scope of the assessment were defined, the target nanomaterials were introduced, and the system boundaries (e.g. occupational settings) were identified.

This paper analyses the use of a probabilistic RA approach for nanomaterials. Standard (*deterministic*) risk assessment involves determining point values for both the dose-response analysis and the exposure assessment. Typically a no-observed-adverse-effect level (NOAEL) or low-observed-adverse-effect-level (LOAEL) is determined from the dose-response data and used as a POD. Such values are used to communicate the dose at which there is no or low measured (adverse) effect. However the limitations of this approach such as ignoring the shape of the dose response curve and that the NOAEL may not actually reflect a dose at which *no* effect occurs, have been previously documented [28], [29], [30], [31], [32], [33]. Similarly, the deterministic approach uses single value assessment factors (e.g. interspecies factor) to derive lower safety-related human exposure values. Single point values are chosen on a conservative and therefore acceptable basis representing worst case scenarios. Such approaches neglect the likelihood, uncertainty and variability of the risk assessment process, which are important concepts when dealing with the human health risks from ENMs.

In this study we proposed a probabilistic approach [34] by deriving a distribution for the DNEL, which was directly related to the output of the dose-response analysis, as well as distributions for each ES, which were used as inputs into the risk characterization step [35]. The DNEL distributions take into consideration the uncertainty, such as a lack of relevant toxicological data, and variability that are inherent to the field of nano-toxicology [36]. Uncertainty in the exposure assessment may arise from a lack of complete knowledge on ENM fate and transport in air and incomplete description of the exposure environment (i.e. local exposure controls), while variability in inhalation exposure arises from differences in population based toxicokinetics. Although it would be prudent to differentiate uncertainty from variability, due to data gaps we consider only a single dimension of total uncertainty in this study in order to demonstrate the overall application of the probabilistic approach.

* 1. Hazard Identification

The review of literature pertaining to toxicity posed by nano-TiO2 were limited to those for inhalation exposure pathways. A search in Scopus (www.scopus.com) for the terms “inhalation nano TiO2” and “inhalation ultrafine TiO2” was conducted. But because we wanted to consider other non-journal sources of information (e.g. white papers), using the same search terms above, we consulted Google and Google Scholar due to its ability to identify so-called grey literature [37]. Our search was also limited to long-term studies as these provide more relevant exposure scenarios (i.e. repeated dosing, prolonged exposure) for the occupational risk context.

* 1. Dose-Response Analysis

The benchmark dose (BMD) approach to dose-response modeling was implemented in this study. The BMD approach quantifies a point of departure (POD) using a toxicologically or biologically pre-defined *benchmark* response (BMR), an unacceptable toxicological response level at or above which would cause an undue presence of hazard. In contrast to using a NOAEL or LOAEL [29], the BMD approach avoids many of the disadvantages confronted when using the NOAEL/LOAEL as the POD (*Section 2.1*). In particular, the NOAEL/LOAEL un-intuitively will decrease in value as the duration of a study increases [38], however the BMD will increase in value as a longer-term study should provide greater observational power and response data [30].

Calculation of BMDvalues were completed using the Netherland’s National Institute for Public Health and the Environment’s (RIVM) PROAST model. PROAST is a software package specifically designed for dose-response analysis, which particularly uses the BMD approach. It is suitable for handling *in-vivo* animal dose-response data and is capable of determining statistical differences in data sub-groups such as the species, gender, and study durations. PROAST is also capable of fitting multiple mathematical models to the dose-response data to further assess the statistical uncertainty of the modeling procedure.

PROAST was used to first calculate the BMDanimal (BMDa) based on the toxicological animal data defined from the hazard assessment (see *Results*). Uncertainty was introduced into the BMDa results by creating normal distributions for each BMD’s sub-group and fitted-model (i.e. that passed its goodness-of-fit test) using PROAST’s parametric bootstrap simulation option[[5]](#footnote-5) over 10,000 simulations. Because of this, we did not find it necessary to calculate the lower-bounds of those values (e.g. 90% lower-bound) as this would introduce an unnecessary level of precaution to the POD [32], [39]. POD distributions were converted to DNELs using Equation 1

|  |  |  |
| --- | --- | --- |
| DNEL = | POD | eq. 1 |
|  | EFinter ● EFintra |  |
| Assessment Factors: extrapolation….route-to-route (exposure); interspecies (uncertainty in extrapolating animal data to humans), intraspecies (variation in responses among the human population); exposure duration (extrapolation data with a less-than lifetime exposure); POD; quality of database (completeness, consistency, reliability) [40], [41]  Modifying Factors: bioavailability adjustment; route-to-route extrapolation; exposure duration adjustment; allometric scaling | | |

where EFinter and EFintra are the inter- and intra-species extrapolation factors (EF). For this, distributions for each EF were introduced using the same approach presented by Slob et al. [42]. Briefly, this involved defining log-normal distributions for each EF. In deterministic risk assessment procedures, it is typical to use factors of 10 to extrapolate to conservative yet acceptable human equivalent doses (i.e. DNEL). For the sake of transparency and in agreement with the conservative nature of deterministic values of the EF, we defined log-normal distributions for each EF such that a value of 10 is one order of magnitude greater than the mean and represents the 99th-percentile (i.e. a rare event). In this regard, the use of 10 as a conservative approach to extrapolation in traditional risk assessment was kept [39].

