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ARTICLE

Utilizing literature-based rodent toxicology data to derive potency estimates for quantitative risk assessment

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ABSTRACT

Evaluating the potential occupational health risk of engineered nanomaterials is an ongoing need. The objective of this meta-analysis, which consisted of 36 studies containing 86 materials, was to assess the availability of published *in vivo* rodent pulmonary toxicity data for a variety of nanoscale and microscale materials and to derive potency estimates via benchmark dose modeling. Additionally, the potency estimates based on particle mass lung dose associated with acute pulmonary inflammation were used to group materials based on toxicity. The commonalities among the physicochemical properties of the materials in each group were also explored. This exploration found that a material's potency tended to be associated primarily with the material class based on chemical composition and form (e.g. carbon nanotubes, TiO_2, ZnO) rather than with particular physicochemical properties. Limitations in the data available precluded a more extensive analysis of these associations. Issues such as data reporting and appropriate experimental design for use in quantitative risk assessment are the main reasons publications were excluded from these analyses and are discussed.

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KEYWORDS
Meta-analysis; quantitative risk assessment; toxicology; potency estimation; benchmark dose modeling

Introduction

In the past two decades, the use of engineered nanomaterials (ENMs) has grown rapidly in a variety of industries from pharmacological and medical to technological (Valavanidis and Vlachogianni 2016). With the increasing use comes a concomitant increase in manufacturing of these ENMs. A 2012 survey of ENM production companies across the globe revealed that anywhere from 10 to 10,000 tons of material is produced per year, depending on the type of ENM being produced (Piccinno et al. 2012). In addition to the overall quantity of engineered material production increasing, the diversity within types of the materials being produced is also climbing as the research for novel applications expands (Rodriguez-Ibarra et al. 2020). Most of these materials do not have specific exposure limits (Mihalache et al. 2017).

Among the highest produced nanomaterials are carbon black, synthetic amorphous silica, aluminum oxide, barium titanate, titanium dioxide, cerium dioxide, zinc oxide, carbon nanotubes/nanofibers, and silver (WHO 2017, citing a 2012 EU report). Epidemiological data in workers are available for some of these materials (carbon black, synthetic amorphous silica, titanium dioxide, zinc oxide, and carbon nanotube/nanofibers), mostly from cross-sectional studies (Schulte et al. 2019). Some evidence of adverse effects in the respiratory and cardiovascular systems were reported, including changes in some biomarker levels compared to controls, although limited exposure data and a relatively short period since first exposure may have affected the incidence of observed adverse effects (Schulte et al. 2019).

As the variety of engineered nanomaterials and their micro-scale counterparts grows, the need to address the safety involved with the manufacturing of these materials continues. Occupational exposure limits (OELs) have been developed for specific ENMs or categories, including many of the high production nanomaterials, although these OELs often vary considerably for a given material, in part due to differences

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Supplemental data for this article can be accessed here.

in the methods used (ISO 2016; Mihalache et al. 2017). Quantitative risk assessment methods provide the basis for many OELs, including NIOSH recommended exposure limits (RELs) (NIOSH 2020). Quantitative risk assessment involves in-depth assessments of specific chemical or physical hazards using quantitative dose and response data from animals or humans, following established methodologies (NAS 1983, 2009, US EPA 2012; NIOSH 2020). The materials with OELs are typically those that are most commonly used in a manufacturing setting (NIOSH 2011, 2013, 2020). However, this approach has been unable to address the "vast number of chemical substances in commerce" including ENMs and their microscale counterparts that do not have OELs, which means that many existing newly created materials may not have adequate safety information for the workers (NIOSH 2019). Epidemiological data in humans, if available, is used, and in the absence of sufficient data in humans, data from experimental animals have typically been used in deriving OELs (NIOSH 2020). However, the need to develop alternative testing approaches has been widely recognized (Oberdörster et al. 2005; NAS 2007; Nel et al. 2013; US EPA 2014; OECD 2014b; OECD 2016; NAS 2017; NIOSH 2020). Newer approaches include a classification-based approach focused on grouping materials based on toxicity (Bates et al. 2016; Drew et al. 2017; Landsiedel et al. 2017; Lamon et al. 2018; Ramchandran and Gernand 2019; Ramchandran and Gernand 2020; Sheehan et al. 2018). These grouping methods could potentially be used along with read-across methods to classify new manufactured materials, estimate their toxicity, and provide safety guidance recommendations without extensive levels of testing (OECD 2016; ECHA 2017; Landsiedel et al. 2017). Currently, none of these methods is easily aligned with quantitative risk assessment methods to derive OELs or OEBs, in part due to differences in the purposes of the frameworks, differences in the experimental data utilized (e.g. short-term rodent or cellular toxicity studies), and limited validation compared with benchmark (well-studied reference) materials (Oberdörster et al. 2005; Kuempel et al. 2012; Nel et al. 2013; OECD 2014b; OECD 2016).

A goal of this meta-analysis was to use summary data collected from the current literature pertaining to *in vivo* pulmonary toxicity in rats or mice for a variety of nanoscale and microscale materials to calculate quantitative estimates of toxicity. These data were systematically collected from the literature for particulate materials that vary in chemical composition, size, shape, specific surface area, solubility, crystallinity, density and other properties. These physicochemical properties are among those considered to be important in describing the toxicity of ENMs (OECD 2014a; Rasmussen et al. 2018). Acute pulmonary inflammation is the endpoint evaluated in these analyses, which is measured in the rodent studies as increased polymorphonuclear leukocytes (PMN) cells in bronchioalveolar lavage fluid (BALF). Due to the different techniques of obtaining and counting PMN cells from BALF, percent PMN was chosen to normalize across studies. This endpoint was selected as being a relatively commonly reported response in rodent *in vivo* studies and because recruitment of proinflammatory cells (including PMNs) is a key event in the adverse outcome pathways (AOPs) of inhaled particles in the development of lung diseases (Bos et al. 2019; Halappanavar, Ede, et al. 2020). Acute inflammatory responses in lung cells are also measured in cell cultures (*in vitro*) (Donaldson et al. 2008; Rushton et al. 2010; Zhang et al. 2012; Barosova et al. 2020) and may provide a link to *in vivo* outcomes and the further development and use of alternative assays. In this study, the materials were grouped based on hazard potency estimates derived from dose-response data of rodent *in vivo* pulmonary inflammation, where hazard potency is defined as the inverse of dose. In addition, the commonalities among the physicochemical properties of the materials within each group were explored. A secondary goal was to compare the accuracy of two common techniques used for calculating quantitative estimates of potency: statistical model-based estimates of benchmark dose (BMD) versus the study design-based dose measures of no observed adverse effect levels or lowest observed adverse effect levels (NOAELs or LOAELs). The strengths and limitations of literature-based data for use in quantitative risk assessment are discussed in the context of developing alternative methods for deriving OELs or OEBs for ENMs.

Materials and methods

Literature searches & data collection

Literature searches

Data for these analyses were derived from four separate searches of the scientific literature by NIOSH

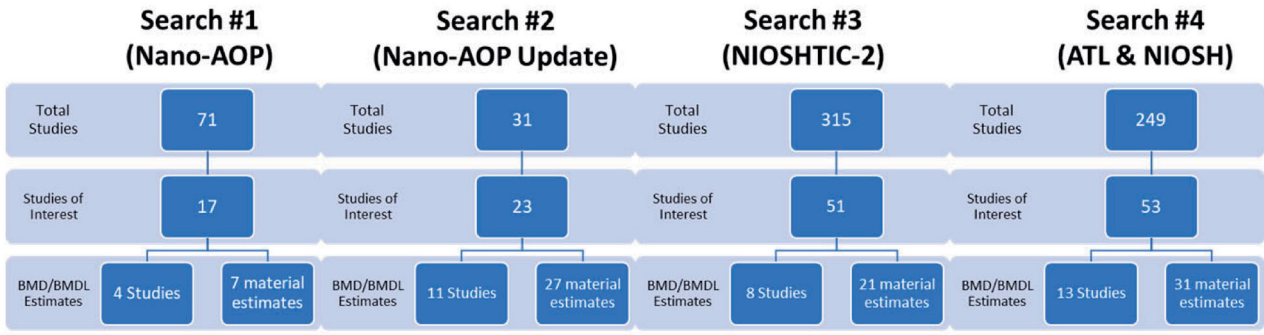


Figure 1. The flowcharts display the number of studies selected and ultimately used from each literature search. "Total Studies" represents the number of studies from the respective search that investigated *in vivo* lung inflammation from exposure to nano-/micro-scale materials. The "Studies of Interest" for each literature search represent the number of studies which reported (directly or with enough information to calculate) the percentage of PMNs from BALF cell differential assays. Additionally, the "Studies of Interest" were restricted to a dose-response study design with an acute ($\leq$3 days) post-exposure time period and reported sufficient summary data for dose-response modeling. The final row of each flowchart represents the number of studies as well as the corresponding number of materials from those studies in which a parametric dose-response model was adequately fit, resulting in a reliable estimate of a benchmark dose (BMD), which is a maximum likelihood estimate, or the 95% lower confidence limit of the BMD (BMDL). Databases utilized: Nanomaterial adverse outcome pathway (Nano-AOP) [Halappanavar, van den Brule, et al. 2020]; NIOSHTIC-2 [https://www2a.cdc.gov/nioshtic-2/AdvSearch2.asp.]; Advanced Technologies and Laboratories (ATL) International, Inc. through a contract with NIOSH [ATL 2015].

