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# Nanotoxicity: challenging the myth of nano-specific toxicity

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The analysis of nanoparticle (NP) hazard is currently a major research pre-occupation for particle toxicologists since there is a pressing requirement for a comprehensive understanding of nanoparticle hazard because of the wide spectrum of NP varying in composition, shape and size that require testing for risk assessment. The Biologically Effective Doses (BEDs) of nanoparticles, the dose entity that drives toxicity include charge, solubility, contaminants, shape and the ability to translocate from the site of deposition in the lungs. We point out here that all of these modes of toxicity are relevant and described for conventional pathogenic particles. There is no evidence that particles below 100 nm, the threshold definition of a NP, show any step-change in their hazard meaning that there is no evidence of novel 'nano-specific hazard'. Therefore conventional particle toxicology data are useful and relevant to the determination of the nanoparticle hazard. Emphasis away from 'nano-specific effects' and the availability of hazard data from conventional particles will focus limited resource towards a full understanding of the NP hazard. This will lead to improved ability to identify and test for their effects and measure their toxicokinetics and so contribute to their risk assessment.

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## Introduction

Nanotechnology offers great potential for the future in terms of enabling technological advances across a wide range of industries [1]. However, the acceptance of nanotechnology and hence nano-enabled products is reliant on public and consumer confidence in the human and environmental safety of this new technology. In addition, both governments and regulators attempt the difficult balancing act between promoting rapid technological development and business, whilst at the same time making sure that it is done safely and regulating new

technology effectively. To this end, various governments, regulators and international organisations such as the OECD, ISO and BSI have been proactive in trying to understand nanotechnology and how best to facilitate its safe development and use. There have been numerous reports and documents published by various bodies addressing different attributes of nanotechnology and one of the early and most interesting was the joint study by Royal Society and Royal Academy of Engineering entitled '*Nanoscience and nanotechnologies: opportunities and uncertainties*' [2]. There are abundant examples of other reports and opinions which have been more specific in their remit, for example, addressing nanoparticle terminology and definitions [3], inhalation toxicity testing for nanomaterials [4], risk management of carbon nanotubes [5] and the effectiveness of specific regulatory frameworks such as REACH for nanoparticles [6,7], all with the aim of improving the safe handling of nanotechnology. However, despite the obvious hard work, funding and good intention that is being focused towards the safe development of nanotechnology there is still considerable doubt and uncertainty.

This is, at least in part, because there is concern that nanomaterials may harbour an unknown mode of toxicity or 'nano-specific effects' and a great deal of effort has been directed towards understanding these 'nano-specific effects' which have led to the identification of several modes of action of nanoparticle toxicity. Not all nano-sized particles harbour novel size-dependent properties and indeed it has been argued by Auffan and colleagues that that evidence for novel size-dependent properties, rather than particle size, should be the primary criterion in any definition of nanoparticles in relation to health and safety [8]. In a recent review by Fubini *et al.* this argument was considered further and it was acknowledged that this position does fulfil the need to state *when* and *why* a material at the nano-level should be considered 'new' [9]. This is an important consideration since the definitions available for a nanoparticle, based around a threshold dimension(s) of 100 nm (e.g. see [3,10]), are *not* derived from toxicological evidence of a step-change in toxicity at 100 nm. Indeed much of the evidence for 'nano' effects is far more gradual as acknowledged by Fubini *et al.* who noted that where biological response is related to surface area (which forms the interface of insoluble particle/biological interactions), nanoparticles will of course show effects orders of magnitude greater than that of bulk particles at the same mass dose due to vastly greater surface area [9]. Taking this into account, it becomes apparent that in terms of mode of toxicity at least, passing below this threshold into the nano-realm

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does not necessarily infer any new and specific properties; therefore the arbitrary assumption of different and ‘nano-specific’ toxicity appear to be unfounded. Instead there is likely to be a more gradual magnification of the intrinsic hazard. Indeed this is echoed in the statement by Norppa *et al.* that it cannot be generally assumed that nanoscale size would increase the genotoxicity of nanomaterials or that nanoparticles are generically genotoxic [11]. Indeed the view of ‘nano-specific’ toxicity could be seen as intrinsically unhelpful as it has the effect of labelling *all* nanomaterials as hazardous or potentially so, thereby prejudicing against their use despite the fact that in most cases, as for conventional particles, nanoparticles in common use show a range of inherent toxicities, the majority being low toxicity. In addition, the focus on the search for ‘nano-specific’ may have the effect of ‘re-inventing the wheel’ of what is already known for conventional particles and thereby delaying the important issue of ensuring that nanoparticle hazard can be adequately tested for, quantified and regulations put in place that can facilitate this in an efficient and proportional manner.