Monte Carlo (MC) assessment was conducted using Microsoft Excel to complete the extrapolation of BMDa to DNEL distributions. 10,000 MC combinations between BMDa values and EFinter were completed. The output was a human-equivalent BMD (BMDh) [42] – one BMDh distribution for each animal species under consideration. These new BMDh values were merged into a single BMDh distribution. 10,000 more MC simulations were performed to combine BMDh values with an EFintra to derive final (DNEL) values.

* 1. Exposure Assessment

Exposure was estimated for nine occupational scenarios dealing with production of pristine TiO2 nanoparticles: ES1 (Laser Ablation), ES2 (Manufacturer Manual Loading Trays in Booth), ES3 (Manufacturer Dumping into Mixing Tank), ES4 (Lab Transfer During Weighing and Solution Preparation), ES5 (Lab Creating Stock Solutions Fume Hood), ES6 (Dumping Large Amount of Powder In Vessel), ES7 (Bag Bin Filling), ES8 (Laser Ablation) and ES9 (Weighing of Powder). ES1 was described using occupational exposure parameters derived from the MARINA (http://www.marina-fp7.eu) database. Exposure scenarios 2-8 were described using occupational exposure parameters derived from the NANEX (http://nanex-project.eu) database. MARINA was an EU FP7 project aimed at developing risk management methods for ENMs, including development of occupational release and exposure scenarios for ENMs. NANEX was also an EU FP7 which aimed to catalogue potential exposure to ENMs across the life-cycle including manufacturing and industrial use of ENMs.

The exposure assessment was completed using an interim, un-released[[6]](#footnote-6) update to the NanoSafer 1.0 control banding exposure algorithm, titled NanoSafer v1.1β [43]. Version 1.1β uses a two-box aerosol model to assess near-field (NF) and far-field (FF) exposure *potential* in mass concentration [44]. The work room volume (Table##) is equal to the combined space of the FF and NF volumes (VFF and VNF, respectively). For each exposure scenario, the NF is defined as having a volume of 12.17 m3, while the NF to FF ventilation rate and aerosol decay functions are based on calibrated indoor-outdoor particle entrainment models [45].

The emission source rate was quantified using Equation 2:

|  |  |  |  |
| --- | --- | --- | --- |
| Ei = | Mwc | ● Hi | eq. 2 |
| twc |

where Mwc (kg) is equal to the mass of nanomaterial handled per work cycle, twc (min) is the duration of each work cycle and Hi is the handling energy factor, which relates the mechanical energies of process *i* and the dustiness index measurement (classification of dust producing capacity) [46]. The air-exchange-rate (AER) defines the transfer of clean air between the NF and FF and is (QNF, m3/min) defined by Equation 3:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| QNF = | QFF · | VNF | · *κ* | eq. 3 |
| VFF |

OR

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| QNF = | AER· | VNF | · *κ* | eq. 3 |

OR

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| QNF = | QFF · | VNF | + *10* | + 10 log | (Vtotal) | eq. 3 |
| VFF | 38 |

where QFF (m3/min) is the ventilation rate of clean air equal to the AER divided by VFF, and *κ* is a (constant) equal to 10 m3/min. The minimum value of Vtotal has been set to 38 m3.

The potential resulting NF and FF airborne concentrations (CNF, CFF) of TiO2 were calculated using Equations 4 and 5:

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cinh,NF (ti) | = | Ei | (ti) | + | Minh, FF🡪NF (ti-1) | - | Minh, NF🡪FF (ti-1) | + | Cinh, FF (ti) | eq. 4 |
| VNF | VNF | VNF |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Cinh,FF (ti) | = | Minh, NF🡪FF (ti-1) | - | Minh, FF🡪NF (ti-1) | + | Rinh(ti) | eq. 5 |
| Vtotal | Vtotal |

where Minh, NF🡪FF (ti), Minh, FF🡪NF (ti) and Rinh (ti) are defined by Equations 6, 7, and 8:.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Minh, NF🡪FF (ti) | = | [ | QNF | · | Cinh,NF (ti-1) | ] | · | [ | Δt · | QNF | + | e | Δt | ·QNF | + | 1 | ] | eq. 6 |
| VNF | VNF | |
|  | Δt · Q | 2 | VNF |  | |
| VNF |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Minh, FF🡪NF (ti) | = | [ | QNF | · | Cinh,FF (ti-1) | ] | · | [ | Δt · | QNF | + | e | Δt | ·QNF | + | 1 | ] | eq. 7 |
| VNF | VNF | |
|  | Δt · QNF | 2 | VNF |  | |
| VNF |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Rinh (ti) | = | 0.5 | [ | Cinh,FF (ti-1) | + | Cinh,FF (ti-1) | ] | · | [ | 1 | - | e |  |  | ] | eq. 8 |
| Δt · QFF | |
|  | Δt · QFF |  | VFF | |
| VFF |

Localized controls (e.g. emission source ventilation) and/or aerosol dynamics (e.g. aggregation) were not considered, resulting in an exposure *potential* (i.e.an upper limit of exposure in a specified compartment with specified ventilation). The hypothetical models were assessed using occupational exposure parameters obtained from the MARINA (E1) (www.marina-fp7.eu) and NANEX (E2-E8) (www.nanex-project.eu) databases (Table##).