et al. (Figure 1). The aim of this study was to capture toxicology studies on both nanoscale and microscale materials that have reported sufficient data for dose-response modeling and analysis of the role of physicochemical properties on the hazard potency of the material. Three of the four literature searches (Searches #1, 2, and 4) utilized the online databases of PubMed, Web of Science, Toxline, and/or Scopus, which index publications in many U.S. and international scientific journals related to toxicology and occupational health. The final literature search (Search #3) was conducted using NIOSHTIC-2 (available at: https://www2a.cdc.gov/nioshtic-2/default.asp), a bibliometric database of peer-reviewed, published research studies conducted through the National Institute of Occupational Safety and Health (NIOSH). All four searches were conducted using a combination of standardized search strings focused on capturing toxicity studies involving nanomaterials (Table S-5). Studies involving microscale materials were not discarded if they appeared in a set of search results of nanoscale materials.

Searches #1 and #2 were a part of the Nano-AOP database effort to conduct a comprehensive literature search of toxicological studies pertaining to adverse outcome pathways involving nanomaterials (Halappanavar, van den Brule, et al. 2020). The NIOSHTIC-2 literature search (Search #3) aimed to include all NIOSH research studies on pulmonary toxicology of nanoscale or microscale particles published through December 2019. Search #4 was an earlier search conducted by ATL through a contract with NIOSH. The aim was to include all published toxicology studies of ENMs and other respirable particles and fibers of similar chemical composition, focusing on acute to chronic lung effects in rodents. Search #4 included all studies (beginning date not restricted) published until the search end date from June 23, 2015 through March 11, 2016 (Table S-5). The Nano-AOP searches restricted publication dates to 2000–2015 (Search #1) and 2015–2017 (Search #2), respectively (Figure 1). Additional information about the strategies and criteria for each of these searches is provided in Supplemental Table S-5. The studies selected from each of these searches were those that provided quantitative dose-response data on pulmonary inflammation in rodents.

Data collection

From the combined results of the four searches, a subset of studies was used to collect data for studies of interest (Figure 1). These studies were selected as providing quantitative dose-response data on pulmonary inflammation following exposure to nanoscale or microscale particles in rats or mice. The

pulmonary inflammation measure used was the percentage of polymorphonuclear leukocytes (PMN) from bronchoalveolar lavage (BAL) fluid. Studies that reported PMN count and total cell count from BAL were also included if the percent PMN could be calculated. The data were recorded from each publication to the extent it was provided included: experimental design parameters (e.g. exposure regimen, dosages, species/sex/strain/age of groups), material characterization (e.g. particle dimensions, surface area), and summary statistics (sample mean, sample standard deviation/error, and sample size) from assays of interest (including PMNs in BAL). The studies that reported the percentage of PMNs (or from which it could be calculated) were subsequently selected if the experimental design was amenable (lung exposure via inhalation, intratracheal instillation, or pharyngeal aspiration in rats or mice; BAL was performed within 3 days of exposure (i.e. $\leq$3 days post-exposure); and the summary statistics reported were adequate for dose response-modeling. In addition, a minimum of two dose groups and a control (unexposed) group were required for modeling. Post-exposure timepoints of three days or less were selected to represent an acute post-exposure assessment of inflammation prior to potential tissue injury. Further information about the data that was collected and used can be found in Supplemental Table S1.

Potency estimate calculations

No observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL)

NOAELs and LOAELs were recorded as a type of potency estimation. If not explicitly defined in the publication, the NOAEL was recorded as the highest dose group that did not result in a statistically significant increase in PMN (count or percentage) from the control group. Similarly, the LOAEL was recorded as the lowest dose group that did result in a statistically significant increase in PMN (count or percentage) from the control group. Statistical significance was assessed as reported in the publication.

Benchmark dose modeling

The BMD and the lower 95% confidence level of the BMD (BMDL) were calculated using dose-response modeling conducted with the continuous modeling suite in Benchmark Dose Software (BMDS v2.7) (freely available at: www.epa.gov/bmds/benchmark-dose-software-bmds-version-27-materials) using an added 4% PMNs above control (absolute deviation) as the benchmark response (BMR). This BMR was chosen because an added 4% PMNs in BAL fluid has been shown to be a biologically-relevant early adverse effect in rats that is also of relevance to workers (NIOSH 2011, Section 3.5.2.2), and was assumed to be applicable to mice. In addition to being biologically relevant, a BMR of an added 4% is close to the recommended default BMR of an added 5% for continuous variables generally (EFSA Scientific Community 2017). Model selection followed the US EPA (2012) guidance on model selection using the lowest Akaike Information Criterion (AIC) that passed all four hypothesis tests. The four hypothesis tests for continuous models included: evaluation of a dose-response trend, presence of constant variance, adequacy of the chosen variance model, and overall model fit. A level of significance of 5% was used in the first three hypothesis tests, while a level of significance of 10% was used in the test for overall model fit. These significance levels were based on the US EPA (2012) guidance. Additional considerations in these analyses included: for models that passed all hypothesis tests, each model was investigated for any boundary parameters and an adjustment of $+2$ to the AIC was made for each boundary parameter. These boundary parameter-adjusted AICs were then used for model selection. Also, saturated models were considered for model selection. BMD estimates from each selected model were used for further analyses (see Data Analysis subsection).

Normalization

All BMD estimates were normalized to the particle mass deposited in the lungs (μg/g lung) in order to account for variation in animal models (species/strain/sex) and exposure route and duration. Deposited doses (in mg) were calculated based on route of exposure. For inhalation studies, the deposited dose was calculated using Equation (1), where dose represents the administered dose concentration, duration represents the total duration of exposure, ventilation rate is the standard rate of ventilation by species, and the deposition fraction

of particles in the lung was calculated using the Multiple Path Particle Dosimetry modeling for that species/strain (MPPD, v3.04) (ARA 2015). The calculated deposition fractions ranged from 0.97 to 23.23% and utilized ventilation rates according to the EPA (US EPA 1994) guidance. Particle size distribution inputs include median aerodynamic diameter and geometric standard deviation. Equation (1):

$$\begin{aligned} deposited\ dose = dose\ \left({}^{mg}/_{m^3}\right) \\ \times duration\ (hr \times day \times weeks) \\ \times ventilation\ rate\ \left({}^{L}/_{min}\right) \\ \times 0.0001\ \left({}^{m^3}/_{L}\right) \times 60\ \left({}^{min}/_{hr}\right) \\ \times deposition\ fraction \end{aligned} \quad (1)$$

The pulmonary (alveolar) deposited dose was assumed to be 80% for pharyngeal aspiration (similar to 81% reported in Mercer et al. (2010) and 78–88% reported in Rao et al. (2003)). For intratracheal instillation, the full administered dose was assumed to reach the pulmonary region.

If reported in the publication, the average lung weight of the control group before treatment (in grams) was used in the dose normalization. If lung weight was not reported in the paper, the deposited dose was normalized using control group lung weights that we compiled from the literature and matched based on the same species, strain, sex, and age as the individuals used in the study. The lung weights used, if not specifically reported, were: mouse age 6–8 weeks: 0.14 g female, 0.15 g male; rat age 8–10 weeks: 0.8 g female, 0.9 g male (Fischer 344); 1.0 g female; 1.3 g male (Sprague Dawley).

Data analysis

Hierarchical clustering using a Euclidean distance metric with complete linkage was performed on the full set of materials to uncover initial patterns in groupings based on the materials' potency estimates (represented by the material BMD estimates, which are the particle mass lung doses associated with an added 4% PMNs above background in BALF). A lower BMD is indicative of a greater potency for a given material.