The purpose of this article is to discuss this general basis of toxicity for nanoparticles as shown in recent studies and to demonstrate that the mode of action is in most, if not all, cases the same as that shown for conventional bulk particles. Generally speaking, to understand the basis of toxicity is to understand the driving component and how this can be a variable entity between materials of the same as well as differing physico-chemical characteristic and this is described below in relation to the Biologically Effective Dose (BED) [12\*\*].

### Biologically Effective Dose (BED)

In conventional particle toxicology the dose is defined by mass or concentration of particles per unit tissue or per number of cells or surface area of cells in cell culture. Mass is the way that particles are measured in workplace and the environment for risk management purposes, with the key exception of fibres that are counted by number. However, it is self-evident that mass cannot be the entity that is toxic, it is merely the simplest, most convenient practical physical measure of the quantity of particles that is present. Toxic effects are subtle and complex and invariably depend on molecular effects at the cellular level that depend on the physico-chemistry of the particles. Therefore mass is a surrogate for the important dose and increasing mass increases the delivery of the dose that drives the toxic effect. The Biologically Effective Dose (BED) is defined by Povey *et al.* as ‘the active dose of the agent of interest’ and that ‘the nearer the dose specified can be to the active dose of the real agent of interest (the BED), the more likely it is that an association may exist between an agent and a disease’ [13]. To be more direct we have defined the BED in particle toxicology as ‘the entity within any mass dose of particles that drives a critical pathophysiologically relevant form of

toxicity in tissue, such as inflammation, genotoxicity or cellular proliferation’ [12\*\*]. If only a portion of the total mass dose is BED then the implication is that a quantity of the retained mass dose is *not* biologically effective. This is consistent with the presence of a high degree of pigmentation in the lungs of normal subjects with no disease at autopsy [14] and in workers in dusty trades that have increased amounts of dust in their lung [15] but no lung disease (Figure 1).

The BEDs of some of the best known pathogenic particles have been identified; for example in the case of quartz it is the unpassivated (active) surface area and in the case of asbestos it is the long, biopersistent fibres [12\*\*,16]. It is notable that despite knowledge of the BED for some particles, they are still measured by mass since the variability in the BED between particle types and the difficulty in measuring the BED currently precludes it. However, no doubt in the future the development of measuring instrumentation that directly measure the BED will allow the BED to be the metric and so improve epidemiological dose:responses and thereby improve risk management. Indeed this is already the case with advancements in portable, on-line measurements of particle number and size.

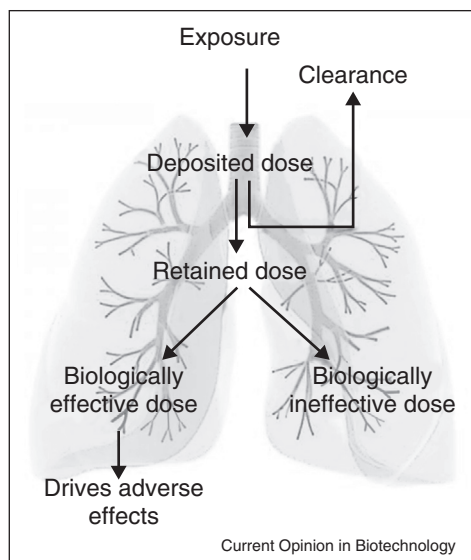
### Known modes of toxicity for nanomaterials

As described, there are various modes of toxicity identified for nanomaterials and these vary in terms of their potency yet are all intrinsically related to the physico-chemical properties of the nanoparticle. However, whilst these are physicochemical properties of a nanoparticle, they are not ‘nano-specific’ as they have long been identified as playing a role in the toxicity of more conventional particles. These modes of toxicity are outlined below including more historical references demonstrating the lack of nano-specificity.

#### Surface area–size cut-off

Evidence that surface area is a BED for low toxicity particles came from the original studies with low-toxicity low solubility (LTLS) particles in the eighties. These studies arose from concern that chronic exposure to LTLS or nuisance particles at around the nuisance dust standard produced disease in rats due to the phenomenon of ‘rat lung overload’. The metric that drives rat lung overload was initially considered to be volume of particles in the lungs [17,18]. However, surface area was later found to be better related to overload pathology [19] and onset of overload inflammation [20]. Nanoparticles, termed ultrafine particles in these studies, have a greater surface area per unit mass than micron-sized particles but both micron and nanoparticles fall on the same dose/response line relating surface area to tumourigenic potential [21] as well as inflammation [20,22]. Therefore the BED of surface area is relevant to the pathogenic effects of both nanoparticles and micron-sized particles.

Figure 1



Particle Biologically Effective Doses [descriptive overarching figure].

