Approach toward In Vitro-Based Human Toxicity Effect Factors for the Life Cycle Impact Assessment of Inhaled Low-Solubility Particles

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ABSTRACT: Today’s scarcity of animal toxicological data for nanomaterials could be lifted by substituting in vitro data with in vivo data to calculate nanomaterials’ effect factors (EF) for Life Cycle Assessment (LCA). Here, we present a step-by-step procedure to calculate in vitro-to-in vivo extrapolation factors to estimate human Benchmark Doses and subsequently in vitro-based EFs for several inhaled nonsoluble nanomaterials. Based on mouse data, the in vitro-based EF of TiO₂ is between 2.76·10⁻⁴ and 1.10·10⁻³ cases/(m²/g·kg intake), depending on the aerodynamic size of the particle, which is in good agreement with in vivo-based EFs (1.51·10⁻⁴–5.6·10⁻² cases/(m²/g·kg intake)). The EF for amorphous silica is in a similar range as for TiO₂, but the result is less robust due to only few in vivo data available. The results based on rat data are very different, confirming the importance of selecting animal species representative of human responses. The discrepancy between in vivo and in vitro animal data in terms of availability and quality limits the coverage of further nanomaterials. Systematic testing on human and animal cells is needed to reduce the variability in toxicological response determined by the differences in experimental conditions, thus helping improve the predictivity of in vitro-to-in vivo extrapolation factors.

KEYWORDS: titanium dioxide, silica, cerium oxide, extrapolation factor, dosimetry, LCA

INTRODUCTION

Nanotechnology has been recognized as one of the Key Enabling Technologies of the 21st century, thanks to its revolutionary applications in multiple sectors, ranging from energy to healthcare.¹ According to the International Organization for Standardization (ISO), nanomaterials are defined as materials “with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale.”² In parallel to the enthusiasm for their novel functions, the inclusion of nanomaterials in products has also raised concerns about their potential impacts on the health of workers, consumers, and in general humans exposed to them along the product life cycle.³

Life Cycle Assessment (LCA) is the preferred methodology to assess the environmental impacts of nanoenabled products and compare them with existing alternatives, accounting for the negative but also positive impacts that a new technology may have on the overall environmental profile of the product (e.g., increased toxicity for humans but reduced greenhouse gas emissions).⁴,⁵

In LCA, impacts are calculated by linking all emissions occurring during a product life cycle to their corresponding characterization factors, which define the incidence of negative health/ecological effects caused by the emission of a substance. For toxicological impacts (on humans as well as on the ecosystem), the LCA community agreed on the use of USEtox as a common consensus model.⁶ Within USEtox, a characterization factor is calculated as a combination of 1) a fate factor, which indicates how a substance is distributed in the environmental compartments following its emission; 2) an exposure factor, which describes the human uptake of the substance from the environmental compartments via multiple exposure pathways; 3) and an effect factor (EF), which relates the uptake of the substance to potential negative health effects.⁷ USEtox and its calculation principles have been developed for organic chemicals and metal ions⁶,⁸ and is thus not adequate for nanomaterials in its original setting.⁹ A nanospecific fate model has been developed to calculate the fate factor for nanomaterials,¹⁰ while the exposure factor is either calculated according to existing methodologies or disregarded.¹¹ The EF is calculated from animal toxicological studies using those extrapolation factors (e.g., the interspecies extrapolation factor) needed to convert the animal results to a

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human chronic ED50, i.e., the lifetime dose generating a 50% increase in disease probability for humans. Since these extrapolation factors have been obtained based on data for organic chemicals, their validity for nanomaterials is yet to be proven. However, a bigger challenge lies upstream: animal testing is being reduced in favor of alternative methods, resulting in a scarcity of toxicological data compared to the number of newly developed nanomaterials.

A potential solution to this could be to use in vitro data, i.e., the results of toxicological studies conducted on human cells, as the data pool for the calculation of human toxicity EFs, as suggested by several authors. Salieri et al. proposed an approach to calculate EFs for soluble nanoparticles from in vitro data, based on the fact that the toxic effects are mainly caused by the dissolved ions rather than the particle itself. This approach is though not fit for nonsoluble particles.

Recently, we proposed that a combination of models could be used for the calculation of EFs from in vitro data, and we developed a model to ease the application of this strategy for the specific case of inhaled spherical nanomaterials and their effects on the lung. In this paper, we provide a proof of concept of the estimation of in vitro-to-in vivo extrapolation factors, and we use these preliminary factors to calculate in vitro-based EFs for titanium dioxide, amorphous silica, and cerium oxide.

### MATERIALS AND METHODS

**Overview of Methodology.** The calculation of in vitro-to-in vivo extrapolation factors and in vitro-based EFs follows multiple steps, depicted in Figure 1. The first step is the collection of toxicity data from animal studies and from in vitro studies using animal and human cells. Then dosimetry models are applied to find the deposited doses per cm² of cell culture or lung corresponding to the in vitro and in vivo doses. The obtained doses are transformed into surface area doses, and a Benchmark Dose (BMD) is then calculated for each dose-response data set. The in vitro-to-in vivo extrapolation factors are then calculated as the ratio between the in vivo and in vitro animal BMD values and use of the extrapolation factors to calculate the human BMD from the in vitro human BMD values following the traditional extrapolation procedure.
sugesting that in vitro tests may be able to measure early events leading to acute lung inflammation. Acute inflammation may become chronic if the exposure is not halted and the inflammation resolved, and more serious diseases such as lung fibrosis may develop.

**Data Collection. In Vitro Data.** A literature search was conducted using Google Scholar and Scopus, using various combinations of the following keywords: "nanomaterial name", "in vitro", "inflammation", "toxicity", "macrophages"; to find data for human or animal cells, these additional keywords indicating the species or macrophages cell line were used: "mouse", "rat", "murine", "THP-1", "RAW264.7", "J774A.1", "HMDM", "NR8383". Moreover, the data set published in Romeo et al. was also used as a data source.