Principal component analysis (PCA) was performed between and within material classes using the BMD and BMDL potency estimates and the most prevalently reported quantitative physicochemical properties. The term "material class" is used here to indicate differences in the chemical composition and/or physical form that are typically reported in the literature. In most cases, materials are grouped by chemical composition (e.g. TiO_2) including any different physical forms (e.g. sphere, wire, or belt) or coatings (Table S-1). In some cases, the materials are divided by physical form as well as chemical composition (e.g. carbon nanotubes, graphene, carbon spherical particles). Another consideration in creating these the material classes was to balance the need for reducing heterogeneity to the extent feasible within a class vs. having an adequate number of studies within a material class for analysis. Relevant qualitative variables were used to display groupings of materials on a PCA biplot since only quantitative variables are allowable in PCA . PCA was performed on all materials that provided a measure of mean diameter (in nm) and specific surface area (in m^2/g) to investigate the potential of correlations between these physicochemical properties of a material and its potency estimation. In addition, PCA was performed within material classes for titanium dioxide (TiO_2) and carbon nanotubes (CNTs), which are the two classes with the most studies in this database. The most commonly reported physicochemical properties within each of these classes were selected for PCA. Materials with missing physicochemical data for these quantitative properties were excluded from the analyses. Comparisons within the TiO_2 material class only used specific surface area in analyses and within-material class CNT PCA analysis utilized the physicochemical properties specific surface area and length (in nm). While length was not commonly reported across the full set of materials, it was commonly reported within the subgroup of CNT materials and thus was used in the CNT-specific analysis.

A bootstrapping approach was used to assess the significance of the correlations from the above PCA analyses (Efron and Tibshirani 1986). A random sample of size equal to the original dataset used in the corresponding PCA (86 for the full set of materials, 26 for the subset of TiO_2 materials, and 20 for the subset of CNT materials) was taken with replacement. The Pearson correlation coefficient between the BMD estimate and its corresponding

Table 1. Percentage of materials recorded in the 144 "Studies of Interest" that reported (or information was able to be obtained for) a variety of physicochemical properties.

Property**	% of Materials
Primary particle size	94.2
Diameter	90.7+
Specific surface area	86
Density	20.9+
Other properties*	<10%

*Other properties recorded include: length, surface charge, dissolution rate, zeta potential, shape, structure, surface reactivity, hydrophobic/hydrophilic.
**Obtained additional information from external sources (e.g. manufacturer, CRC Chemical Handbook) for some properties when not reported within paper.
+A variety of types of measurement reported across papers.

physical property was computed for each random sample. This process was repeated 10,000 times and an empirical distribution was created from the simulated correlation coefficients. A 90% confidence interval was constructed from the lower 5th and upper 95th percentiles of each distribution. All data analyses were performed in R (v 3.6.0) using RStudio (v 1.2.1335) (R Core Team 2019).

Results

Literature searches and data collection

The four literature searches resulted in a total of 666 studies that investigated lung inflammation in rodents from exposure to nanoscale or microscale particulate materials; of these, 144 were identified as studies of interest and were used for data collection (Figure 1). Ultimately, BMD/BMDL estimates were able to be calculated from adequate model fit for 36 of the 144 studies of interest, which resulted in a total of 86 materials from individual experiments (Figure 1). When available in the publication, the corresponding NOAELs and LOAELs for these 86 materials were recorded.

Across the 86 materials, the extent to which the physicochemical properties were reported in the publication varied. Primary particle size was the most reported property for the materials (94.2% of materials), followed by diameter measurements (90.7%), specific surface area (86%), and density (20.9%) (Table 1). Primary particle size was recorded in this dataset only if it was explicitly reported as such in the journal article. Any type of diameter measurement that was reported was recorded, along with the measurement type used (e.g. average, median, hydrodynamic, other). Density was for the "bulk" material and if not reported in the journal article was obtained from external sources (e.g. manufacturer, CRC Chemical Handbook). Among these commonly reported properties, mainly diameter and density, a variety of measurement techniques were reported and recorded. The measurements from these different techniques were assumed to be equivalent in these analyses for simplicity. Other properties were reported in less than 10% of materials (Table 1).

Between & within material potency trends

Between materials

Hierarchical Clustering of materials by their BMD estimates displayed distinct groupings by material class (Figure 2). The most noticeable groupings of materials included: zinc oxide (ZnO), carbonaceous materials (CNT, Graphene, and Carbon), and titanium dioxide (TiO_2). Although a few TiO_2 isoforms were spread throughout the clusters, most of this material was clustered into a few tightly clustered groups (Figure 2). Between these material classes, the BMD and BMDL estimates varied by orders of magnitude (Figure 3(A–C), Supplemental Table 4). The potency estimates within the graphene and carbon material groups were less variable than the potency estimates of the TiO_2 and ZnO material classes. The CNT material class potency estimates were more variable than those of ZnO and less than those of TiO_2 (Figure 3(A–C), Supplemental Table 4).

PCA provides information about the potential correlations between the physicochemical properties of a material and its potency estimation. The two particle properties of diameter and specific surface area were chosen for investigation in PCA because they were two of the most common quantitative properties reported across all 86 materials As expected, there was a strong positive correlation (0.938) between a material's BMD and BMDL estimate and a moderate negative correlation (−0.389) between a material's diameter and specific surface area (Figure 4(A,E)). This result is expected as the specific surface area and the particle diameter of a substance mathematically have an inverse relationship. As seen in Figure 2, the material grouped by material class in Figure 4(A), with TiO_2 having the most variation within material. However, there was

Table 2. Example of a data table template for providing summary data for single assay result where each row represents results from a different treatment condition.

Treatment condition	Dose (units provided)	Sample size	Mean	Error measurement*
MWCNT – 1 day post exposure	20 μg	4	24	2.7
MWCNT – 7 day Post exposure	20 μg	4	30	3.1
…	…	…	…	…

*Error measurement could be either standard deviation or standard error but should be specified.

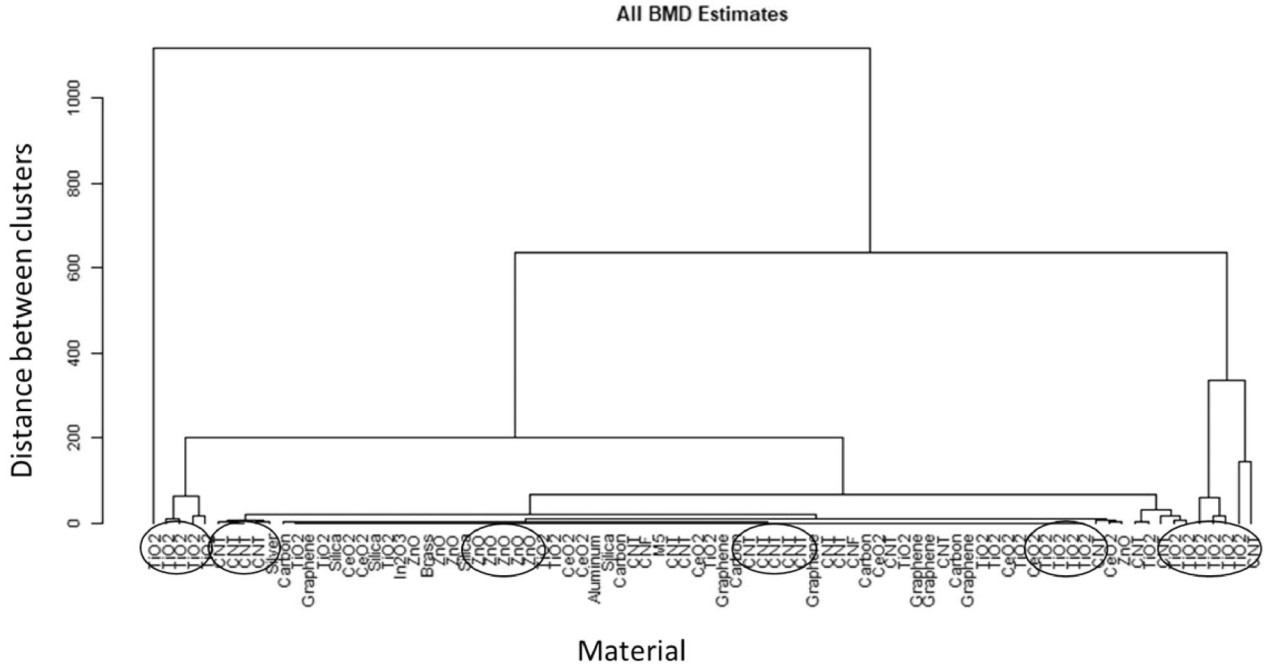


Figure 2. The tree displays the results of Hierarchical Clustering that was performed on 86 BMD estimates. The y-axis represents the max Euclidean distance of clusters before merging. Circles indicate instances of materials of the same material class that grouped together based on their potency estimates.

essentially no correlation between a material's potency estimate (BMD, BMDL) and either its mean diameter (-0.111, −0.118) or specific surface area (-0.129, −0.093) across all materials (Figure 4(A,E)). In other words, more of the variability in the BMD estimates can be explained by the material class (e.g. CNT, TiO_2, ZnO, etc.) than by the particle properties of diameter and surface area based on the ranges in the data examined.