The surface of a particle is also the site from which any soluble material is delivered to the biological system. Surface area may therefore have a linear relationship with toxicity for a soluble material associated with, or comprising any nanoparticle, although the surface area is then only a surrogate, being the ‘Trojan horse’ that delivers the soluble toxin to the biological system rather than the actual BED. The above discussion concerns LTLS particles and nanoparticles but if a particle has reactive surface then the toxicity per unit surface area is likely to be increased, a so called ‘double hazard’ [22]. The prime example of this is quartz which shows a much steeper SA/inflammation curve than the LTLS particles [22]. It remains to be seen whether NP with a reactive surface will be produced but *pro-rata* such particles would have a very high toxicity per unit mass.

#### Surface charge/reactivity

The surface charge, or zeta potential ( $\zeta P$ ), of microscopic particles has been related to various measures of toxicity. Ambient particles larger than 2  $\mu m$  were shown to stimulate IL-6 release as a consequence of their zeta potential [23] and asbestos has been reported to be cytotoxic in relation to its charge [24,25]. Micron-sized polymer particles have also been reported to have toxicity that depends on the particle charge [26]. The  $\zeta P$  is very dependent on pH and this is relevant to events in the phagolysosomes after uptake into macrophages. A range of metal oxide NP were found to have inflammatory potential that was related to the zeta potential under acid conditions and a hypothesis relating to the ‘stripping off’ of the corona in the lysosome to reveal the charged surface

in an acid milieu was advanced for the mechanism [27<sup>••</sup>]. Other NPs have been shown to have charge-related toxicity including polymer NP [28] and silica NP [29].

#### Composition and contaminants

This issue relates to the nature of the composition of nanoparticle and how this may influence the reactivity of particle. The composition of the particle may be considered in two ways, firstly if the particle is composed primarily of a biologically active substance, such as a redox active metal or if the nanoparticle contains a biologically active substance as minor constituent or contaminant. If the particle is composed of or contains an active component such as a redox active transition metal, this would infer that the nanomaterial itself is likely to be redox active. For example, a nanomaterial composed of copper oxide (II) is likely to be redox-active which may pose a hazard, especially considering the large surface area of nanomaterials over which redox reactions can take place (e.g. 23 m<sup>2</sup>/g for a 42 nm powder vs. 1.5 m<sup>2</sup>/g for a 3  $\mu m$  powder [30]) potentially meaning the nanomaterial is far more reactive than the parent compound. Indeed this combination of high surface area and high reactivity may lead to the formation of a ‘double hazard’ in inflammatory potential [22,30]. This link to transition metals and reactivity leading to toxicity is well established within particle toxicology and suggested to play a substantial role in the pathogenesis of several particle mediated diseases. For example, as stated by Aust *et al.* [31], there is increasing evidence suggesting that transition metals present in particles generate reactive oxygen species (ROS) that may be involved in producing some observed respiratory symptoms. Of these metals, iron is often cited as having a leading role in ROS generation and subsequent oxidative stress and damage [31–37]. Aust summarised the hypothesis behind the toxic effect of transition metal contamination of particulates as ‘bioavailable transition metals from inhaled airborne particulates catalyse redox reactions in human lung epithelial cells, leading to oxidative stress and increased production of mediators of pulmonary inflammation’ [31]. In addition, they also hypothesised that the size as well as the transition metal content of particulates could affect their ability to cause these effects. Using cell-free as well as cellular measures of oxidant production and damage, Aust *et al.* demonstrated that coal fly ash (CFA) activity could be reduced by over 90% after the addition of the metal chelator desferrioxamine which they concluded strongly suggests that transition metal(s), probably iron, were responsible [31]. They also demonstrated that the iron in CFA was also responsible for inducing the inflammatory mediator interleukin (IL)-8 in the human alveolar type II cell line, A549. Interestingly and highly relevant to the consideration of the effect of transition metals on the toxicity of nanoparticles, by using size fractionated CFA, they found that the amount of iron mobilised was inversely related to the size of particles (as well as dependent

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on the source of coal indicating the importance of source variability) [31].

As well as iron, other transition metals have been implicated in the reactivity and subsequent toxicity of particulates. Welding fumes are a complex mix and are known to be rich in transition metals as well as being a known occupational health hazard with exposure leading to respiratory conditions such as bronchitis, chemical pneumonitis and metal fume fever (MFF) [38]. In their 2004 study, McNeilly and colleagues examined the role of soluble transition metals using *in vitro* and later, *in vivo* models. Chemical analysis of standard welding fume samples showed high levels of several transition metals such as iron, nickel, and chromium and chelation of these metals or reduction via washing reduced the toxicity *in vitro* and *in vivo* markedly [38,39]. Specifically, in their 2004 study the authors showed that the pro-inflammatory effects (NF- $\kappa$ B and AP-1 activation and IL-8 release) of three different nickel and cobalt based welding fumes were all driven by transition metals that were rapidly released and that caused oxidative stress to the epithelial cells [39].