The criteria for inclusion of data from a study were as follows: a) used a monoculture of human, rat, or mouse macrophages; b) tested spherical particles; c) tested the release of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8, MIP-2); d) included at least two doses plus negative control; e) included all parameters needed for the use of the Combined Dosimetry model CoDo, as described in Romeo et al.

From 26 publications, we extracted 141 dose—response data sets, 59 using human cells, 35 for rat cells, and 47 for mouse cells.

**In Vivo Data.** In vivo data was collected from the literature and from the data set published in Romeo et al. using a combination of the following keywords: "nanomaterial name", "rat", "mouse", "in vivo", "toxicity", "lung", "inhalation". The inclusion criteria were as follows: a) rat or mouse as the animal; b) at least two doses tested in addition to the negative control; c) neutrophils (PMN) influx as a number or percentage in Bronchoalveolar Lavage Fluid (BALF) as the end point; d) the exposure time lasted at maximum 1 week; e) the postexposure time was at maximum 72 h if the particles were delivered via an intratracheal instillation; f) either the specific surface area of the particles or the primary particle diameter was reported.

155 dose—response data sets, 109 using rats and 46 using mice, were extracted from 30 publications.

**Simulation of Particle Deposition and Retention.** For in vitro data, the Combined Dosimetry model CoDo was used to simulate the deposition of the particles on the cells, determined by sedimentation and diffusion processes. For in vivo data when the particles were administered via inhalation, the Multiple-Path Particle Dosimetry model (MPPD) was used to calculate the amount of particles retained in the animal alveoli, while for instillation, we assumed 100% deposition in the lung. The parameters used for both models are reported in the Supporting Information. Whenever possible, the retained dose was preferred to the deposited dose as it has been shown to better correlate with the effects measured in the animal.

Both in vitro and in vivo deposited/retained doses were normalized by the surface area of the cell culture well or the animal alveoli, respectively.

**Calculation of Benchmark Doses.** The Benchmark Dose (BMD) approach was chosen due to its recognition in the risk assessment and LCA communities as a way to determine toxicological dose descriptors from dose—response data sets. In this approach, a dose—response curve is fit on the data, and the dose causing a certain response over the control (the Benchmark Response (BMR)) is identified, together with the uncertainty of such a value.

Since the surface area was identified in multiple studies as a more relevant dose metric than mass, the deposited/retained doses were transformed from mass to surface area doses using the specific surface area (SSA) of the particles; when not reported, the SSA was calculated from the primary particle diameter of the particles by assuming a perfectly spherical shape. A Benchmark Response (BMR) of 20% was chosen for the BMD calculation, done with the PROAST software. The percentage of neutrophils in BALF was considered a quantal response, while other end points were considered continuous responses. Whereas Pennington et al. proposed the use of the ED10 or BMD10 for the linear extrapolation of risk at low doses when calculating an EF (in place of the ED50), we chose a BMD20 equivalent to the ED20 since such change is considered a sign of low inflammation and still resides in the low-dose region of the dose—response curve.

**Calculation of In Vitro-to-in Vivo Extrapolation Factors.** The calculation of in vitro-to-in vivo extrapolation factors is done in parallel for each nanomaterial, for rat and mouse animal and cell lines and for the two in vivo end points (number of PMN and PMN percentage). For each group of data, we calculated the ratio between each combination of in vivo and in vitro BMD20 values. A nonparametric bootstrapping procedure was applied to estimate the distribution of the median in vivo—in vitro ratio. The use of the median is more robust compared to the mean for non-normal distributions. Then, we removed the outliers according to the 1.5 IQR rule, which identifies as outliers those points that have a distance from the 0.25 and 0.75 quantiles of at least 1.5 times the interquartile range (IQR).

The in vitro-to-in vivo extrapolation factor estimated via the bootstrapping procedure is the arithmetic mean of the estimated population of ratios, after the removal of outliers.

**Calculation of Human Toxicity EFs from In Vitro Data.** For each particle, the calculation of the in vitro-based EFs was done following these steps:

1. Calculate the median BMD20 from in vitro human data via nonparametric bootstrapping;
2. Multiply by the in vitro-to-in vivo extrapolation factor to obtain the human BMD20 in dose per cm2 lung;
3. Multiply by the human alveoli surface area to obtain the total retained dose in the lung;
4. Divide by the retention rate to find the intake dose. The retention rates were calculated via the MPPD model for particles with an aerodynamic diameter between 10 nm and 1 μm; since the retention rate is not constant over time, a 7-day continuous exposure was chosen (same exposure limit as for the selection of animal studies). The maximum and minimum rates were then used to obtain a range of intake doses;
5. Divide by 7 to find the daily intake dose;
6. Divide by 5 to extrapolate from subacute BMD20 to chronic BMD20 with the extrapolation factor from Vermeire et al.;
7. Convert to lifetime intake by multiplying by 365 days and 70 years;
8. Convert the lifetime chronic BMD20 from cm2 particle lifetime intake to (m2 particle/μm particle) kg intake so that the unit of the EF will be consistent with the one of published EFs (see the SI for conversion steps);
9. Calculate the EF as 0.2/human BMD$_{20}$ (similar to Pennington et al., where EF = 0.1/ED$_{10}$).

**Calculation of Human Toxicity Effect Factors from Animal Data.** As a comparison, EFs were calculated from the collected animal data:

1. Calculate the median BMD$_{20}$ from animal data via nonparametric bootstrapping;
2. Multiply the median animal BMD$_{20}$ by the animal alveolar surface area to find the total retained dose;
3. Extrapolate to the retained dose in human using the ratio between the human alveoli surface and the animal alveoli surface, as in Fransman et al.