Uncertainty was introduced into the exposure assessment by creating log-normal distributions for each exposure scenario. This was done using the assumption that the single-point exposure values derived from NanoSafer v1.1β represent worst-case values. Log-normal distributions were constructed by specifying that the worst-case exposure results were one order of magnitude greater than the mean and situated at the 99th-percentile of the distribution.

* 1. Risk Characterization

Risk was defined using the risk characterization ratio (RCR) (eq. 1). A risky scenario involves a RCR value greater than or equal to 1 (i.e. exposure levels higher than the DNEL). In order to evaluate RCR distributions for each of the 9 exposure scenarios, the DNEL distribution was sampled and combined with each of the 9 ES distributions over 10,000 MC-simulations, resulting final RCR distributions.

|  |  |  |
| --- | --- | --- |
| Risk Characterization Ratio = | EXPi | (eq. 1) |
| RfC |

1. **Results**

3.1 Problem Formulation

The goal and scope of this risk paper was demonstrate a probabilistic assessment of inhalation risks for the population of workers involved in the production and handling of nano-TiO2. TiO2 has a molecular weight of 79.9 grams, appears as white powder at room temperature and has two representative crystal structures: rutile and anatase. TiO2 nanomaterials are largely defined as particles and/or aggregates with a primary size ranging from 1 to 100 nm. Compared to bulk TiO2, nano-TiO2 have a larger surface area to volume ratio, greater degree of absorption of ultraviolet radiation, decreased scattering of the visible light, and an enhanced photo-catalytic activity. Besides the functional advantages ENMs possess, smaller sized particles may also influence the fate/transport, exposure, and hazard potential of the material.

TiO2 was chosen because it is a high production volume nanomaterial with significant relevance in many industrial and consumer product sectors [7].Nano-TiO2 production in the U.S. is expected to grow from 30,000 metric-tons in 2012 to an estimated 2.4 million metric-tons in 2026, accounting for approximately 99% of the total U.S. TiO2 production [9]. Similarly because of its wide acceptance into countless consumer products, TiO2 presented an appropriate case study to assess potential human health risks. Furthermore, we chose to focus on occupational inhalation exposure due to a plausible assumption of exposure to high, concentrated levels of pristine TiO2 powder compared with consumer exposures and end-of-life (EOL) scenarios whose exposure potential to pristine ENM is less well-understood. Inhalation is also likely to be an important exposure pathway for not only TiO2 but many nanomaterials, particularly spherical, metal oxides produced as powders, and thus contributes to a general understanding of occupational risks to inhalation of ENMs.

* 1. Hazard Identification

Relevant inhalation toxicity studies demonstrate that inflammation results from exposure to nano- TiO2 [[2](#_ENREF_2)]. In these cases, exposure to nano-TiO2 can lead to increased white blood cell counts, particularly macrophages [20], [21], [22], [47], lymphocytes [20], [21], [22], [48], and the polymorphonuclear (PMN) cell family [20], [21], [22], [48], [47] including neutrophils, basophils and eosinophils. Additionally, non-threshold effects such as increase rates of lung tumors were observed in rats in a chronic 2-year study to ultrafine TiO2 particles [18].

We chose to focus on long-term, inflammation studies. Long-term studies provide the most meaningful exposure testing conditions compared with occupational exposure scenarios where prolonged exposure to repeated doses will be found, while inflammation is a sensitive and robust endpoint that can be an important pathological hallmark of diseases including cancer [49]. Although more importantly, this study is not advocating an REL limit and thus was not bound by the types of considerations such as the feasibility of emissions reductions that a governmental agency such as NIOSH has to justify.

The results of our literature review found only a single study involving sub-chronic or chronic inhalation exposure of ultra-fine or nano-TiO2 to multiple dosing groups (Table ##) suitable for use in the dose-response analysis [20]. Bermudez et al. exposed rats, mice and hamsters to uncoated, nano-TiO2 obtained from Evonik (formerly DeGussa) with an average particle size of 21 nm as supplied by the manufacturer [20]. The mass median aerodynamic diameters (MMAD) per tested species were 1.29 +/- 0.30 um (Hamster); 1.45 +/- 0.49 um (Mice); and 1.44 +/- 0.57 um (Rat). The particular size range of the Evonik material could be a principle link with the exposure concentration and any observed effects seen locally in the lung. Nanomaterials tend to lodge deeper and more uniformly in the lung (i.e. alveoli) compared to bulk materials of the same composition [49], leading to increased interaction with the epithelium and ultimately inflammation. Although the primary particle size is likely to increase due to aggregation and agglomeration over time and increasing particle concentration [49]. Free primary particles of dry powders of ENMs, when aerosolized, will tend to aggregate quickly, thus exposure to primary or small “nano” aggregates might be most relevant close to the emission source [49].