As well as the role of transition metals in the toxicity of numerous 'conventional' particles such as asbestos [40,41], air pollution, welding fumes, this has also been suggested for nanomaterials such as nanofibres [42] and there have also been studies showing toxicity from nanoparticles composed of, or contaminated with transition metals. For example, a study by Fahmy and Cormier looked at the effects of three metal oxide nanoparticles (silicon oxide, ferric oxide and copper oxide) on airway epithelial cells. Whilst all metal oxides generated ROS in the exposed cells, they found that the copper oxide was more able to overwhelm antioxidant defences of the cells leading to cytotoxicity and concluded that the variability of effects may involve sustained oxidative stress possibly due to redox cycling [43]. In terms of transition metals being present as a contaminant yet influencing the toxicity of a nanomaterial, an important study was that of Kagan *et al.* where the role of iron in toxicity towards a macrophage cell line was investigated. Using an iron-rich, non-purified single walled carbon nanotube (SWCNT) sample containing 26 wt% of iron they compared its ability to cause oxidative stress in macrophages with that of an iron-stripped (purified) SWCNT sample with 0.23 wt% of iron. They found that iron-rich SWCNTs were more effective in generating hydroxyl radicals in activated macrophages than purified SWCNT as well as reducing levels of antioxidants indicating a role of iron in oxidative stress and toxicity [34]. In a later study, Turci *et al.* produced synthetic chrysotile nanofibres and progressively loaded them with iron ranging from 0% to 1.78% and found that even the smallest iron contamination imparted radical reactivity and therefore potential toxicity [44]. They also suggested that their findings

could be useful in the design of safer nanofibres, indicating the potential role and importance of transition metal contamination in engineered nanoparticle toxicity [44]. Such a reduction in metal impurities, either pre-production or more commonly post-production (e.g. via acid treatment) has also been suggested as a method of reducing metal-mediated toxicity [45]. The importance of metallic impurities and in particular defining the level of impurity required to affect toxicity was the subject of a 2009 article by Pumera and Miyahara. Using carbon nanotubes containing different amounts of impurities they examined the reduction of hydrogen peroxide and the oxidation of hydrazine as a measure of carbon nanotube activity. They found that the middle-ppm range marked the borderline between being redox active/inactive for iron-based impurities [46]. This study was later followed by study by one of the authors (Pumera) also emphasising that presence of residual metal-catalyst impurities in carbon nanotubes is responsible for their toxicity [46–48]. However, they also stressed the importance of differentiating between the total amount of impurities and the redox-active (bioavailable) impurities because it is bioavailable impurities that drive toxic effects (and therefore the BED) and they also described methods for detecting bioavailable nickel [47] and molybdenum [48].

### Shape

Particle shape and its role in toxicity are certainly of relevance to nanoparticles and this has been addressed within several studies looking at the role of size and shape of nanoparticulates in toxicity, primarily towards the lung and pleural cavity. Shape is often considered in relation to high aspect ratio (i.e. length to width ratio) which infers a fibrous morphology. However, if we consider the term 'aspect' in relation to its dictionary definition of 'a particular part or feature of something' [49] then a high aspect particle in this instance is simply a particle which has 1 or more dimensions (aspects) which is large enough to cause difficulties in clearance from the lung/pleura.

In terms of lung physiology, the progressively narrowing and bifurcating airways present a barrier preventing larger particles penetrating deep into the lung and depositing beyond the ciliated airways. Indeed, when looking at the International Commission on Radiological Protection (ICRP) model of particle deposition in the lung, we see that for larger particles ( $\sim 10 \mu\text{m}$ ) the majority of deposition occurs within the pharyngeal region of the respiratory system without penetrating the conducting airways. As particle size reduces into the respirable range ( $< 4.25 \mu\text{m}$ ) [50], deposition in the pulmonary region increases. The greatest fractional deposition is at the smaller submicron size range, with particles able to penetrate to the tracheo-bronchial and alveolar regions. When particle size decreases further ( $< 0.2 \mu\text{m}$ ), deposition by

Brownian diffusion increases and a larger proportion of particles deposit in the upper respiratory tract [51].