Within material class

The lack of association between a material's physical dimensions such as diameter or specific surface area and potency estimate across material groups prompted further investigation into associations that may be present within a material class. For this, PCA was performed individually on the two most frequently reported material groups (CNT and TiO_2) using the most frequently reported quantitative properties from each group. CNT and TiO_2 were the only material groups that contained enough data for a relevant PCA analysis. Within the CNT grouping, specific surface area and mean length were used in the analysis. Our results indicate a slightly negative correlation between the specific surface area of the CNT materials and their BMD or BMDL estimates (-0.280, −0.278, respectively), suggesting higher potency (i.e. lower BMD or BMDL) with higher specific surface area (Figure 4(B,F)). However, a weakly positive association was observed between BMD or BMDL and mean length (0.202, 0.114, respectively), suggesting a lower potency (higher BMD or BMDL) with increasing length of CNTs (Figure 4(B,F)). Additionally, there appears to be slight grouping of materials by their surface functionalization or modification (e.g. acidification, polymerization, etc.), suggesting that the surface properties of a CNT may also be a factor affecting a material's potency (Figure 4(B)).

Within the TiO_2 grouping, specific surface area was reported most frequently. Diameter measurements for

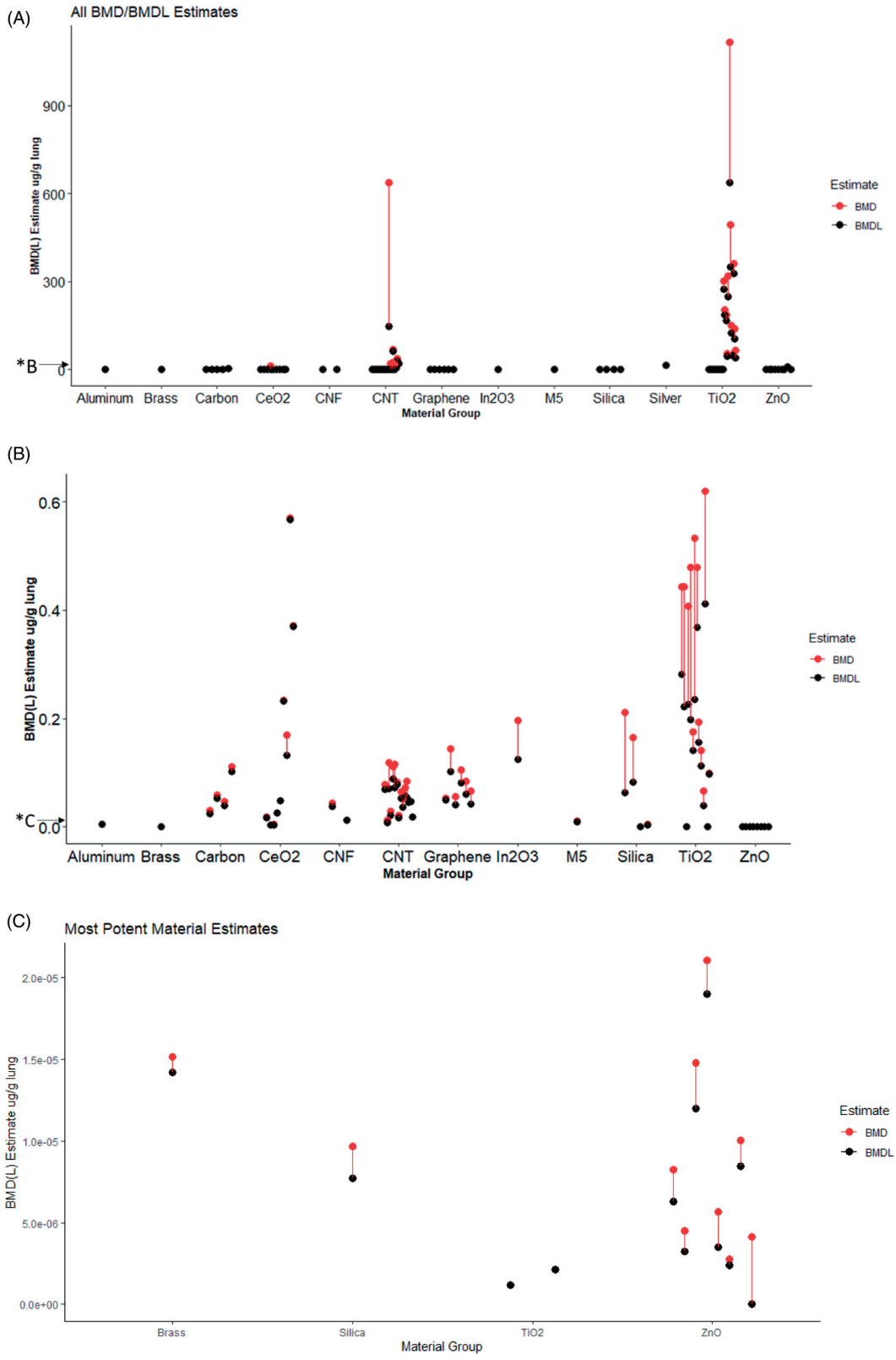


Figure 3. (A) Plot displaying the potency estimates of the 86 materials displayed in Figure 2 stratified by material class. BMD estimates (top dot) are connected by a line to their corresponding BMDL estimate (bottom dot) to represent the lower 95% confidence interval for the potency estimate. The lower the BMD/BMDL estimate, the more potent a material is. (B) Zoomed in version of (A) with all benchmark dose (BMD) and 95% lower confidence limit (BMDL) estimates <1 μg/g lung. (C) Most potent material estimates (i.e. lowest BMD and BMDL) <0.0001 μg/g lung. *The arrows along the y-axis in Figures A and B indicate a visual for the cutoffs used for the following zoomed-in figure.