The animals were exposed to nano-TiO2 at concentrations of 0.5, 2.0 and 10 mg/m3 for 6 hours/day, 5 days/week, for 13 weeks, while the control group received filtered air only. Bronchoalveolar lavage (BAL) was completed on the lungs of the sacrificed animals in that study, measuring the counts of macrophages, neutrophils, eosinophils and lymphocytes. Their study reported that statistically significant changes in the BAL results were limited to the macrophages, neutrophil and lymphocyte cell types in the highest dose-groups [20]. Furthermore, only data from the mice and rats were found to have statistically significant changes in *percent* cell counts over the control and thus the hamster data from Bermudez et al. was not considered in our paper [20]. Multiple post exposure animal groups were sacrificed to assess recovery time. Only the effects at post exposure time zero (i.e. immediately following completion of exposure) will be considered for the risk assessment. In accordance with the hazard identification, the dose-response assessment was completed for neutrophil, macrophage and lymphocyte cell counts which are both associated with inflammation in the lung [20].

3.3 Dose-Response Analysis

In general, a BMR should approach a lower limit of reasonably measured effects and has been previously defined as a 10% change over the background response [30]. However, due to uncertainty differences in measuring percent cell changes compared with total cell count, we use a BMR of 20% to determine a significant response in macrophage, neutrophil and lymphocyte percent change. Modeling errors related to the lymphocyte data prohibited us from continuing analysis on this endpoint and its results were not included in this paper. Species covariation among mice and rat dose-response data was tested, resulting in distinct dose-response relationships for the two species. For mice, PROAST fit 7 valid mathematical models to the neutrophil dose-response data (Table##, see supporting information for figures). Bootstrapping over 10,000 simulations resulted in normal distributions whose summary statistics are in The bootstrap results for each model were aggregated into a single normal distribution defined by a mean and standard deviation of 12.44 and 1.2, respectively. Additionally, PROAST fit 4 valid mathematical models to the macrophage[[7]](#footnote-7) dose-response data (Table##). The BMDa values ranged from 9.10 – 18.77 mg/m3 and were normally distributed (11.9 +/- 1.1).

Similarly,7 valid mathematical models were fit to the rat neutrophil data (Table ##, see supporting information for figures), with BMDa values ranging from 2.11 – 5.54 mg/m3 and were normally distributed (3.71 +/- 0.56). Four valid mathematical models were (Table ##) fit to the rat macrophage data with BMDa ranging from 2.12 – 5.01 mg/m3 and were normally distributed (3.47 +/- 0.56).

The distributions for the neutrophil and macrophage data are similar. This is expected given that the decrease in macrophage count was due to the increases in other inflammatory cell types, and neutrophils were the only other inflammatory cell type measured with a significant increase.

Between the neutrophil and macrophage dose-response data, neutrophils resulted in a more sensitive BMDa distribution (i.e. the minimum neutrophil BMDa value for both mice and rats was lower than their respective macrophage values). Further dose-response modeling (i.e. extrapolation) was only considered for this more conservative, neutrophil BMDa distribution.

Biological mechanisms in a population such as latency periods in diseases and survival times after disease onset are generally asymmetrical as opposed to being described by a typical “bell-shape” curve [50]. For this reason we defined lognormal distributions for the inter- and intra-species EF with a geometric mean and geometric standard deviation of 1 and 2.7, respectively. The final distribution for the neutrophil response after taking into account the inter- and intra-species extrapolation factors ranged from 0.003 – 1779.52 mg/m3. These values were log-normally distributed with a geometric mean and geometric standard deviation of 6.72 and 4.62, respectively.

3.4 Exposure assessment

Due to data gaps in the case of ES1, we assumed that all material was dispersed into the air. For ES4, the mass handled during work cycle was changed from 10 kg to 0.1 kg and *Atransfer* (i.e. the amount of material transferred at one time during a work cycle) from 10 kg to 0.02 kg.

The emission rates ranged from a low of 2.0E-8 kg/min (ES5) to a high of 44.8 kg/min (ES6) (Table##). Potential exposure time series are shown in Figure 1 which were used to calculate potential exposure concentrations in the near-field and far-field. Large variations in the potential exposure concentrations were caused by differences in the air dilution and the amount of materials used. The results of the exposure assessment for each exposure scenario are displayed in Table ##. Single-point values for the near-field exposures ranged from a low of 0.001 mg/m3 (ES9) to a high of 36.2 mg/m3 (ES6). ES6 (i.e. dumping powder in a vessel) involved pouring a large quantity of TiO2 (8,960 kg) inside of a small room (75 m3) with a low ventilation rate (4 h-1). Far-field exposures were lower, as expected and ranged from a low of 0.0003 mg/m3 (ES9) to a high of 11.8 mg/m3 (ES6).