After obtaining the human BMD$_{20}$ as retained dose, the EF was calculated following steps 3 to 8 from the previous section.

**Calculation of Uncertainties.** The uncertainty of LCIA extrapolation factors is expressed by the dispersion factor $k$, which indicates how much a factor $x$ might deviate from the median ($M$), with a 95% probability ($P$):

$$P\left\{ \frac{M}{k} < x < M \cdot k \right\} = 0.95$$

For the in vitro-to-in vivo extrapolation factors, we calculated the dispersion factors from the 95th percentile of the bootstrap distribution, after the removal of outliers, with the formula from Huijbregts et al., which does not require any assumption on the shape of the data distribution:

$$k = \sqrt{\frac{97.5\text{th percentile}}{2.5\text{th percentile}}}$$

The uncertainty of the final EF was calculated as a combination of the dispersion factors of the extrapolation factors, according to Slob.

**RESULTS AND DISCUSSION**

**Benchmark Dose Values.** 109 BMD$_{20}$ values were obtained from the in vitro data, most of them regarding human and mouse cells; the values ranged over multiple orders of magnitude, in particular for the larger data sets, i.e., amorphous silica and titanium dioxide (Figure 2 and Table S1). Such differences were due to the collected data rather than the deposition simulations: the particle concentrations used in the studies ranged from $1 \times 10^{-5}$ to 1.7 mg cm$^{-3}$, and the deposited doses ranged from $1 \times 10^{-6}$ to 0.7 mg cm$^{-2}$ or $1.27 \times 10^{3}$ to $8.8 \times 10^{3}$ cm$^{-2}$/cm$^{2}$ when using the surface area dose. We did not observe any trend based on the cytokine considered (TNF-$\alpha$, IL-1$\beta$, IL-6, IL-8, MIP-2), supporting our choice of aggregating them in a unique end point called “cytokine release”.

For in vivo data, we obtained 103 BMD$_{20}$ values, 59 considering the absolute number of neutrophils as end point (Figure 3) and 44 considering the percentage of neutrophils as end point (Figure S1). Also in this case, the BMD$_{20}$ values had a very broad range (Table S2), which is linked to the original
data rather than the deposition calculation, since the deposition rate was set as constant in the case of instilled nanomaterials and only spread over an order of magnitude for the administration via inhalation.

The wide range of both in vitro and in vivo BMD20 can only be explained by the differences in material properties and experimental conditions of the original studies. The impact of such factors on the biological response has been highlighted in multiple publications.69-72

While the nanomaterials we considered are the most studied, our constraints for the inclusion of data are quite stringent. Only studies with a comprehensive characterization of the particle physicochemical properties were included, since this information was necessary for the simulation of the particle behavior in the in vitro system. For example, multiple studies had to be discarded because they did not report the diameter of the agglomerated particle in the media. For the calculation of the BMD20, at least two doses plus the control were needed to fit a dose–response curve over the data, thus excluding those studies where only one dose was tested (This was often the case for in vivo studies.). Last, those data sets without a clear dose–response relationship were discarded as well by the BMD modeling process. This explains why some nanomaterials and species only have a few data points.

**In Vitro-to-In Vivo Extrapolation Factors.** Table 1 reports the in vitro-to-in vivo extrapolation factors calculated from rat and mouse data and considering the number of PMN (polymorphonuclear leukocytes) as events counted as the endpoint. The extrapolation factors used in this manuscript are the EFs that correctly represent the in vivo toxic potential as well as the sensitivity of the human lung to inhaled nanomaterials.

| Nanomaterial | Species | In Vitro-to-In Vivo Extrapolation Factor | k |
|--------------|---------|-----------------------------------------|---|
| Crystalline SiO2 | Rat | 3.24 × 10⁻⁴ | 8.89 |
| Amorphous SiO2 | Rat | 4.73 | 14.5 |
| Amorphous SiO2 | Mouse | 5.32 × 10⁻² | 2.77 |
| TiO2 | Rat | 1.00 × 10⁻³ | 1.61 |
| TiO2 | Mouse | 4.55 | 1.78 |

From rat and mouse data and considering the number of PMN as end point. Cerium oxide had to be excluded since it did not have corresponding in vitro and in vivo data. The extrapolation factors calculated for neutrophilic events are presented in Table S3.

The extrapolation factors obtained from the ratios of in vivo and in vitro BMD20 values (Figures S2 and S3) via bootstrapping are different for each particle and show a level of uncertainty dependent on the number and variability of the BMD20 values. Depending on the species considered, amorphous silica and titanium dioxide follow opposite trends. Such a difference does not support the hypothesis that a unique extrapolation factor might be valid for low-toxicity low-solubility particles; however, given the wide spread of BMD20 values, some data sets (e.g., for amorphous silica) are so small that it is questionable whether they correctly represent the distribution of the BMD20. A more reliable approach to test this hypothesis would be to have triads of in vivo data, in vitro data using mouse cells, and in vitro data using human cells obtained by testing in (as much as possible) the same exposure conditions and using the same nanomaterial. In this case, the comparison of the ratios of multiple nanomaterials would not suffer from the large variability of the BMD20 values. Unfortunately, the current lack of such fit-for-purpose data prevents us from applying this approach today.

**Human Toxicity Effect Factors from In Vitro Data.** The EFs have been calculated from in vitro human data as ranges (Table 2), to account for the effect that the aerodynamic particle size (which in our case was unknown) has on the retention of the particles in the human lung. Considering particles with an aerodynamic diameter between 10 nm and 1 μm, the retention rate ranged between 6% and 24% of the intake dose considering 7 days of continuous exposure.