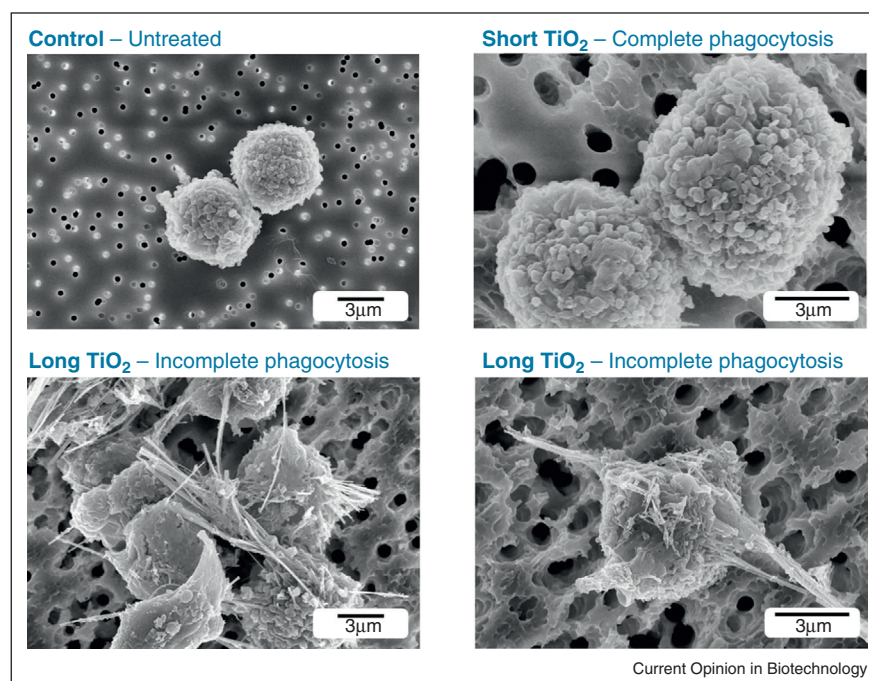
On the basis of the ICRP model showing the elutriating effect of the lung, we would expect that large or *high aspect* particles would not be able to penetrate into the distal lung, but we know that some large particles, namely fibres, can penetrate this region despite having an aspect (length) greater than  $5\ \mu\text{m}$  [52–55]. This is because penetration into the lung is dictated by aerodynamic diameter ( $D_{\text{ae}}$ ) defined as the diameter of a unit density ( $1\ \text{g/cm}^3$ ) sphere with the same settling speed as the particle of interest [56]. Therefore, whilst a particle may have a larger physical diameter than the respirable cut-off of  $4.25\ \mu\text{m}$  [50], if it has an aerodynamic diameter of less than  $4.25\ \mu\text{m}$  then it will be respirable. For most particles this is not an issue but due to a peculiarity of aerodynamics in which the fibre length imparts greater drag, fibres can possess a small  $D_{\text{ae}}$  yet have a very long length. The World Health Organisation (WHO) definition of a fibre is a structure having a diameter  $<3\ \mu\text{m}$  [57], but with a length many times this. This is because the aerodynamic diameter is proportional to the fibre *diameter* not the length [58].

The reason this paradoxical respirability of high aspect particles might be pathogenic relates to the clearance mechanisms and sensitivity of the alveolar region of the lung. Here, clearance of exogenous material is facilitated

by alveolar macrophages which engulf particulates, bacteria, etc. and clear them from the lung via the mucociliary escalator or towards lung associated lymph nodes. However, if the macrophage encounters a high aspect respirable particle (HARP) larger than itself then it will engage the particle and attempt to engulf it but will fail and as a result, the particle will persist in its location and with repeated exposure, may accumulate to a toxic dose. In addition, this inability to fully enclose a particle leads to a condition of frustrated phagocytosis, which can lead to the release of various toxic components, crucial to the microbiocidal activity of the cells as part of the innate immune defence [59\*\*]. The concept of frustrated phagocytosis was first identified by Kushner and Wright with respect to glass fibres and asbestos in guinea pigs when they suggested that frustrated phagocytosis might lead to fibrosis [60]. Later this idea was expanded into a hypothesis for the carcinogenicity of fibres by Archer in which he proposed that frustrated phagocytosis lead to the release of oxygen radicals such as superoxide ( $\text{O}_2^{\bullet-}$ ) and through its dismutation, hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) which could damage surrounding cells [61].

This issue of length-dependent retention and effects was shown for carbon nanotubes whereby MWCNT  $> 10\ \mu\text{m}$  long persisted in the peritoneal cavity and pleural cavity of mice after injection (as a surrogate for pleural retention) leading to substantial inflammation and fibrosis [62\*,63]. However, tightly curled MWCNT which did not possess

**Figure 2**



Effect of particle shape/length on macrophage uptake. Images kindly provided by Ray Hamilton [65].