The single-point exposure results were transformed into exposure distributions (Table#) such that the single-point values represented a worst-case scenario defined as one-order of magnitude greater than the mean and correlated with the 99th-percentile of a log-normal-distribution distribution. The overall ranking order of each exposure distribution was the same as for the single-point values, with a low of 1.00E-7 +/- 2.66 (ES9) and a high of 3.58 +/- 2.70 (ES6) for the NF exposures and a low of 3.00 +/- 2.72 (ES9) and a high of 1.17 +/- 2.65 (ES6) for the FF exposures. This was anticipated since the mean and standard deviations of all distributions were derived from extrapolation of the single point exposure values.

3.5 Risk Characterization

Tables ## and ## display the results of the risk characterization for the NF and FF ES, respectively. The RCR values for each ES were log-normally distributed. Scenario ES6 had a particularly high probability of risk compared to the other scenarios, with nearly 24% of the risk distribution values being greater than 1. ES2, ES3 and ES7 only had roughly 0.02, 0.21 and 0.25% of their RCR being greater than 1. Four out of the 9 ES resulted in RCR distributions ≥ 1. For sake of seeing if conservative values for both the DNEL and exposure still resulted in a risk greater than 1, the values of their lower-95%CI for each were considered and used in (eq. 1). ES6 was the only scenario where such a conservative calculation of risk exceeded 1.

Similarly for the FF scenarios, ES3, ES6 and ES7 resulted in RCR distributions RCR ≥ 1. In total, approximately 9% of the results for scenario ES6 were greater than 1, while ES3 and ES7 only had 0.02% of their risk distributions greater than 1. No exposure scenario had a RCR ≥ 1when the 95th percentile values of exposure and hazard were compared.

1. **Discussion**

This paper discusses using a probabilistic approach for assessing the occupational human health risk to nano-TiO2. In place of single-point values of a DNEL and exposure, distributions were defined to express the overall uncertainty contained within the RA process. The RA of nanomaterials contains many sources of uncertainty arising from, for instance, the physico-chemical variation of the ENM, dose-metric used during dose-response analysis, the toxicokinetic and toxicodynamic considerations given when extrapolating between tested species and the human population, and lack of relevant toxicity and/or exposure data (e.g. testing conditions) [36].

An important consideration not explicitly included in this paper was the uncertainty arising from the use of a mass-based dose-metric. Ideally, RA of ENMs should define a dose-metric that is the best indication of toxicity as well as exposure [51]. ENMs toxicity and exposure are determined by a set of characteristics that might depend on the size, surface area, or aspect ratio, among others [52]. For inhalation of ENMs, particle sizes are a key determinant in where particle deposition in the lung will occur [53]. However it is not evident which dose-metric most appropriately captures this property, thus consideration of a multi-metric approach including mass-concentration, number of particles, or surface area, should be considered [53], [54]. In some cases, extrapolation of one dose-metric to another may be possible, for instance when estimating surface area from particle size distribution of the number concentration for spherical ENMs, but this may not be applicable for ENMs with other types of aspect ratios [53].

The exposure assessment in this study was determined for worst-case scenarios, as modifying factors (e.g. emission controls, efficiency of local exhaust ventilation) that may reduce airborne EM concentrations were not considered. For example, Fransman et al. (2011) provides protection factors for common localized controls and personal protective equipment using assigned protection factors (APF). Although these can deviate significantly from measured protection factors (Koivisto et al. *in submission*). Additionally, the dustiness index used in this study does not correspond well to the size-distributions and masses of the respirable fraction which on average makes around only 15% wt. of the total (inhalable) dust content. Therefore, overestimations for powder handling could be significant whereas the estimations derived for spray or fugitive-type releases might be more reasonable. Other sources of uncertainty in the exposure estimation may result from the consideration of external doses in this study compared with an estimation of internal dose. As discussed above, particle sizes are an important characteristic influencing where deposition of ENM in the lung will occur. Such an approach could be used with the NanoSafer model but would require size-resolved concentration data (e.g. mass median aerodynamic diameters (MMADs)) [18], [20], [47].

Exposure distributions should also be ideally based on the inherent uncertainty contained in the model and associated parameters (e.g. handling energy factor). For instance, ventilation rate and the volume of the workplace might impact the fate, transport and exposure of some ENMs more than ENM-specific manifestations such as agglomeration/aggregation [55].

In practice, expert judgement is an important factor in the RA process and can have important influences on the final results [41]. This is true for regular chemicals but potentially more importantly for ENMs, given the previous points discussed about the current knowledge on toxicity and exposure to ENMs as well as the state-of-the-science for RA approaches [52], [53]. Particularly, where data and methods are uncertain, transparency of such ambiguity should not be sacrificed. For example, NIOSH previously reported anon-carcinogenic REL[[8]](#footnote-8) for nano-TiO2 of 0.004 mg/m3. In context of our results, the NIOSH value represents a DNEL value with 0.05% frequency of occurrence. Furthermore, we derived a NOAEL value of 2mg/m3 from the same toxicity data that informed our DNEL BMD values [20], and compared it to the BMD distributions for mice and rats. Respectively, the NOAEL value had a probability of occurrence of 4.2E-78% and 0.17%. And if default inter- and intra-species extrapolation factors of 10 are applied, the resultant DNEL equals 0.02 mg/m3. Compared with our results, this represents a value with 0.98% frequency of 0.98%.