The calculated EFs significantly differ depending on which species was used to calculate the in vitro-to-in vivo extrapolation factors. In the case of rat, the calculated EFs correctly represent the higher toxicity of crystalline silica, but amorphous silica and titanium dioxide, both considered low-toxicity materials, show a great difference in potency, with the latter multiple orders of magnitude more toxic. Looking at the EFs using mouse data, both particles show a similar low toxicity, though no data is available to compare it with crystalline silica. This difference is explained by the fact that titanium dioxide is reported in the data we collected as very inflammogenic for rats in vivo, while the same effect was not observed for amorphous silica or for mice.

The higher susceptibility to inhaled nanomaterials of rats compared to mice due to a faster lung overload and a stronger inflammatory response is well-known.51,52 This suggests that, despite being frequently used in animal studies, the rat might be a precautionary choice rather than a representative one for the effects of particles on human lungs.

**Comparison between In Vitro- and In Vivo-Based Human Toxicity Effect Factors.** Table 3 and Figure 4 show the comparison between the in vitro-based EFs, the in vivo-based EFs calculated from the same animal data used for the in vitro-to-in vivo extrapolation factors (see also Table S4), and the EFs available from the literature and obtained from chronic or subchronic animal studies.

Table 2. Human Toxicity EFs Calculated from In Vitro Data, Expressed as cases/(m²/g·kg intake), Based on the In Vitro-to-In Vivo Extrapolation Factors Respectively Obtained from Rat and Mouse Data.

| Nanomaterial | Type | No. of Data Points | Median in Vitro Human BMD20 | EF Extrapolated from Rat Data | k_{rat} EF | EF Extrapolated from Mouse Data | k_{mouse} EF |
|--------------|------|--------------------|-----------------------------|-------------------------------|-----------|-------------------------------|-------------|
| Crystalline SiO2 | 3 | 0.132 | 4.60 × 10⁻³–1.84 × 10⁻⁴ | 38.5 | 4.07 × 10⁻³–1.63 × 10⁻² | 14.7 |
| Amorphous SiO2 | 25 | 0.954 | 1.10–1.10 | 12.6 | 12.8 |
| Titanium O2 | Anatase + Rutile | 13 | 0.160 | 1.30–5.18 | 12.6 | 12.8 |
| Titanium O2 | Anatase | 7 | 0.361 | 0.58–2.30 | 13 | 12.8 |
| Titanium O2 | Rutile | 6 | 0.430 | 0.48–1.93 | 12.6 | 12.8 |
| Titanium O2 | P25 | 1 | 0.0185 | 11.2–44.9 | 12.6 | 12.8 |

The uncertainties k of the EF represent the combination of the uncertainties of all extrapolation factors used to calculate the EF.
The results show a good agreement between the *in vitro* EFs and the *in vivo* EFs we calculated; while these values are partially correlated, since the *in vivo* BMD$_{20}$ values are used to calculate the *in vitro*-to-*in vivo* extrapolation factors, it is also true that the extrapolation factor depends also on the *in vitro* animal BMD$_{10}$ values and that different extrapolation procedures are used for the two data sources to calculate the EFs. When comparing also with the published EFs for titanium dioxide, both our *in vitro* and *in vivo* EFs fall in the same range when mouse data is used, while the EFs based on rat data confirm the strong response this species has to this nanomaterial. No EFs have been published for the other materials, but the good correspondence between our calculated *in vitro* and *in vivo* EFs for amorphous silica and titanium dioxide from mouse data suggests this might be a representative result as well.

Another interesting point is that while our data was restricted to short-term inflammation, published data referred to longer term studies looking at a variety of effects such as alveolar epithelial cell hypertrophy, cell necrosis, histopathological findings, and neutrophil levels in BALF and often considered the No Observed Adverse Effect Level (NOAEL) or the Lowest Observed Adverse Effect Level (LOAEL) rather than an ED$_{50}$. This seems to suggest that short-term effects might be predictive of more chronic effects (for which they are suggested to be necessary but not sufficient precursors, see.

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**Table 3. Comparison between the *In Vitro*-Based EFs (Highlighted in Light Blue), the *In Vivo*-Based EFs Calculated from Our Data Set, and the EFs Available from the Literature$^{a}$**

|                  | TiO$_2$ | Amorphous SiO$_2$ | Crystalline SiO$_2$ | CeO$_2$ |
|------------------|---------|-------------------|---------------------|---------|
| *In vitro*-based EF extrapolated from rat data | 1.30 - 5.18 | 4.60·10$^{-5}$ - 1.84·10$^{-4}$ | 4.85 - 19.4 | - |
| *In vitro*-based EF extrapolated from mouse data | 2.76·10$^{-4}$ - 1.10·10$^{-3}$ | 4.07·10$^{-3}$ - 1.63·10$^{-2}$ | - | - |
| *In vivo*-based EF from rat data | 0.181 - 0.723 | 1.05·10$^{-3}$ - 4.22·10$^{-3}$ | 0.630 - 2.52 | 0.646 - 2.58 |
| *In vivo*-based EF from mouse data | 8.87·10$^{-3}$ - 3.55·10$^{-2}$ | 9.88·10$^{-3}$ - 3.95·10$^{-2}$ | - | - |
| EF from published studies | 5.6·10$^{-2}$ from$^{53}$ | - | - | - |
|                 | 1.51·10$^{-4}$ from$^{54}$ | - | - | - |
|                 | 2.40·10$^{-2}$ from$^{55}$ | - | - | - |

$^{a}$All EFs are reported as cases/(m$^2$/g·kg intake). When the EF was not reported with respect to the particle surface area, a default specific surface area of 48 m$^2$/g was used, as in Buist et al.$^{53}$ Crysts. SiO$_2$ = crystalline silica.