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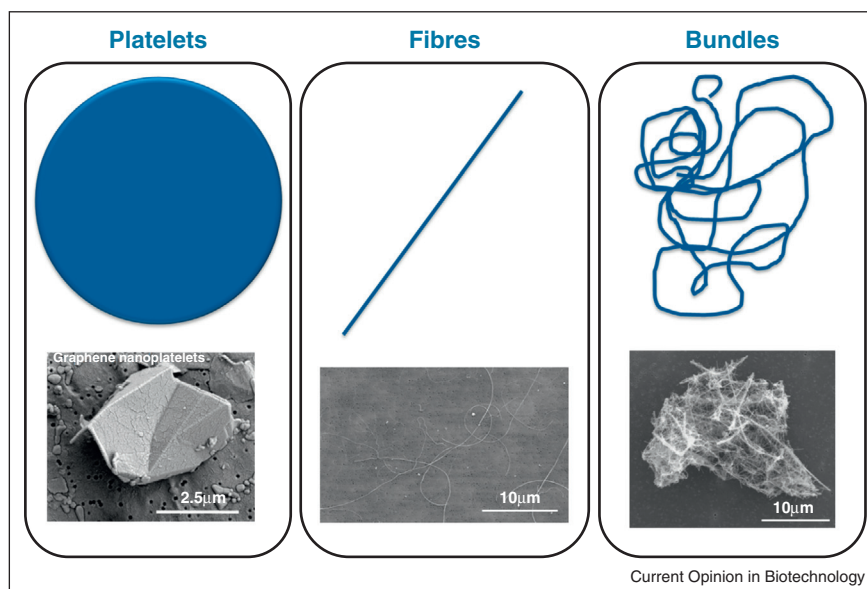
an appreciable straight length were rapidly cleared from the cavity via peritoneal stomata and did not cause inflammation or fibrosis indicating a role for length in toxicity. This has been confirmed for nickel nanofibres also [64]. In 2009, Hamilton *et al.* using an elegant study design showed the effect that the fibrous shape and fibre length can have on the toxicity of a material. Here they compared the toxicity of TiO<sub>2</sub> presented as a spherical nanoparticle (60–200 nm), short fibre (0.8–4 µm) or long fibre (15–30 µm) towards primary mouse macrophages [65<sup>••</sup>]. As demonstrated in Figure 2, the effect of the difference in particle shape/length is striking with both the spherical and short TiO<sub>2</sub> particles leading to complete phagocytosis whilst the long fibres caused distortion of the cell. This led the authors to conclude that the alteration of the anatase TiO<sub>2</sub> nanomaterial into a fibre structure of greater than 15 µm creates a highly toxic particle [65<sup>••</sup>].

Whilst it is clear that ‘long’ fibres can cause a significant problem with clearance from the lung and toxicity, the question has often remained as to the threshold length at which a fibre becomes pathogenic? Because of the polydispersity in length of naturally occurring mineral fibres and MMVF, this has always been difficult to ascertain although a length >8 µm was suggested by Stanton in a landmark study [66]. However, in a recently published paper, Schinwald *et al.* used manufactured nanofibres of differing composition and tight length ranges to examine this question. Here they injected a range of fibres into the pleural space of mice and examined the resultant effects. They found a clear demarcation, with fibres 5 µm long and greater causing a potent response whilst those

particles less than 5 µm caused no such response and were cleared rapidly to the mediastinal lymph nodes [67<sup>••</sup>]. The reason for this tight definition was hypothesised to be due to the clearance routes from the pleural space which consist of pore-like stomata in the parietal pleura [68] of 3–10 µm in diameter [63] which imposes a size restriction on clearance from the space. In a later study, Schinwald *et al.* also examined the cut-off length in the lung and found this to be larger with a 14 µm threshold for fibre-induced pulmonary inflammation after aspiration exposure and an *in vitro* threshold for inhibition of macrophage locomotion of 5 µm [69]. The difference in threshold length relates to the difference in clearance routes from the two regions. In the lung it is mediated by macrophages which can engulf particles greater than 5 µm (with reduced motility) yet cannot clear particles of similar size to themselves. However, in the pleural cavity there is the additional size restriction of stomata in the parietal pleura meaning that the cut-off for retention and hence elicitation of a response is considerably smaller. Therefore, as the pleura is a route of movement of particles out of the lung as shown recently for carbon nanotubes by Mercer and colleagues [70], it would be prudent to define a cut-off of 5 µm and above.

Whilst this issue of fibre length and paradoxically small  $D_{ac}$  is well understood for fibres, the fundamental principles of this hazard may have wider relevance to other particles with a similar small  $D_{ac}$  yet one or more aspects that is large enough to cause difficulties with clearance. An example of three shapes or HARPs that this may be relevant to is given in Figure 3, with fibres already discussed in depth.

Figure 3



Particle forms potentially presenting a high aspect yet low aerodynamic diameter meaning that they are potentially respirable.

One of these particles which have received a great deal of attention recently is graphene/graphite platelets. These flat, thin particles can have a large physical diameter of greater than 5  $\mu\text{m}$  (and as such may cause problems with clearance) yet due to being very thin, often in the nanoscale, they can have a very low aerodynamic diameter which could lead to inhalation and substantial deposition in the human respiratory tract [71]. Recently the aerodynamic diameter of graphene/graphite nanoplates was calculated based on a range of dimensions and these are shown in Table 1.