If the goal of an REL is to estimate a precautionary and safe limit, our results confirm the conservative nature of the NIOSH results, for example. However, the deterministic approach cannot communicate to what *degree* a DNEL value is conservative because it leaves out any estimation regarding the *probability* of uncertainty. In the greater context of ENM development, communicating the probability explicitly during risk characterization may help more effectively convey human health risks of ENMs, alleviating any undue concerns that may exist, and provide improved pathways to technological innovation.

The heterogeneity of ENMs poses an immediate problem for RA due to excessive variability in physico-chemical properties and associated effects or release/exposure potentials. In this study, this issue is avoided as the authors focus on a specific nanomaterial to perform case-specific RA. This approach is compliant with current regulations (REACH), but it is clear that performing it for each variation of the material is cost- and resource-prohibitive. In order to avoid excessive case-by-case testing of EMN it seems to be possible to assign certain biological effects or release potentials to specific material properties and group them on this basis. A strategy for grouping of EMN could be based on physico-chemical, release, exposure, bio-kinetic or toxicological information or on a combination thereof [56]. Based on such data, grouping can facilitate the RA process through waiving of testing, or to highlight issues that need additional testing. The approach could also allow for read-across between EMN and non-EMN, thus reducing testing and the use of experimental animals.

1. **Conclusion**
2. **Acknowledgements**

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**Tables**

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| **Table ##** Summary of Bermudez et al. used to characterize the dose-response relationship of nano-TiO2 with neutrophil and macrophage count | | | | | | |
| **Nanomaterial** | **Species** | **Exposure** | **Dose (mg/m3)** | **Effects** | **BMR** | **Other** |
| 21nm P25 Degussa (Evonik) | Rat, Mice | 13 weeks (6 hrs/day; 5 days/wk) whole-body inhalation | Control (0.0), 0.5, 2.0, 10 | Neutrophil and macrophage cell counts | 20% change | **[**[**16**](#_ENREF_16)**]** |
| Control: Filtered air  BMR: Benchmark response | | | | | | |

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| **Table ##.** Parameters used in NanoSafer v1.1β where *Hi* = handling energy factor, *twc* is work cycle time, *pwc* is pause between work cycles, *nwc* is number of work cycles, *Atransfer* is amount of material transferred at one time during the work cycle, *V* is the room volume, and *AER* is the air exchange ratio. | | | | | | | | | |
| No. | **Exposure scenario** | ***Hi*** | ***Mwc*, [kg]** | ***twc*, [min]** | ***pwc*, [min]** | ***nwc*** | ***Atransfer*, [kg]** | **V, [m3]** | **AER, [h-1]** |
| ES1 | **Laser Ablation (MARINA Data)** | NA /1 | 4.00E-04 | 8 | NA / 0 | NA / 1 | NA / 4.00E-04 | 150 | NA / 4 |
| ES2 | **Manufacturer Manual Loading Trays In Booth** | 0.25 | 10 | 10 | 10 | 24 | 10 | 43.2 | 4 |
| ES3 | **Manufacturer Dumping Into Mixing Tank** | 0.8 | 10 | 10 | 10 | 24 | 10 | 100 | 8 |
| ES4 | **Lab Transfer During Weighing and Solution Prep** | 0.1 | 0.1 | 5 | 25 | 16 | 0.02 | 100 | 8 |
| ES5 | **Lab Creating Stock Solutions Fume Hood** | 1.00E-03 | 2.00E-04 | 10 | 20 | 16 | 2.00E-04 | 100 | 4 |
| ES6 | **Dumping Large Amount of Powder In Vessel** | 0.8 | 560 | 10 | 20 | 16 | 560 | 75 | 4 |
| ES7 | **Bag Bin Filling** | 0.8 | 250 | 480 | 0 | 1 | 250 | 706 | 4 |
| ES8 | **Laser Ablation** | 0/1 | 3.00E-03 | 8 | 52 | 8 | 3.00E-03 | 250 | 8 |
| ES9 | **Weighing of Powder** | 0.1 | 1.00E-04 | 3 | 12 | 24 | 1.00E-04 | 70 | 8 |

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| **Table ##.** BMD results (**mg/m3)** and models fit for a 20% increase in neutrophil count in mice dose-response data. | | | | | | | | |
| **Model** | **Two-Stage** | **Log-logistic** | **Weibull** | **Log-probabilistic** | **Gamma** | **EXP4** | **Hill5** | **Integration of All Models** |
| Median | 12.76 | 12.54 | 13.04 | 12.39 | 12.77 | 11.44 | 11.61 | **12.32** |
| Mean | 12.84 | 12.62 | 13.15 | 12.47 | 12.87 | 11.48 | 11.66 | **12.44** |
| Standard Deviation | 1.15 | 1.05 | 1.24 | 1.06 | 1.11 | 0.64 | 0.99 | **1.20** |
| Minimum | 9.25 | 9.58 | 9.60 | 9.37 | 9.66 | 9.57 | 8.82 | **8.82** |
| Maximum | 18.20 | 19.54 | 21.01 | 18.39 | 18.85 | 14.66 | 17.78 | **21.01** |