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**Figure 4. Comparison between the ranges of EFs (on a log scale) calculated from *in vitro* rat and mouse data, *in vivo* rat and mouse data (our data sets), and the EFs available from the literature. When the EF was not reported with respect to the particle surface area, a default specific surface area of 48 m$^2$/g was used, as in Buist et al.$^{53}$**

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e.g., the proposed Adverse Outcome Pathway for lung fibrosis\textsuperscript{56}, even though more analyses are needed to confirm this hypothesis.

**Implications for Further Research.** Published experimental studies suggest that inflammation might be a promising predictive end point to be tested in vitro.\textsuperscript{22,57–59} Other studies point out that the surface area better correlates with the lung effect of nanomaterials compared to mass doses.\textsuperscript{44,56,60,61} Multiple studies address the importance of considering the deposited dose in vitro instead of the nanomaterial concentration for a better characterization of the dose–response relationship.\textsuperscript{62–64} We put together these pieces of information in developing our EF calculation strategy and tested it with data collected from the literature. The goal was to calculate in vitro-to-in vivo extrapolation factors, which can then be used similarly to any other extrapolation factor for the estimate of human toxicity EFs. Ideally, once an in vitro-to-in vivo extrapolation factor has been estimated and its predictive power confirmed for multiple nanomaterials, there would be no need for animal and in vitro animal data but only for in vitro human data.

The comparison with published EFs can be used as a benchmark for the in vitro-based EFs, to understand whether this new data pool provides comparable results. This was the case for titanium dioxide when using the in vitro-to-in vivo extrapolation factor based on mouse data, where the EF is in the same range as published values. The good coverage of the in vivo and in vitro data used to calculate the extrapolation factor for titanium dioxide makes the factor more robust, since the real distribution of the BMD\textsubscript{20} values is better approximated by our samples.

Despite this promising result, the difficulty in calculating the extrapolation factors for the other nanomaterials shows the limitations of applying our approach with the currently available data. The main challenge we face is the quality and consistency of the toxicological data. For example, only a few BMD\textsubscript{20} values were available for amorphous silica in vivo, which questions the reliability of the extrapolation factor; even worse, for cerium oxide there were no corresponding in vivo and in vitro data, preventing the calculation of any factor. The wide toxicity range of in vitro and in vivo data confirms that the particle properties and the experimental conditions can have a huge impact on the results, hindering their comparison. Using a median BMD\textsubscript{20} obtained from a large data set is a better choice than using a single value from a specific study and allows keeping track of the BMD\textsubscript{20} uncertainty, but it can produce skewed results when the data is scarce and is not a representative sample of the BMD\textsubscript{20} distribution.

For the in vitro-to-in vivo extrapolation factors, coupled in vitro and in vivo data (i.e., obtained using similar particles and experimental conditions) for animals, animal cells, and human cells are needed to verify the parallelogram approach and investigate whether a single extrapolation factor might be valid for multiple particles. Moreover, removing the variability connected to the differences in experimental conditions would reduce the amount of data required to describe the distribution of the BMD\textsubscript{20} values, as we would expect the values to be more precise. However, on a provisional level and keeping in mind the factor 10 uncertainty, an in vitro-to-in vivo extrapolation factor of ca. $5 \times 10^{-1}$ (halfway between the TiO\textsubscript{2} and amorphous silica factors) might be used to compare non-soluble particles, especially if the in vitro human data has a high level of comparability (e.g., same experimental conditions).

In conclusion, we are not yet there for a consistent and systematic calculation of in vitro-based EFs. However, we showed a promising method to calculate these factors and identified which further steps are needed to reduce the uncertainty and improve and expand the results. One example above all, the conduction of fit-for-purpose in vitro experiments on human and animal cells, could be done systematically for multiple particles, thus providing a way to refine and test further our procedure without the need for animal testing.

We believe that our work not only can help direct future interdisciplinary efforts to tackle the critical aspects of the use of in vitro data in LCIA but also be of interest for the Risk Assessment community, which is facing similar challenges in extrapolating human responses without the use of animal data.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c01816. Additional methodological details and formulas, figures, and tables reporting result details (PDF)

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**Notes**

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The authors declare no competing financial interest.

The data underlying this study are openly available in Zenodo and may be accessed at DOI: 10.5281/zenodo.5845476.