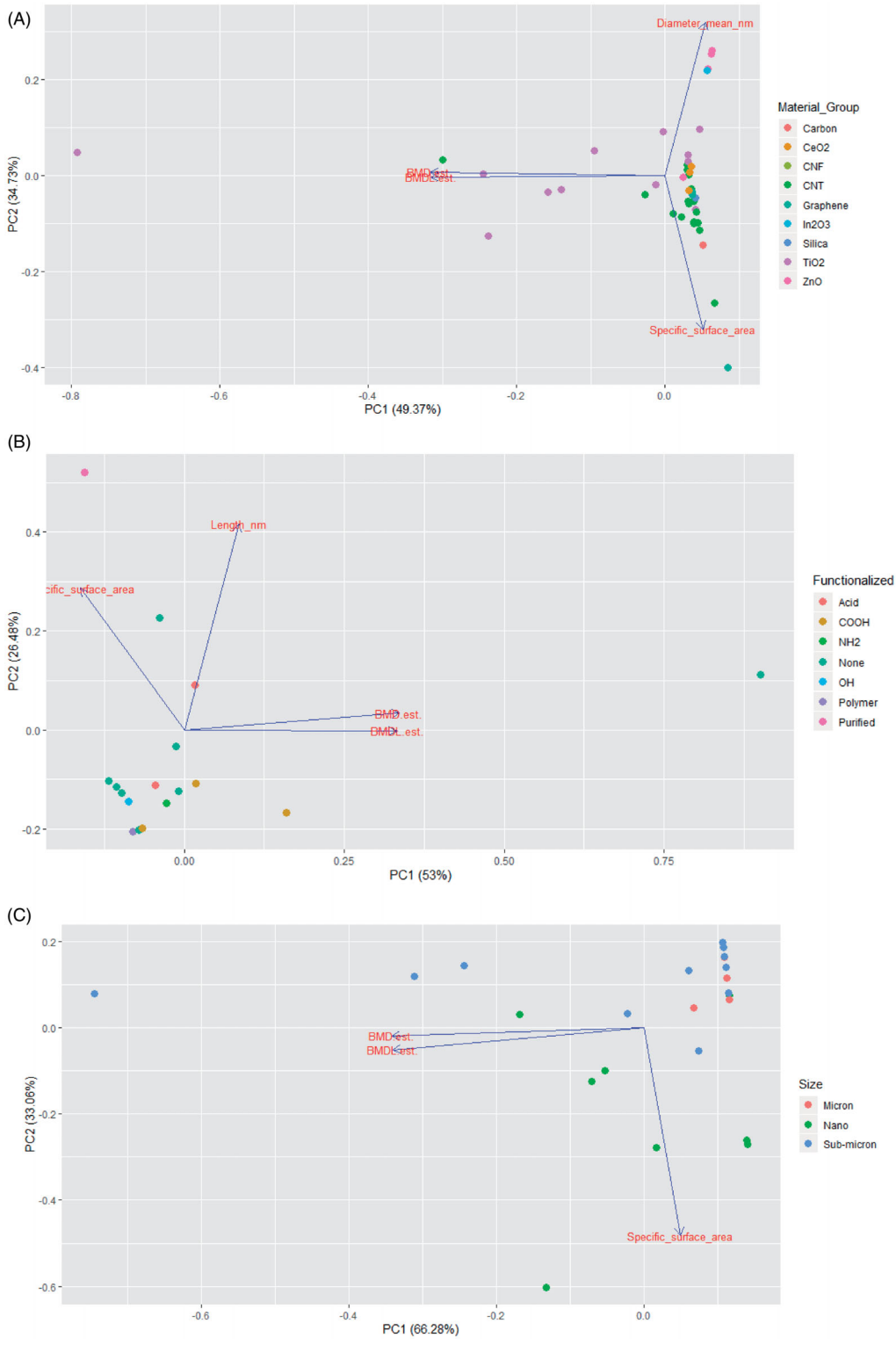
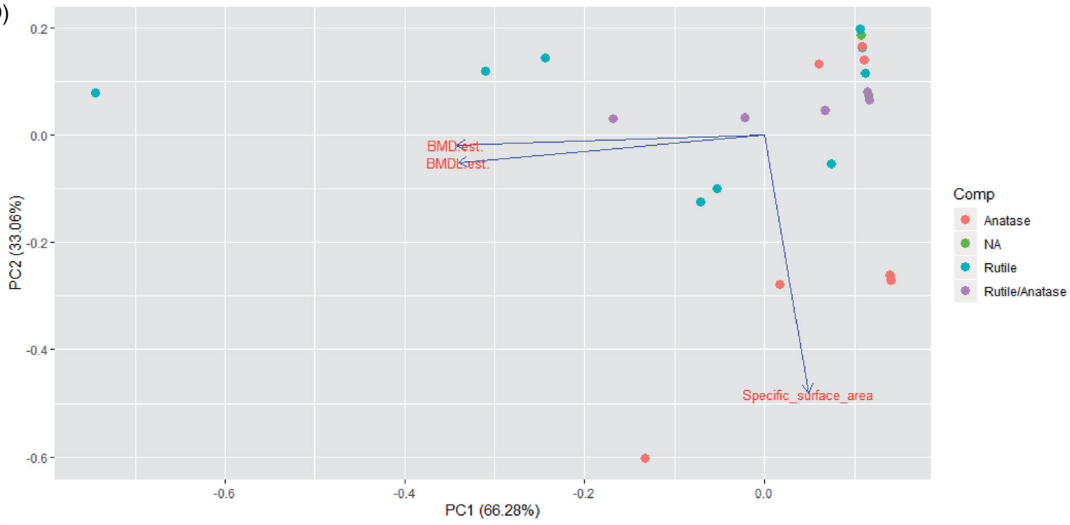


Figure 4. (A) Principal Component Analysis (PCA) between the two most reported quantitative physicochemical properties (Diameter in nm and Specific Surface Area in m^2/g) and the potency estimates (benchmark dose [BMD] and 95% lower confidence limit of the BMD [BMDL]) of all 86 materials. Material labels are colored by corresponding material class from Figure 3. Angles between vectors indicate relative associations such that angles less than 90° indicate a positive correlation between variables, angles approximately 90° indicate little-to-no correlation, and angles greater than 90° indicate a negative correlation. (B) PCA between potency estimations and the two most reported quantitative physicochemical properties of the 20 CNT materials (Length in nm and Specific Surface Area). Material labels are colored by functionalization applied to surface of the material. (C) PCA between potency estimations of the 26 TiO_2 materials and the most reported physicochemical property (Specific Surface Area) within this material. Material labels are colored by particle size category based on reported diameter. (D) Same PCA as shown in Part C, but material labels are colored by material composition (Anatase, Rutile, or a mixture). (E) Table of exact Pearson correlation values between quantitative variables used in PCA in Figure 4(A). (F) Table of Pearson correlation values between quantitative variables used in PCA in Figure 4(B). (G) Table of exact Pearson correlation values between quantitative variables used in PCA in Figure 4(C,D).

(D)



(E)

	Diameter (nm)	Specific Surface Area (m^2/g)	BMD	BMDL
Diameter (nm)	1.000	-0.38889310	-0.1108619	-0.11792612
Specific Surface Area (m2/g)	-0.38889310	1.000	-0.1290954	-0.09321501
BMD	-0.1108619	-0.1290954	1.000	0.93759870
BMDL	-0.11792612	-0.09321501	0.93759870	1.000

(F)

	Length (nm)	Specific Surface Area (m2/g)	BMD	BMDL
Length (nm)	1.000	-0.2881883	0.2014735	0.1137057
Specific Surface Area (m2/g)	-0.2881883	1.000	-0.2802297	-0.2783356
BMD	0.2014735	-0.2802297	1.000	0.9444243
BMDL	0.1137057	-0.2783356	0.9444243	1.000

(G)

	Specific Surface Area (m^2/g)	BMD	BMDL
Specific Surface Area (m2/g)	1.000	-0.1040731	-0.03870686
BMD	-0.1040731	1.000	0.97794369
BMDL	-0.03870686	0.97794369	1.000

Figure 4. Continued.

Table 3. The 90% confidence intervals from bootstrap simulations of benchmark dose (BMD) potency estimates and numeric physicochemical properties displayed in Figure 4.

Material	Variables	Correlation point estimate	Lower 5% quantile	Upper 95% quantile
All	BMD-diameter	−0.1108619	−0.167091	−0.06507012
All	BMD-specific surface area	−0.1290954	−0.2526543	−0.01100498
CNT	BMD-length	0.2014735	−0.565607	0.8271885
CNT	BMD-specific surface area	−0.2802297	−0.498568	0.09752068
TiO_2	BMD-specific surface area	−0.1040731	−0.3638893	0.3777734

The correlation point estimate is the correlation calculation from principal component analysis (PCA) results in Figures 4(E–G).

Table 4. Material groups ranked highest to lowest in potency for all material groups with two or more studies evaluated.

Material[a]	Number of studies	Rodent median BMD estimate (μg/g lung)	Estimated human-equivalent lung dose (μg)[b]	Estimated worker single-day (8-hr TWA) airborne particle concentration (μg/m^3)[c]
ZnO	9	0.0000083	0.0099	0.00345
Carbon nanofibers	2	0.028	33.85	11.8
Carbon	5	0.059	71.30	24.8
Graphene	6	0.075	90.36	31.4
Silica	4	0.085	102.39	35.6
Carbon nanotubes	20	0.11	136.10	47.3
CeO_2	9	0.17	204.15	70.9
TiO_2	26	0.58	692.23	240

Median BMD estimate from each group was used to rank the material groups as well as estimate the human-equivalent lung dose, and subsequently the corresponding estimated worker single day (8-hour) time-weighted average (TWA) airborne particle concentration.
[a]May include nanoscale and/or microscale particles; different crystal structures, shapes, and/or surface modification or functionalization.
[b]1,200 g whole lung weight in adult humans [ICRP 2002].
[c]Worker single day (8-hour) time-weighted average (TWA) airborne particle concentration:
μg/m^3: μg deposited in lungs/(9.6 m^3 air inhaled × 0.3 alveolar deposition fraction). Air inhaled by reference worker from ICRP [2015]; alveolar deposition fraction estimated for nanoscale particles (~50–100 nm) from Multiple-Path Particle Dosimetry (MPPD) model 3.04 [ARA 2015]).

each material were categorized into "Micron", "Sub-Micron", and "Nano" for diameters of >1000 nm, 100–999 nm, and < 100 nm, respectively. A material was categorized by the diameter measurement unless unreported, in which case the primary particle size was used. This was done to reduce the effect of the inconsistent diameter measurement reporting that was seen within the TiO_2 grouping of materials. All other quantitative properties were not frequently or uniformly reported, and thus specific surface area was the only quantitative property used in this analysis. Figures 4(C,D) display the TiO_2 PCA results with materials grouped by the categorical diameter variable "Size" and by Crystallinity (Rutile, Anatase, or a mixture), respectively. Within TiO_2 isoforms, specific surface area had essentially no correlation with the potency estimate (-0.104 BMD, −0.039 BMDL); however, both size and composition had a slight effect, as illustrated by the loose grouping of materials on each plot (Figure 4(C,D,G)). Many of the outliers within the TiO_2 group came from a specific subset of studies, indicating that study design and interlaboratory variability also likely play a role in the heterogeneity seen in the BMD and BMDL estimates, although these affects were not specifically tested (Supplemental Figure 1, Supplemental Table 1).