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| **Table ##.** BMD results (**mg/m3)** and models fit for a 20% increase in neutrophil count in mice dose-response data. | | | | | | | | |
| **Model** | **Two-Stage** | **Log-logistic** | **Weibull** | **Log-probabilistic** | **Gamma** | **EXP4** | **Hill5** | **Integration of All Models** |
| Median | 4.26 | 3.81 | 4.25 | 3.56 | 3.89 | 3.00 | 3.17 | **3.76** |
| Mean | 4.28 | 3.82 | 4.26 | 3.57 | 3.90 | 3.02 | 3.11 | **3.71** |
| Standard Deviation | 0.29 | 0.27 | 0.29 | 0.24 | 0.25 | 0.21 | 0.49 | **0.56** |
| Minimum | 3.26 | 2.99 | 3.38 | 2.84 | 3.03 | 2.42 | 2.11 | **2.11** |
| Maximum | 5.48 | 5.17 | 5.54 | 4.69 | 5.08 | 4.24 | 4.58 | **5.54** |

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| **Table ##** BMD results (**mg/m3)** and models fit for a 20% change in macrophage count in mice dose-response data. | | | | | |
| **Model** | **Log-Log** | **Log-probabilistic** | **Gamma** | **Hill5** | **Integration of All Models** |
| Median | 4.26 | 3.81 | 4.25 | 3.56 | 3.89 |
| Mean | 4.28 | 3.82 | 4.26 | 3.57 | 3.90 |
| Standard Deviation | 0.29 | 0.27 | 0.29 | 0.24 | 0.25 |
| Minimum | 3.26 | 2.99 | 3.38 | 2.84 | 3.03 |
| Maximum | 5.48 | 5.17 | 5.54 | 4.69 | 5.08 |

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| **Table ##** BMD results (**mg/m3)** and models fit for a 20% change in macrophage count in rat dose-response data. | | | | | |
| **Model** | **Log-Log** | **Log-probabilistic** | **Gamma** | **Hill5** | **Integration of All Models** |
| Median | 3.73 | 3.52 | 3.87 | 2.66 | **3.60** |
| Mean | 3.74 | 3.53 | 3.89 | 2.73 | **3.47** |
| Standard Deviation | 0.26 | 0.23 | 0.25 | 0.50 | **0.56** |
| Minimum | 2.94 | 2.74 | 3.06 | 2.12 | **2.12** |
| Maximum | 4.88 | 4.62 | 5.01 | 4.48 | **5.01** |

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| **Table ##** Mean exposure concentration during the work day. | | | |  | |
| No. | **Exposure scenario** | **Ei [kg m-1]** | ***CNF*, [µg m-3]** | | ***CFF*, [µg m-3]** | |
| ES1 | **Laser Ablation – MARINA Data** | 5.00E-05 | 0.002 | | 0.001 | |
| ES2 | **Manufacturer Manual Loading Trays In Booth** | 2.50E-01 | 278 | | 89 | |
| ES3 | **Manufacturer Dumping Into Mixing Tank** | 8.00E-01 | 893 | | 259 | |
| ES4 | **Lab Transfer During Weighing and Solution Prep** | 2.00E-03 | 0.745 | | 0.217 | |
| ES5 | **Lab Creating Stock Solutions Fume Hood** | 2.00E-08 | 0.002 | | 0.001 | |
| ES6 | **Dumping Large Amount of Powder In Vessel** | 4.48E+01 | 36.2×103 | | 11.8×103 | |
| ES7 | **Bag Bin Filling** | 4.17E-01 | 825 | | 160 | |
| ES8 | **Laser Ablation** | 3.75E-04 | 0.103 | | 0.024 | |
| ES9 | **Weighing of Powder** | 3.33E-06 | 0.001 | | 0.0003 | |

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| **Table ##** Geometric means and geometric standard deviations defining the exposure distributions for each ES. | | | | | | | | | |
| **Exposure Scenario** | **ES1** | **ES2** | **ES3** | **ES4** | **ES5** | **ES6** | **ES7** | **ES8** | **ES9** |
| **Geometric Means** | 1.98E-07 | 2.81E-02 | 8.95E-02 | 7.53E-05 | 1.96E-07 | 3.58E+00 | 8.27E-02 | 1.04E-05 | 1.00E-07 |
| **Geometric Standard Deviations** | 2.67 | 2.69 | 2.70 | 2.69 | 2.70 | 2.70 | 2.68 | 2.70 | 2.66 |