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REFERENCES

(1) Tegart, G. Nanotechnology: the technology for the twenty-first century. *Foresight* 2004, 6, 364−370.
(2) ISO, *Nanotechnologies--Vocabulary--Part 1: Core Terms*, 2015.
(3) Srivastava, V.; Gusain, D.; Sharma, Y. C. Critical review on the toxicity of some widely used engineered nanoparticles. *Ind. Eng. Chem. Res.* 2015, 54, 6209−6233.
(4) Rebiter, G.; Ekvall, T.; Frischknecht, R.; Hunkeler, D.; Norris, G.; Rydberg, T.; Schmidt, W.-P.; Suh, S.; Weidema, B. P.; Pennington, D. W. Life cycle assessment: Part 1: Framework, goal and scope definition, inventory analysis, and applications. *Environ. Int.* 2004, 30, 701−720.
(5) Klöpffer, W.; Curran, M. A.; Frankl, P.; Heijungs, R.; Köhler, A.; Olsen, S. I. *Nanotechnology and Life Cycle Assessment*. A systems approach to Nanotechnology and the environment: Synthesis of Results Obtained at a Workshop Washington, DC 2–3 October 2006; European Commission, DG Research, jointly with the Woodrow Wilson International Center for Scholars, 2007.
(6) Rosenbaum, R. K.; Bachmann, T. M.; Gold, L. S.; Huijbrugs, M. A. J.; Jolliet, O.; Juraské, R.; Koehler, A.; Larsen, H. F.; MacLeod, M.; Margni, M.; McKone, T. E.; Payet, J.; Schuhmacher, M.; van de Meent, D.; Hauschild, M. Z. USEtox-the UNEP/SETAC toxicity model: recommended characterisation factors for human toxicity and freshwater ecotoxicity in life cycle impact assessment. *International Journal of Life Cycle Assessment* 2008, 13, 532−546.
(7) Fantke, P.; Bijster, M.; Guignard, C.; Hauschild, M. Z.; Huijbrugs, M. A. J.; Jolliet, O.; Kounina, A.; Magaud, V.; Margni, M.; McKone, T. E.; Posthuma, L.; Rosenbaum, R. K.; van de Meent, D.; van Zelm, R. In USEtox 2.0 Documentation (Version 1); Fantke, P., Ed.; USEtox International Center hosted at the Technical University of Denmark, 2017; p 208.
(8) Huijbrugs, M. A.; Steinmann, Z. J.; Elshout, P. M.; Stam, G.; Verones, F.; Vieira, M.; Zijp, M.; Hollandar, A.; van Zelm, R. ReCiPe2016: a harmonised life cycle impact assessment method at midpoint and endpoint level. *International Journal of Life Cycle Assessment* 2017, 22, 138−147.
(9) Romeo, D.; Hschier, R.; Nowack, B.; Jolliet, O.; Fantke, P.; Wick, P. In vitro-based human toxicity effect factors: challenges and opportunities for nanomaterial impact assessment. *Environmental Science: Nano* 2022, DOI: 10.1039/D1EN01014J.
(10) Salieri, B.; Hschier, R.; Quik, J. T.; Jolliet, O. Fate modelling of nanoparticle releases in LCA: An integrative approach towards USEtox4Nano. *Journal of Cleaner Production* 2019, 206, 701−712.
(11) Salieri, B.; Turner, D. A.; Nowack, B.; Hschier, R. Life cycle assessment of manufactured nanomaterials: Where are we? *NanoImpact* 2018, 10, 108−120.
(12) Rosenbaum, R. K.; Huijbrugs, M. A. J.; Henderson, A. D.; Margni, M.; McKone, T. E.; van de Meent, D.; Hauschild, M. Z.; Shaked, S.; Li, D. S.; Gold, L. S.; Jolliet, O. USEtox human exposure and toxicity factors for comparative assessment of toxic emissions in life cycle analysis: sensitivity to key chemical properties. *International Journal of Life Cycle Assessment* 2011, 16, 710−727.
(13) Laurent, A.; Harkema, J. R.; Andersen, E. W.; Owsianiak, M.; Vea, E. B.; Jolliet, O. Human health no-effect levels of TiO2 nanoparticles as a function of their primary size. *J. Nanopart. Res.* 2017, 19, 130.
(14) Burden, N.; Aschberger, K.; Chaudhry, Q.; Clift, M. J.; Fowler, P.; Johnston, H.; Landsiedel, R.; Rowland, J.; Stone, V.; Doak, S. H. Aligning nanotoxicology with the 3Rs: What is needed to realise the short, medium and long-term opportunities? *Regul. Toxicol. Pharmacol.* 2017, 91, 257−266.
(15) Walser, T.; Meyer, D.; Fransman, W.; Buist, H.; Kuipers, E.; Brouwer, D. Life-cycle assessment framework for indoor emissions of synthetic nanoparticles. *J. Nanopart. Res.* 2015, 17, 245.
(16) Fantke, P.; Chiu, W. A.; Aylward, L.; Judson, R.; Huang, L.; Jang, S.; Gouin, T.; Romberg, L.; Aurisano, N.; McKone, T.; Jolliet, O. Exposure and toxicity characterization of chemical emissions and chemicals in products: global recommendations and implementation in USEtox. *International Journal of Life Cycle Assessment* 2026:5 2021, 26, 899−915.
(17) Salieri, B.; Kaiser, J.-P.; Rosslein, M.; Nowack, B.; Hschier, R.; Wick, P. Relative potency factor approach enables the use of in vitro information for estimation of human effect factors for nanoparticle toxicity in life-cycle impact assessment. *Nanotoxicology* 2020, 14, 275.
(18) Romeo, D.; Salieri, B.; Hschier, R.; Nowack, B.; Wick, P. An integrated pathway based on in vitro data for the human hazard assessment of nanomaterials. *Environ. Int.* 2020, 137, 105505.
(19) Romeo, D.; Nowack, B.; Wick, P. Combined in vitro-in vivo dosimetry enables the extrapolation of in vitro doses to human exposure levels: A proof of concept based on a meta-analysis of in vitro and in vivo titanium dioxide toxicity data. *Nanomaterials* 2022, 5, 100376.
(20) Eisenbrand, G.; Pool-Zobel, B.; Baker, V.; Balls, M.; Blaauwhoer, B. J.; Boobis, A.; Careere, A.; Kevekordes, S.; Lluguenot, J. C.; Pieters, R.; Kleiner, J. Methods of in vitro toxicology. *Food Chem. Toxicol.* 2002, 40, 193−236.
(21) Teegarden, J. G.; Milkeve, V. B.; Minard, K. R.; Forsythe, W. C.; Wang, W.; Sharma, G.; Karin, N.; Tilton, S. C.; Waters, K. M.; Asgharian, B.; Price, O. R.; Pounds, J. G.; Thrall, B. D. Comparative iron oxide nanoparticle cellular dosimetry and response in mice by the inhalation and liquid cell culture exposure routes. *Particle and Fibre Toxicology* 2014, 11, 46.
(22) Donaldson, K.; Borm, P.; Oberdörster, G.; Pinkerton, K. E.; Stone, V.; Tran, C. Concordance between in vitro and in vivo dosimetry in the proinflammatory effects of low-toxicity, low-solubility particles: the key role of the proximal alveolar region. *Inhalation Toxicology* 2008, 20, 53−62.
(23) Weldon, B. A.; Griffith, W. C.; Workman, T.; Scoville, D. K.; Kavanagh, T. J.; Faustman, E. M. In vitro to in vivo benchmark dose comparisons to inform risk assessment of quantum dot nanomaterials. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* 2018, 10, e1507.
(24) Duffin, R.; Tran, L.; Brown, D.; Stone, V.; Donaldson, K. Proinflammmogenic Effects of Low-Toxicity and Metal Nanoparticles In Vivo and In Vitro: Highlighting the Role of Particle Surface Area and Surface Reactivity. *Inhalation Toxicology* 2007, 19, 849−856.
(25) Weiss, M.; Fan, J.; Claudel, M.; Lebeau, L.; Pons, F.; Ronzani, C. Combined in vitro and in vivo approaches to propose a putative adverse outcome pathway for acute lung inflammation induced by nanoparticles: a study on carbon dots. *Nanomaterials* 2021, 11, 180.
(26) Braakhuis, H. M.; Murphy, F.; Ma-Hock, L.; Dekkers, S.; Keller, J.; Baguley, C.; Lonij, A. G.; Stone, V. An Integrated Approach to Testing and Assessment to Support Grouping and Read-Across of Nanomaterials after Inhalation Exposure. *Applied In Vitro Toxicology* 2021, 7, 112−128.
(27) Wang, X.; Sun, B.; Liu, S.; Xia, T. Structure activity relationships of engineered nanomaterials in inducing NLRP3 inflammasome activation and chronic lung fibrosis. *NanoImpact* 2017, 6, 99−108.
(28) Piguet, P. F.; Collart, M. A.; Grau, G. E.; Sappino, A.-P.; Vassalli, P. Requirement of tumour necrosis factor for development of silica-induced pulmonary fibrosis. *Nature* 1990, 344, 245−247.
(29) Traboulsi, H.; Guerrina, N.; Ju, M.; Maysinger, D.; Ariya, P.; Bagloé, C. J. Inhaled Pollutants: The Molecular Scene behind Respiratory and Systemic Diseases Associated with Utrafine Particulate Matter. *International Journal of Molecular Sciences* 2017, 18, 243.
(30) Anjivel, S.; Asgharian, B. A Multiple-Path Model of Particle Deposition in the Rat Lung. *Fundam. Appl. Toxicol.* 1995, 28, 41−50.
(31) Miller, F. J.; Asgharian, B.; Schroeter, J. D.; Price, O. Improvements and additions to the Multiple Path Particle Dosimetry model. *J. Aerosol Sci.* 2016, 99, 14−26.
(32) Cosnier, F.; Seidel, C.; Valentino, S.; Schmid, O.; Bau, S.; Vogel, U.; Devoy, J.; Gaté, L. Rattened particle surface area dose drives inflammation in rat lungs following acute, subacute, and subchronic inhalation of nanomaterials. *Particle and fibre toxicology* 2021, 18, 29.
(33) Haber, L. T.; Doursoum, M. L.; Allen, B. C.; Hertzberg, R. C.; Parker, A.; Vincent, M. J.; Maier, A.; Boobis, A. R. Benchmark dose (BMD) modeling: current practice, issues, and challenges. Critical reviews in toxicology 2018, 48, 387–415.