Further investigations focused on additional evaluation of the associations between the physicochemical properties of a material and its potency. These additional investigations focused on evaluating the reliability of the correlational results from the above PCA analyses. Bootstrapping was utilized to simulate a distribution of correlation coefficients between BMD potency estimates and the quantitative physical properties investigated in Figure 4 (diameter, specific surface area, and length). The 5th and 95th percentiles from each distribution were used to construct a 90% confidence interval for the correlation point estimates from our analyses, none of which was statistically significantly different from zero in the within material class analyses (Table 3). These findings indicate that none of these physiochemical properties was significantly associated with the BMD estimates in either of the material classes evaluated (TiO_2 or CNT). The confidence intervals from the between material evaluations were statistically significant for the weak negative correlations (Table 3).

Material potency ranking

To assess the plausibility of using these literature-based datasets for comparative quantitative risk

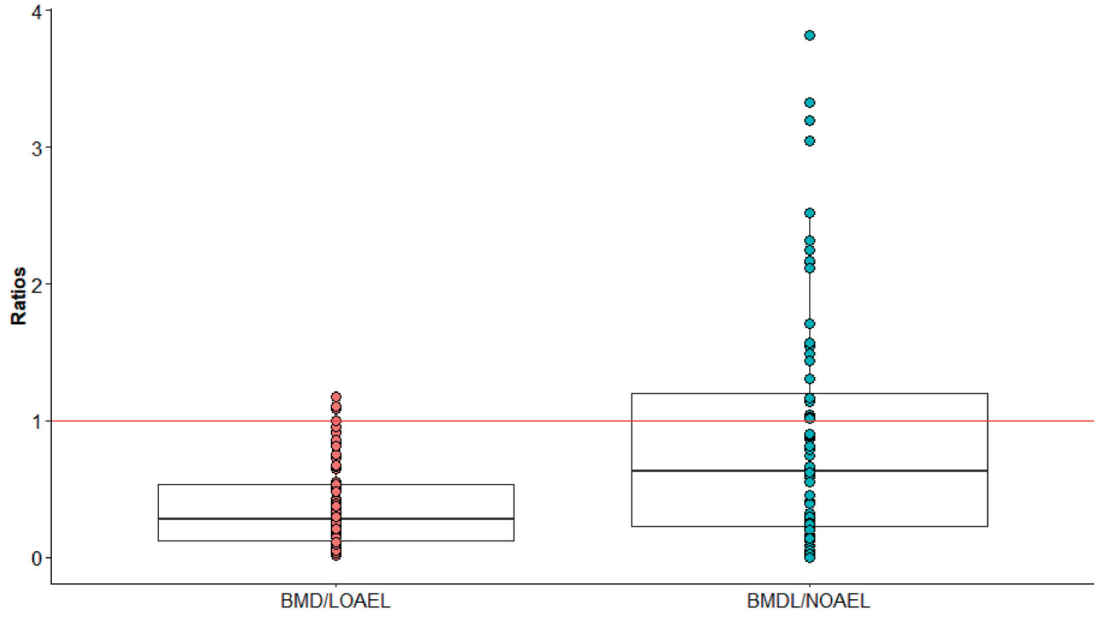


Figure 5. Boxplot displaying ratios comparing the benchmark dose (BMD) and 95% lower confidence limit of the BMD (BMDL) estimates to their corresponding lowest observed adverse effect level (LOAEL) and no observed adverse effect level (NOAEL) doses as reported by the publication. The red line indicates a ratio of 1, in which the BMD and BMDL estimates would be equal to the corresponding LOAEL and NOAEL doses. Data values above this line indicate that the BMD or BMDL was larger than the corresponding LOAEL or NOAEL, respectively, while data values below this line indicate the reverse.

assessment across a variety of materials, we evaluated relative hazard potency for acute pulmonary inflammation by ranking materials by the median BMD estimate for materials with two or more studies included in this literature-derived dataset (Table 4). The median BMD estimate within a material group was chosen as a best estimate of the distribution of BMDs in each group, as those distributions are typically skewed. The most potent material for neutrophilic lung inflammation was ZnO. Materials with intermediate potencies include carbonaceous nanomaterials (CNF, carbon, graphene, and CNT), silica (crystalline and amorphous), and CeO_2. TiO_2 was the least potent material, based on median BMD, although it was also the most variable (Figure 3). Estimated worker-equivalent doses are also shown in Table 4. These comparisons are provided as a type of sensitivity analysis for the plausibility of using this acute inflammation endpoint in rodents to predict human-relevant doses by comparing single day worker exposures to current recommended exposure limits.

Potency estimation comparison

When the specific data and experimental design requirements of benchmark dose modeling are not met (US EPA 2012), other methods for potency estimation may be used such as NOAELs or LOAELs. Ratios were constructed to compare the potency estimates between these two methods. BMD/LOAEL ratios were constructed for all materials where a LOAEL was reported within the study, which excluded 12 of the original 86 materials. Similarly, BMDL/NOAEL ratios were constructed for all materials where a NOAEL was reported, which excluded a separate set of 22 materials from the original set of 86. These ratios were constructed as a method of comparing commonly used points of departure (NOAELs and BMDLs) and estimates of potency (LOAELs and BMDs) (NIOSH 2020). The aim of this analysis was to compare these different methods of estimating points of departure, rather than to derive extrapolation factors as has been done in previous analyses (e.g. Lampe, Fuller, and Kuppusamy 2018).

On average, NOAELs were a more accurate estimate of the corresponding BMDL than the LOAELs were to the corresponding BMD (mean ratio approximately 0.91 and 0.37, respectively). However, the LOAEL was a less varied estimator of the BMD with a standard deviation of only 0.31 compared to a standard deviation of 0.91 for the BMDL/NOAEL ratios. In general, the BMD was about 75% lower

than the corresponding LOAEL (median ratio approximately 0.28), and these ratios were much more symmetrical in distribution compared to the BMDL/NOAEL ratios (Figure 5). In contrast, the BMDL tended to be roughly 50% lower than the corresponding NOAEL (median ratio approximately 0.63), but these ratios varied widely and were heavily skewed by several instances in which the BMDL was higher than the corresponding NOAEL, indicated by a ratio larger than 1 (Figure 5). The implications of the findings for use of these estimates in quantitative risk assessment are discussed later (Discussion, Potency Trends, Differences in Potency Calculations).

Discussion

Potency trends

Material potency trends

The results from this meta-analysis indicated that a material's potency was more associated with its material class (e.g. CNT, TiO_2, ZnO) than specific physical properties of the particle (e.g. diameter or specific surface area). This was shown in the weak estimates of linear association for the most frequently reported physicochemical properties and the BMDs or BMDLs. However, due to data limitations only a small number of physicochemical properties were able to be assessed. In contrast, a more distinct grouping by material class is seen across all materials (Figure 4(A,E)). This pattern of grouping by material class was also seen in the clustering results (Figure 2). This consistency in results from different methods of clustering is further supported by other studies that showed a pattern of grouping by material class (Drew et al. 2017). Similar findings with regard to the sparsity of the physicochemical property data have been reported in other studies. For example, in a comparative *in vitro* study of 24 manufactured nanoparticles, toxicity patterns were primarily based on the elemental composition, with no correlation to size of the particle (Lanone et al. 2009). In another *in vitro* study, nanomaterials were grouped by material class, and iron oxide nanoparticles displayed a different toxicity profile compared to other metal oxides (such as copper, zinc, or titanium dioxide) and carbon nanotubes (Karlsson et al. 2008). However, within a material class (such as CNT or TiO_2), studies have shown differences in toxicity based on their physicochemical properties, including particle size, shape, and crystal structure (NIOSH 2011; Braakuis et al. 2014; Stoehr et al. 2011; Gernand and Casman 2014).