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| **Table ##** Summary of the risk characterization (reported as risk ratios, RR) distributions for each near-field exposure scenario over 10,000 Monte Carlo simulations. | | | | | | | | | |
| **Exposure (Near Field)** | **ES1** | **ES2** | **ES3** | **ES4** | **ES5** | **ES6** | **ES7** | **ES8** | **ES9** |
| **RR GM** | 1.63E-08 | 2.30E-03 | 7.33E-03 | 6.00E-06 | 1.63E-08 | 2.97E-01 | 6.71E-03 | 8.48E-07 | 8.21E-09 |
| **RR GSD** | 1.66E-08 | 2.30E-03 | 7.15E-03 | 6.13E-06 | 1.61E-08 | 2.96E-01 | 6.72E-03 | 8.66E-07 | 8.23E-09 |
| **RR Minimum** | 5.02E-11 | 1.02E-06 | 2.07E-05 | 9.52E-09 | 1.60E-11 | 3.29E-04 | 5.56E-06 | 3.06E-10 | 4.72E-12 |
| **RR Maximum** | 5.98E-06 | 1.27E+00 | 6.81E+00 | 4.77E-03 | 1.07E-05 | 1.33E+02 | 6.07E+00 | 3.67E-04 | 1.29E-05 |
| **Any RR≥1?** | No | **Yes** | **Yes** | No | No | **Yes** | **Yes** | No | No |
| **Probability RR≥1 (i.e. No. RR simulations≥1)** | 0.00% | 0.03% | 0.16% | 0.00% | 0.00% | 23.53% | 0.17% | 0.00% | 0.00% |
| **Lower 95%-CI RR value** | 8.48E-08 | 1.18E-02 | 3.79E-02 | 3.16E-05 | 8.48E-08 | 1.53E+00 | 3.50E-02 | 4.37E-06 | 4.24E-08 |
| **Lower 95%-CI RR≥1?** | No | No | No | No | No | **Yes** | No | No | No |

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| **Table ##** Summary of the risk characterization (reported as risk ratios) distributions for each near-field exposure scenario over 10,000 Monte Carlo simulations. | | | | | | | | | |
| **Exposure (Far Field)** | **ES1** | **ES2** | **ES3** | **ES4** | **ES5** | **ES6** | **ES7** | **ES8** | **ES9** |
| **RR GM** | 8.21E-09 | 7.27E-04 | 2.12E-03 | 1.78E-06 | 8.08E-09 | 9.55E-02 | 1.31E-03 | 1.98E-07 | 2.50E-09 |
| **RR GSD** | 8.15E-09 | 7.27E-04 | 2.08E-03 | 1.75E-06 | 8.19E-09 | 9.53E-02 | 1.29E-03 | 1.98E-07 | 2.52E-09 |
| **RR Minimum** | 1.66E-11 | 2.03E-06 | 3.29E-06 | 3.67E-09 | 1.36E-11 | 1.40E-04 | 1.23E-06 | 4.17E-10 | 5.17E-12 |
| **RR Maximum** | 5.83E-06 | 6.00E-01 | 1.60E+00 | 1.46E-03 | 2.58E-06 | 6.79E+01 | 1.09E+00 | 1.24E-04 | 1.12E-06 |
| **Any RR≥1?** | No | No | **Yes** | No | No | **Yes** | **Yes** | No | No |
| **Probability RR≥1 (i.e. No. RR simulations≥1)** | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 8.62% | 0.01% | 0.00% | 0.00% |
| **Lower 95%-CI RR value** | 4.24E-08 | 3.77E-03 | 1.10E-02 | 9.20E-06 | 4.24E-08 | 5.00E-01 | 6.78E-03 | 1.02E-06 | 1.27E-08 |
| **Lower 95%-CI RR≥1?** | No | No | No | No | No | No | No | No | No |

**Figures**

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| C:\Work\MATLAB\Danail\NF_and FF_exposures.png |
| **Figure ##** Potential exposure time-series in the a) near field and b) far field. |

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| **Figure##** Distributions of BMDh, BMDh/Exposure Ratio, and BMDa/Exposure Ratio |

1. Assessment factors being a generic term that includes uncertainty and extrapolation factors applied in the course of deriving human dose or concentration levels used for risk assessment or regulatory purposes. [↑](#footnote-ref-1)
2. An REL, in principle, represents a DNEL. [↑](#footnote-ref-2)
3. Reported as a time-weighted average for a 40-hour work week while working 10 hours per day. [↑](#footnote-ref-3)
4. Measured as the response in polymorphonuclear neutrophil (PMN) count in a bronchoalveolar lavage (BAL) sample. [↑](#footnote-ref-4)
5. Bootstrapping is a set of methods for random sampling with replacement. [↑](#footnote-ref-5)
6. At the time of final acceptance of this paper, an updated version of NanoSafer 2.0 was not yet publically released. [↑](#footnote-ref-6)
7. Measured as the inverse of the decrease in macrophage count. PROAST does not measure decreasing trends and thus the inverse of the decrease in macrophage count was necessary. [↑](#footnote-ref-7)
8. For inflammation resulting from inhalation to nano-TiO2 [↑](#footnote-ref-8)