As well as the potential for inhalation and deposition in the alveolar region of the lung, several studies have also showed difficulties for macrophages in handling [71,72\*\*] and engulfing these particles as well as retain and inflammation in the lung and pleural space after injection [72\*\*].

Taking this issue of low aerodynamic diameter and high aspect further, it is also possible that open bundle-like fibrous particles may also demonstrate this property. As the  $D_{\text{ac}}$  of a particle is defined by its density as well as its Stokes diameter, it is possible that a very low density particle could be physically large enough to cause problems with macrophage-mediated clearance yet still possess a  $D_{\text{ac}}$  low enough to facilitate deposition in the alveolar region. If this is the case and the particle is sufficiently robust enough to avoid deforming during attempted phagocytosis then it may present the same hazard as long fibres and large plate-like particles.

In summary, it is clear that (nano)particle size and shape can have an important impact on its hazard status and as shown, can render a low toxicity material such as  $\text{TiO}_2$  pathogenic. However in terms of shape, it is not only particles with a high aspect ratio (i.e. fibres) that can be potentially pathogenic but indeed any particle including platelets and possibly low density bundles with the mix of high aspect and reparability. Therefore, a more general focus towards high aspect and respirability rather than high aspect ratio may be prudent and in hazard identification of *all* particulates, consideration of shape and size (as well as aerodynamic diameter) should be made.

**Table 1**

**Calculated aerodynamic diameter of graphene/graphite nanoplates**

| Projected area diameter ( $\mu\text{m}$ ) | Thickness ( $\mu\text{m}$ ) | Aerodynamic diameter ( $\mu\text{m}$ ) |
|-------------------------------------------|-----------------------------|----------------------------------------|
| 5                                         | 0.01                        | 0.42                                   |
| 10                                        | 0.01                        | 0.59                                   |
| 15                                        | 0.01                        | 0.73                                   |

Adapted from [72]

### Translocation/secondary systemic effects

The small size of nanoparticles has led to the concern that they may become systemically available and able to reach specific targets within the body [9] and the ability of some nanoparticles to translocate in small quantities have been shown in several important studies [73–76]. However, the extra pulmonary translocation and accumulation of particulates is certainly not a nano-specific-phenomenon and has been observed for larger particles also. As already stated, asbestos is known to translocate into the pleural cavity after inhalation exposure and has also been known to cause primary mesothelioma in the peritoneal cavity [77] suggesting the ability to translocate even further. This route of movement of particles out of the lung has also been described for air-pollution particles where in a study of 150 consecutive necropsies of urban dwellers in Brussels, ‘black-spots’ or accumulations of particulates was noted on the parietal pleura in 92.7% of cases [78]. Whilst suggested as a route of egress and pleural exposure for nanoparticles and in particular nanofibres [59\*\*], pleural exposure was only demonstrated recently in a study by Mercer *et al.* showing pleural penetrations of carbon nanotubes after lung administration [70] which demonstrates that this route is applicable to both nanoparticles and conventional particles alike.

As well as the pleural translocation of nanoparticles, it has been observed that nanoparticles administered to the lung can translocate to and accumulate in the liver [75,79], a phenomenon observed for more conventional particles. For example, the 1982 study by LeFevre *et al.* describes the moderate or heavy pigment accumulation in coal workers revealing an incidence of 10.4% in liver sections and 19.5% in spleen sections across a cohort of 99 coal miners [80]. Extra-pulmonary accumulation of crystalline silica has also been described with accumulations in the liver [81], spleen [82], peritoneal cavity [83] and bone marrow [82].

Whilst it is clear that extrapulmonary transfer and systemic availability can occur for all particles and is not ‘nano-specific’, studies already cited do show increased translocation for smaller particles and therefore a key difference may be a greater systemic availability at lower exposure concentrations for smaller particles than has been conventionally seen for larger particles which has often been associated with very high lung burdens. Indeed, when considering this issue of exposure and also the nature of BED and how, with smaller particles this can increase dramatically it becomes apparent that perhaps the most urgent and arguably the most important ‘nano-question’ is that of understanding the dose.