When stratified by material class, some material-specific associations between potency estimates and physicochemical properties were shown in this analysis for the CNT and TiO_2 material classes. For TiO_2 materials, while specific surface area was not associated with the potency estimates, the overall size category (i.e. micron-, submicron-, and nanoscale) and crystallinity (i.e. Rutile, Anatase, or Rutile-Anatase mixture) showed a slight effect in grouping (Figure 4(C,D), respectively). The size of a TiO_2 particle has been established as a factor that contributes to the material's potency due to the greater total particle surface area of the smaller sized particles per unit mass. Studies have shown that smaller particles tend to be more potent and cause a larger inflammatory response than larger particles at a given mass dose (Brzicova et al. 2019; Kobayashi et al. 2009; Sager et al. 2008; NIOSH 2011). Additionally, the crystallinity of TiO_2 has been shown to influence the potency of TiO_2 particles, although the form and severity of differential toxic effects based on crystallinity are more variable than in size comparison studies (Brzicova et al. 2019; Yu et al. 2017).

For the CNT material class, a weakly positive, but not statistically significant, association was estimated between their BMD estimates and length (i.e. longer CNT materials were less toxic) (Figure 4(B,F), Table 3). Other meta-analyses of CNTs lung effects in rodents have reported similar findings. Gernand and Casman (2014) found that shorter median length of CNT was associated with increased PMN influx, while Ramchandran and Gernand (2019) found "no evidence of a significant relationship" between the median length (or diameter) of CNTs and the potency of the cluster. Results from individual toxicology studies on the effect of mean length on potency for inflammation endpoints have been mixed (Fujita et al. 2015; Fujita et al. 2016; Hamilton et al. 2013; Hamilton et al. 2018). A recent publication by Fraser et al. demonstrated that the distribution of a carbon nanotube/nanofiber's physical dimensions is a more consistent indicator of toxicity grouping than a

single point measurement, such as mean or median length (Fraser et al., 2020). In addition, our results indicate that modification or functionalization of a CNT material could play some role in the potency of the material (displayed by moderate grouping of the CNT materials seen in Figure 4(B)). These results are consistent with *in vitro* studies investigating the role of functionalization on the toxic effects of MWCNTs, which have shown pro-inflammatory responses, disruption of regular cellular processes, and cell transformation from exposure to functionalized CNTs (Stueckle et al. 2017; Zhang et al. 2015). The lack of statistical significance in the correlations within material class could be attributed to a variety of sources, including the limited (sparse and heterogeneous) data that were able to be obtained and quantitatively used from the literature. It may also be that the relationship between potency and physicochemical properties is more complex than can be described in simple linear models.

Ranking these materials by median BMD estimate shows that ZnO is the most potent in inducing neutrophilic inflammation, and TiO_2 is the least potent overall across material classes (Table 4). The carbonaceous nanomaterials and CeO_2 were intermediate in potency. These findings are consistent with a previous study that compared material hazard potency using the same PMN endpoint in rodents but using a different set of data (Drew et al. 2017). These material relative rankings are also roughly similar to those based on the no observed adverse effect concentrations (NOAECs) from rat short-term inhalation studies (Landsiedel et al. 2017). Estimates of human-equivalent 8-hour workday exposure concentrations (Table 4) are in the ballpark of the few comparable materials with OELs based on subchronic or chronic exposure in rodents. For example, the NIOSH REL is 1 $\mu g/m^3$ for carbon nanotubes and nanofibers (NIOSH 2013), and 300 $\mu g/m^3$ for ultrafine TiO_2 (NIOSH 2013). The roughly comparable estimates between the human-equivalent single day workplace exposure concentrations (Table 4) and these RELs for nanomaterials suggest that the rodent acute pulmonary inhalation endpoint (added 4% PMN in BALF) is relevant for occupational health risk assessment. However, models have not yet been developed to predict long-term health effects from these short-term data. In addition to the differences in the rodent lung effects evaluated here and those used in deriving the RELs, the methods and assumptions also differ. Yet, these findings are encouraging and suggest that the acute pulmonary inflammation endpoint used in this study merits further evaluation in predictive modeling should adequate data become available.

Differences in potency measures

Another aim of this study was to compare the accuracy of two frequently used measures of potency and points of departure in quantitative risk assessment: the NOAEL/LOAEL approach versus modeling to obtain a BMD/BMDL. Overall, these two measures did not appear to have a direct relationship that could be used to easily compare estimates (e.g. to estimate a BMDL if only a NOAEL was available). One of the reasons for the variability, particularly in the BMDL/NOAEL ratio, could be related to the experimental design of a given study (e.g. dose spacing). The cases where the BMDL/NOAEL ratio was greater than 1 were of interest, as this indicates that the NOAEL was smaller (and would be a more sensitive point of departure) than the corresponding BMDL. These cases primarily occurred with materials that were investigated in the same set of studies (thus using the same experimental design and parameters). In those studies, the dose-spacing often had a large range between only two or three dose groups. Choice of number of dose groups and their subsequent spacing had a profound effect on the NOAEL estimate of potency since the NOAEL is restricted to being one of the experimental dose groups (Crump 1984). Thus, a large spacing between the NOAEL dose and LOAEL dose increases the probability that the true potency dose estimate (and the BMD/BMDL) would be between these two dose groups, leading to a BMDL that is larger than the NOAEL. This finding shows a limitation in the experimental design of the dose spacing in those studies that is not ideal for dose-response modeling. The choice of BMR could also possibly influence the BMDL/NOAEL ratios, although this was not investigated in this study. The BMR of added 4% PMN in BALF was selected as a biologically reasonable response of relevance to workers, representing the initial stages of pulmonary clearance overloading in rats and in the range of

response observed clinically in humans (NIOSH 2011).

While the BMD/BMDL modeling approach typically offers more accurate potency estimations since it utilizes the full dose range of data (Bokkers and Slob 2007; Crump 1984), many investigators still rely on the NOAEL/LOAEL approach due to less stringent data and experimental design requirements. This often makes NOAEL/LOAEL potency estimations more readily obtainable than dose-response modeling. If necessary, LOAELs are also used as a replacement for a BMDL estimate, accompanied with the use of an additional uncertainty factor (Dankovic et al. 2015; NIOSH 2019). This is in accordance with the observation that the corresponding BMDL (or NOAEL) is likely a lower and more sensitive estimate of potency. The findings of this analysis suggest caution if using a NOAEL or LOAEL in lieu of a BMDL due to the large variation in the NOAEL and LOAEL estimates across all materials studied relative to the BMDL and BMD estimates, respectively. NOAELs that are considerably larger than the BMDL would be of particular concern, as these would result in an underestimate of the actual potency. These findings reaffirm the utility of BMD estimates for quantitative risk assessment, although many of the published toxicological studies either lack optimal experimental design for dose-response modeling or do not report sufficient statistical information to perform dose-response modeling.

Suggestions for future publications

Minimum data reporting

Of the 144 studies of interest (across all four searches), 36 studies were ultimately successful for BMD modeling (Figure 1). Some of these 108 remaining studies were excluded from modeling because the post-exposure time points (>3 days) exceeded the time point to evaluate acute pulmonary inflammation for these analyses. Several studies met the experimental design criteria but lacked sufficient reporting of the summary statistics required for dose-response modeling. For successful modeling, the means, non-zero measures of standard deviation/error, and an exact sample size must be provided for the control and treatment groups. One common issue encountered among the studies that could not be used for modeling was providing a range of a sample sizes that encompassed all treatment groups rather than the specific sample size per group (if sample size varied across groups). An exact sample size per dose group was considered necessary due to the low number of individuals typically used per group (e.g. 4–6 individuals). That is, slight differences in group sizes could influence the modeling outcome, and thus the potency estimate, of a material. Additionally, for several studies, the summary statistics were not explicitly given in the paper and had to be extracted from the corresponding figure (e.g. a bar graph of the treatment means). While a software was used to extract the values of summary statistics from a graphical figure (https://automeris.io/WebPlotDigitizer/), this could have introduced an unknown amount of extra variation in the data used in modeling. However, some of these studies were not included in modeling due to the absence of visible error bars for each treatment group to estimate standard deviation/error or no control value mean/standard deviation was available.

All of the issues mentioned above could be avoided if authors provide a table of summary data, either supplementary or presented within the publication (e.g. as shown in Table 2). Such a table would allow for specification of exact sample sizes (if not equal across all groups) and exact values for all sample means and standard deviations or standard errors. The latter would not only provide precision for any summary statistics that are too small to display visibly on a figure, but also would reduce any potential uncertainty that stems from extracting summary statistics graphically using software. The summary statistical information needed for BMD modeling is already a part of the experimental findings typically performed in toxicology studies but is not always reported in the publication. The consistent reporting of these basic statistical summary data of the experimental design and results would greatly increase the utility of the toxicological literature for use in hazard and safety assessments.