(34) Pennington, D.; Crettaz, P.; Tauxe, A.; Rhomberg, L.; Brand, K.; Jolliet, O. Assessing human health response in life cycle assessment using ED10s and DALYs: part 2-Noncancerous effects. Risk analysis. Risk Analysis: an official publication of the Society for 2002, 22, 947–63.

(35) EFSA Scientific Committee; Hardy, A.; Benford, D.; Halladorsson, T.; John Jeger, M.; Helle Knutzen, K.; More, S.; Mortensen, A.; Naeghel, H.; Noteborn, H.; Ockleford, C.; Ricci, A.; Rychen, G.; Silano, V.; Solecki, R.; Tauxe, A.; Van den Beuken, E.; Westerhout, J.; Klein Entink, R. H.; Brouwer, D. Anal. Toxicol. 2014, 38, 571–609.

(36) Schmid, O.; Steoger, T. Surface area is the biologically most effective dose metric for acute nanoparticle toxicity in the lung. J. Aerosol Sci. 2016, 99, 133–143.

(37) Monteleer, C.; Tran, L.; MacNee, W.; Faux, S.; Jones, A.; Miller, B.; Donaldson, K. The pro-inflammatory effects of low-toxicity low-solubility particles, nanoparticles and fine particles, on epithelial cells in vitro: the role of surface area. Occupational and environmental medicine 2007, 64, 609–615.

(38) Slob, W. Joint project on benchmark dose modelling with RIVM. EFSA Supporting Publications 2018, 15, 1497e.

(39) Varewycz, M.; Verbeke, T. Software for benchmark dose modelling. EFSA Supporting Publications 2017, 14, 1170e.

(40) Noël, A.; Charbonneau, M.; Cloutier, Y.; Tardif, R.; Truchon, G. Rat pulmonary responses to inhaled nano-TiO2: Effect of primary particle size and agglomeration state. Particle and Fibre Toxicology 2013, 10, 48.

(41) Vranic, S.; Gossens, I.; Jacobsen, N. R.; Jensen, K. A.; Bokkers, B.; Kermanizadeh, A.; Stone, V.; Baeza-Squiban, A.; Cassee, F. R.; Tran, L.; Boland, S. Impact of serum as a dispersion agent for in vitro determinants of particulate toxicity: The dose-metric for poorly soluble dusts; Institute of Occupational Medicine for the Health and Safety Executive: 2003.

(42) Sand, S.; von Rosen, D.; Victorin, K.; Falk Filipsson, A. Identification of a critical dose level for risk assessment: Developments in benchmark dose analysis of continuous endpoints. Toxicol. Sci. 2006, 90, 241–251.