### Consideration of dose

Whilst the mode of toxicity, as this article demonstrates, is unlikely to differ from that of ‘conventional’ particles, it is the level or dose at which an effect may occur at (i.e.

potency) which may differ. This has been most explicitly shown for titanium dioxide particles and the recent Current Intelligence Bulletin (No. 63) published by the US National Institute for Occupational Safety and Health (NIOSH) discusses this in-depth. Indeed within the document, NIOSH describes the basis for the greater toxicity seen with the nano-form of  $\text{TiO}_2$  when compared to larger  $\text{TiO}_2$  particles and relates this to the difference in surface area. This suggests that the most appropriate metric for measuring dose and for setting an exposure limit for non-fibrous  $\text{TiO}_2$  is surface area but as noted by NIOSH, this is currently not practical since surface area cannot be easily and routinely measured in real-time in workplaces. Therefore NIOSH defined two recommended exposure limits of  $2.4 \text{ mg/m}^3$  for fine  $\text{TiO}_2$  and  $0.3 \text{ mg/m}^3$  for ultrafine (including engineered nanoscale)  $\text{TiO}_2$ , as time-weighted average (TWA) concentrations for up to 10 hours/day during a 40-hour work week [84<sup>•</sup>]. This reflects the increased potency of the smaller sized  $\text{TiO}_2$  but crucially (in relation to other occupational exposure limits which only distinguish between total and respirable), both these size definitions are within the respirable range and therefore demonstrates the spectrum of toxicity that can occur within this range due to increased surface area. Indeed one of the only truly nano-specific issues is the high surface area and particle number per unit mass when compared to larger, micron sized particles. However this can and should be seen as a sliding scale rather than as a threshold around the 100 nm and below size range.

### Arguments for nano-specific effects

It is appropriate to mention that, in contrast to the view taken here, there are suggestions in the published literature that nanoparticles do have nano-specific effects. For example Krug and Wick [85] refer to surface composition, size and transport as the factors that contribute to the toxicity of any nanoparticle. They suggest that for any specific nanoparticle these three factors come together to form a unique combination forming ‘... a basis for the description of specific reactions and interactions between nanomaterials/nanoobjects and biological systems...’. These authors argue the obvious corollary of this contention, namely that each nanoparticle ‘must be tested individually’ [85]. This is a ‘counsel of despair’ that we reject above, arguing that the lack of nano-specific toxicity forms a basis for benchmarking to the large amount of available data on conventional particle-mediated pathogenicity. We note that that the final common pathways for pathological effects, that is, oxidative stress, inflammation and genotoxicity are entirely shared by both nanoparticles and conventional particles and no novel pathogenic pathways are anticipated. Therefore whilst the proximate events such as transport of nanoparticles into cells may be unusual even novel, [86], the final common pathways of oxidative stress inflammation and genotoxicity are impacted by all

pathogenic particles. We can see no reason, therefore to invoke nano-specificity to the adverse effects nor should we anticipate novel pathologies. Kreyling has demonstrated that the translocation of NP from the lungs varies depending on the nanoparticle size, with a greater fractional translocation of the smaller nanoparticles [74]. However, the translocation fraction is extremely small and so of questionable significance. That it is not significant is supported by the striking absence of reports of extra-pulmonary pathology in a large number of chronic, high exposure, rat inhalation studies carried out with low solubility, low toxicity nanoparticles in the eighties and nineties, for example [87]. In the case of human epidemiology of ambient combustion-derived nanoparticles (air pollution/PM) exposure, the only clear extra-pulmonary effects — in cardiovascular disease — are now considered most likely to arise from oxidative stress or inflammatory signals from the lungs, although translocation is not completely ruled out [88].

### Conclusion

There is a huge amount of research and regulatory activity in the area of nanoparticle health and safety. Particle toxicologists need to provide a comprehensive understanding of nanoparticle hazard in the context of the huge array of untested NP varying in composition, shape and size for use in risk assessment. This is especially important as the sheer degree of adaptability and variability of engineered nanoparticles precludes against detailed testing of every form produced and so other sources of judgement as to potential toxicity or general mechanisms of toxicity for nanoparticles are required.

The purpose of this article is to point out that conventional particle toxicology data are useful and relevant to the evaluation of nanoparticle hazard and that, so far, there is no evidence that particles below 100 nm show a step-change in their hazard status and no threshold for the onset of novel ‘nano-specific hazard’. This does not mean that there are no changes or quantum size effects for some particles at certain size thresholds. However, the toxicological relevance of these changes must be questioned and identified as the mode of toxicity (and resultant pathobiological reaction such as inflammation) is unlikely to differ from those established for larger particles. Focus away from any postulated ‘nano-specific effects’, as we suggest, will allow research going forward to be focused towards understanding the critical questions of NP dose and its nature for specific NP leading to general rules for nanoparticle structure/toxicity relationships (where there have already been recent advances [89,90<sup>••</sup>]). This implies that the wider particle literature should and must be used more extensively to avoid costly repetition so reducing time and budget towards the goal of good structure/toxicity relationship for NP. This should hasten a fuller understanding of nanoparticle toxicity and how to

identify and test for their effects and measure their toxicokinetics.

### Conflict of interest

KD has carried out consultancy on the toxicology of nanoparticles but not work that is directly relevant to the present paper.

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