Experimental design considerations

Several factors of the experimental design have a large impact on the ability to derive potency estimates. The most evident factor is sample size. Sample size can largely affect both types of potency estimation (NOAEL and LOAEL depending on experimental doses or BMD and BMDL estimates from modeling). The potency estimates from the

NOAEL/LOAEL approach are affected by the sample size since this approach is dictated by statistical significance. Thus, a larger sample size tends to lead to a larger test statistic, increasing the odds of rejecting the null hypothesis and declaring statistical significance (Crump 1984) at a given level of significance. The effect of sample size is less pronounced in BMD/BMDL modeling, with a larger effect on the BMDL than BMD estimate because construction of the 95% confidence interval considers sample size (Davis, Gift, and Zhao 2011). Overall, the effect of sample size is less pronounced on modeling in comparison to the NOAEL/LOAEL approach as changes in small sample size have less of an effect on the BMD/BMDL calculation than it would in a statistical test that determines the NOAEL/LOAEL (Crump 1984, Davis, Gift, and Zhao 2011). A tradeoff can be struck between number of dose groups and the number of individuals needed per group. If multiple dose groups (minimally two dose groups in addition to a control) are included in the experimental design, a smaller number of individuals is more acceptable since modeling can potentially be used to estimate potency. If only a single experimental dose group is used, a larger sample size should be taken into more consideration since the NOAEL/LOAEL approach must be used for potency estimation. However, there is a delicate balance between using enough individuals per treatment group for statistical power and modeling and reducing the overall number of individuals for ethical concerns and maintaining the Tox21 initiative (NAS 2007). In general, more doses with fewer animals per dose group provides more useful data for dose-response modeling for quantitative risk assessment. However, it is a tradeoff as this may not be ideal for determining effect and statistical significance as smaller sample sizes per group can drastically increase error measurements.

A few less apparent factors of experimental design that could influence potency estimates are the spacing and number of dose groups. The spacing of dose groups within an experiment can largely impact the estimates, specifically if the low dose group is only slightly above the control while the high dose group is an order of magnitude or more above the first dose. If using the NOAEL/LOAEL approach, this type of dose spacing can drastically increase the risk of the NOAEL being an under-estimate of the true potency, and thus is less protective. While modeling is much more resistant to the effects of dose spacing than the NOAEL/LOAEL approach, dose spacing can still negatively impact the ability to fit a parametric model if the low dose group response is comparable to the control and the high dose group displays a sharp increase in response compared to the low dose group (Davis, Gift, and Zhao 2011). If using the BMD modeling approach, experiments with a control and one treatment group are insufficient, and without prior mechanistic information it may not be possible to estimate potency. Optimally, the number and spacing of dose groups in an experiment to be useful for dose-response modeling for quantitative risk assessment includes minimally two or three evenly spaced treatment groups in addition to an unexposed control group.

Limitations & future work

The largest limitation faced in this study was the overwhelming heterogeneity of the type of data reported in the literature, mainly regarding experimental design, reported measures of pulmonary response, and physicochemical properties. These sources of variability can influence the detection of the true effects of material properties on toxicity. In addition, within a material class (e.g. TiO_2) considerable heterogeneity in the types of materials was reported, yet the physicochemical property data were not uniformly or completely reported in order to more fully examine the influence of those properties on the toxicity of the material studied. In particular, properties such as diameter and density were recorded as they were reported in the paper and may not be the best representation of the physicochemical properties of the particle during real-world exposure (e.g. singlet versus agglomerates). While there were often multiple observations per material class, within-class heterogeneity tended to be very high, especially among the most frequently studied classes. For example, within the class of TiO_2 materials, there were 19 different TiO_2 isoforms out of a total of 26 observations without complete or uniform physicochemical characterization on each isoform. This heterogeneity makes direct comparisons difficult between potency estimates of the same material isoform and likely

added extra variability in the data that could have precluded the observation of potential associations between the physicochemical properties and potency estimates. In this evaluation, the possible associations of physicochemical properties and pulmonary inflammation potency were primarily examined in a correlational manner via the relationship between component vectors from principal component analysis. The data were too limited relative to the variability to properly conduct more sophisticated statistical or predictive analyses to further estimate the contribution of various factors. The limited physicochemical property data reported in many of these toxicology publications precluded more quantitative estimates of association. More complete and uniform data on physicochemical properties would allow for more sophisticated predictive analyses.

Another source of variation that could have a large effect on this study results from data being collected from multiple sources (e.g. multiple academic labs and research institutions). Even when conducting the replicate experiments using the same experimental design and parameters, inter-laboratory variability was observed *in vivo* (Bonner et al. 2013) and to a great extent *in vitro* (Xia et al. 2013). The magnitude of inter-laboratory variability was not specifically assessed in these studies, but both studies found that the effect of inter-laboratory variability varied across assay and material. Inter-study variability is even more difficult to assess in the broad literature as it is highly correlated with specific materials. Thus, teasing out the role of experimental design/method factors versus the material properties themselves is a challenge with this (and many other meta-analyses driven) datasets; more consistent and compatible data would be required. Lung burden estimation is another source of uncertainty, which depends on the route of exposure. For pharyngeal aspiration and intratracheal instillation, a large fraction (80 or 100%, respectively) of the administered dose was estimated to be deposited in the lungs. For inhalation exposure, the measured lung burdens were used if reported; if not, the MPPD v. 3.04 model (ARA 2015) was used to estimate the deposition fraction in the alveolar region based on the airborne particle size distribution data and the rodent species and body weight. Although not evaluated in this paper, previous comparisons of MPPD model-estimated vs. measured lung burdens in rat inhalation studies of carbon nanotubes have been reported (NIOSH 2013).

As techniques for physicochemical characterization of materials advance, material characterization is more frequently being incorporated as a crucial part of study design. Data availability and curation are still major issues faced by the computational toxicology community (Furxhia et al. 2020). While a definitive link between physicochemical characteristics and potency was not uncovered in this study due to limited data availability, more complete and uniform reporting of this material and toxicological data in future publications will greatly increase the potential for model development in this type of meta-analysis. Coordinated research and experimental can also facilitate the collection of data useful for quantitative risk assessment (e.g. ENPRA and nanoGO, reported in Drew et al. 2017). Accessibility to curated datasets and standardized data templates (e.g. Nanoinformatix; ISO-Tab-Nano) would also facilitate analyses of the physicochemical properties on hazard potency. Additionally, dose-response data for other endpoints in the AOP of lung disease would fill-out the information needs to evaluate alternative assays including in vitro assays for use in hazard assessment or prioritization and grouping/read-across. With more systematic and comprehensive publication results, the methodologies developed in this study could be applied to a variety of different toxicological endpoints and modes of action for predictive model development.

Conclusions

Systematic literature searches yielded quantitative data for 86 materials/experiments from 36 studies, from which estimates could be derived of the deposited particle mass lung doses associated with acute pulmonary neutrophilic inflammation in rodents exposed to nanoscale or microscale particles. Clear differences in hazard potency were observed by material class. Variability within material class was also observed, but no clear patterns emerged to explain that variability. Particle size, specific surface area, crystallinity, and surface functionalization appeared to play a role, but none of these factors were statistically significant. More

complex relationships among these physicochemical properties, combined with sparse or heterogeneous data and other experimental differences, may explain the difficulty in quantifying the role of properties beyond material class in describing the acute inflammation hazard of ENMs in this meta-analysis.

As shown in previous studies, acute pulmonary inflammation provides a promising link to *in vitro* assays for the development of predictive models. More research is needed to link the acute responses to those from chronic exposures. Findings from this analysis suggest that the acute pulmonary inflammatory response in rodents can be used to describe the differences in hazard potency by material class of particles. Further investigations are needed to understand the role of physicochemical properties beyond material class in comparisons across studies, and to develop predictive models describing acute (*in vitro* and *in vivo*) to chronic dose-response. Until then, alternative strategies to rodent bioassays for quantitative risk assessment and OEL development for ENMs are yet to be fully realized.

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Disclosure statement

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention. No potential conflict of interest was reported by the author(s).

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Data availability statement

The full set of potency estimates (BMDs, BMDLs, NOAELs, and LOAELs) used within this manuscript can be found in Table S-1 in Supplemental Material. Data used to calculate these potency estimates can be found in the corresponding publications.

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