(43) Bakker, A.; Gravemeijer, K. P. An historical phenomenology of mean and median. Educational Studies in Mathematics 2006, 62, 149–168.

(44) Upton, G.; Cook, I. Understanding statistics; Oxford University Press: 1996; p 55.

(45) Vermeire, T.; Pieters, M.; Rennen, M.; Bos, P. Probabilistic assessment factors for human health risk assessment 2001, 1–29.

(46) Fransman, W.; Buist, H.; Kuijpers, E.; Walser, T.; Zondervan, A.; Westerhout, J.; Klein Entink, R. H.; Brouwer, D. Comparative Human Health Impact Assessment of Engineered Nanomaterials in the Framework of Life Cycle Assessment. Risk Anal. 2017, 37, 1358–1374.

(47) Slob, W. Uncertainty Analysis in Multiplicative Models. Risk Anal. 1994, 14, 571–576.

(48) Huijbregts, M. A.; Rombouts, L. J.; Rags, A. M.; van Meent, D. Human-toxicological effect and damage factors of carcinogenic and noncancerogenic chemicals for life cycle impact assessment. Integrated Environmental Assessment and Management: An International Journal 2005, 1, 181–244.

(49) Murugadoss, S.; Brassinne, F.; Sebahi, N.; Petry, J.; van Landuyt, K. L.; Godderis, L.; Mast, J.; Lison, D.; Hoet, P. H.; van den Brule, S. Agglomeration of titanium dioxide nanoparticles increases toxicological responses in vitro and in vivo. Particle and Fibre Toxicology 2020, 17, 10.

(50) Bahadar, H.; Maqbool, F.; Niaz, K.; Abdollahi, M. Toxicity of nanoparticles and an overview of current experimental models. Iranian biomedical journal 2016, 20, 1.

(51) Donaldson, K.; Poland, C. Inhaled nanoparticles and lung cancer - what we can learn from conventional particle toxicology. Swiss Medical Weekly 2012, 142, W13547.

(52) Dekkers, S.; Ma-Hock, L.; Lynch, I.; Russ, M.; Miller, M. R.; Schins, R. P.; Keller, J.; Römer, I.; Kühler, K.; de Jong, W. H.; Landsiedel, R.; Cassee, F. R.; Strauss, V.; Strauss, V.; Differences in the toxicity of cerium dioxide nanomaterials after inhalation can be explained by lung deposition, animal species and nanoforms. Inhalation Toxicology 2018, 30, 273–286.

(53) Buist, H.; Hischier, R.; Westerhout, J.; Brouwer, D. Derivation of health effect factors for nanoparticles to be used in LCIA. NanoImpact 2017, 7, 41–53.

(54) Pini, M.; Salters, B.; Ferrari, A. M.; Nowack, B.; Hischier, R. Human health characterization factors of nano-TiO2 for indoor and outdoor environments. International Journal of Life Cycle Assessment 2016, 21, 1452–1462.

(55) Ettrup, K.; Kounina, A.; Hansen, S. F.; Meesters, J. A. J.; Vre, E. B.; Laurent, A. Development of Comparative Toxicity Potentials of TiO2 Nanoparticles for Use in Life Cycle Assessment. Environ. Sci. Technol. 2017, 51, 4027–4037.

(56) OECD, Aop-kb. https://aopkb.oecd.org/index.html (accessed 2019-06-20).

(57) Han, X.; Corson, N.; Wade-Mercer, P.; Gelein, R.; Jiang, J.; Sahu, M.; Biswas, F.; Finkelstein, J. N.; Elder, A.; Oberdörster, G. Assessing the relevance of in vitro studies in nanotoxicology by examining correlations between in vitro and in vivo data. Toxicology 2012, 297, 1–9.

(58) Kim, Y. H.; Boykin, E.; Stevens, T.; Lavrich, K.; Gilmour, M. I. Comparative lung toxicity of engineered nanomaterials utilizing in vitro, ex vivo and in vivo approaches. J. Nanobiotechnol. 2014, 12, 47.

(59) Rushton, E. K.; Jiang, J.; Leonard, S. S.; Eberly, S.; Castranova, V.; Biswas, P.; Elder, A.; Han, X.; Gelein, R.; Finkelstein, J.; Oberdörster, G. Concept of Assessing Nanoparticle Hazards Considering Nanoparticle Dosimetric and Chemical/Biological Response Metrics. Journal of Toxicology and Environmental Health, Part A 2010, 73, 445–461.

(60) Faux, S.; Tran, C.-L.; Miller, B.; Jones, A.; Monteleer, C.; Donaldson, K. In vitro determinants of particulate toxicity: The dose-metric for poorly soluble dusts; Institute of Occupational Medicine for the Health and Safety Executive: 2003.

(61) Monteleer, C.; Tran, L.; MacNee, W.; Faux, S.; Jones, A.; Miller, B.; Donaldson, K. The pro-inflammatory effects of low-toxicity low-solubility particles, nanoparticles and fine particles, on epithelial cells in vitro: the role of surface area. Occupational and environmental medicine 2007, 64, 609–15.

(62) Teeguarden, J. G.; Hinderliter, P. M.; Orr, G.; Thrall, B. D.; Pounds, J. G. Particokinetics In Vitro: Dosimetry Considerations for In Vitro Nanoparticle Toxicity Assessments. Toxicol. Sci. 2007, 95, 300–312.

(63) Pal, A. K.; Bello, D.; Cohen, J.; Demokritou, P. Implications of in vitro dosimetry on toxicological ranking of low aspect ratio engineered nanomaterials. Nanotoxicology 2015, 9, 871–885.

(64) DeLoid, G. M.; Cohen, J. M.; Pyrgiotakis, G.; Demokritou, P. Preparation, characterization, and in vitro dosimetry of dispersed, engineered nanomaterials. Nat. Protoc. 2017, 12, 355–371.