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The Biologically Effective Dose in Inhalation Nanotoxicology

KEN DONALDSON,*,† ANJA SCHINWALD,† FIONA MURPHY,‡ WAN-SEOB CHO,§ RODGER DUFFIN,⊥ LANG TRAN,∥ AND CRAIG POLAND∥

†University of Edinburgh, Edinburgh, U.K., ‡Dong-A University, Busan, South Korea, §MRC Toxicology Unit, Leicester, U.K., ⊥CXR Biosciences, Dundee, U.K., and ∥Institute of Occupational Medicine, Edinburgh, U.K.

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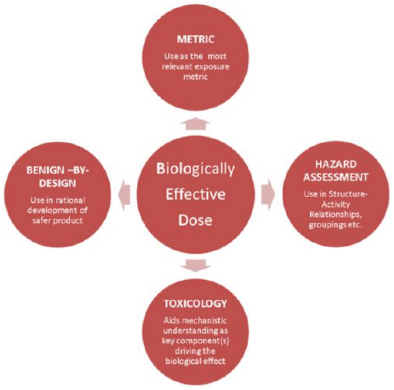
CONSPECTUS

In all branches of toxicology, the biologically effective dose (BED) is the fraction of the total dose of a toxin that actually drives any toxic effect. Knowledge of the BED has a number of applications including in building structure—activity relationships, the selection of metrics, the design of safe particles, and the determination of when a nanoparticle (NP) can be considered to be "new" for regulatory purposes. In particle toxicology, we define the BED as "the entity within any dose of particles in tissue that drives a critical pathophysiogically relevant form of toxicity (e.g., oxidative stress, inflammation, genotoxicity, or proliferation) or a process that leads to it."



In conventional chemical toxicology, researchers generally use the mass as the metric to describe dose (such as mass per unit tissue or cells in culture) because of its convenience. Concentration, calculated from mass, may also figure in any description of dose. In the case of a nanoparticle dose, researchers use either the mass or the surface area. The mass of nanoparticles is not the only driver of their activity: the surfaces of insoluble particles interact with biological systems, and soluble nanoparticles can release factors that interact with these systems. Nanoparticle shape can modify activity.

In this Account, we describe the current knowledge of the BED as it pertains to different NP types. Soluble toxins released by NPs represent one potential indicator of BED for wholly or partially soluble NPs composed of copper or zinc. Rapid dissolution of these NPs into their toxic ions in the acidic environment of the macrophage phagolysosome causes those ions to accumulate, which leads to lysosome destabilization and inflammation. In contrast, soluble NPs that release low toxicity ions, such as magnesium oxide NPs, are not inflammogenic. For insoluble NPs, ζ potential can serve as a BED measurement because the exposure of the particle surface to the acidic milieu of the phagolysosome and interactions with the lysosomal membrane can compromise the integrity of the NPs. Researchers have explored oxidative potential of NPs most extensively as an indicator of the BED: the ability of an NP to cause oxidative stress in cells is a key factor in determining cell toxicity, inflammogenicity, and oxidative DNA adduct formation. Finally we discuss BEDs for high aspect ratio nanoparticles because long fibers or nanoplatelets can cause inflammation and further effects. These consequences arise from the paradoxically small aerodynamic diameter of fibers or thin platelets. As a result, these NPs can deposit beyond the ciliated airways where their extended dimensions prevent them from being fully phagocytosed by macrophages, leading to frustrated phagocytosis. Although knowledge is accumulating on the BED for NPs, many questions and challenges remain in understanding and utilizing this important nanotoxicological parameter.

In conventional chemical toxicology, the mass is the metric generally used to describe dose (e.g., mass per unit tissue or cells in culture) because of its convenience, although concentration, calculated from mass, may also figure in any description of dose. In the case of nanoparticles, the mass or the surface area dose is used. However, nanoparticles are unlikely to have their effect as a consequence simply of their mass since the quantity that interacts with the biological system is the surface for insoluble particles or any soluble factors released in the case of soluble particles

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and may be modified by shape. Our aim here is to describe current knowledge on the biologically effective dose (BED) as it pertains to different nanoparticle (NP) types. Soluble toxins released by NPs represent a potential BED for wholly or partially soluble NPs such as copper or zinc NPs. Rapid dissolution into ions in the acidic milieu of the macrophage phagolysosome leads to accumulation of the toxic ions, which causes lysosome destabilization leading to inflammation. In contrast, soluble NPs that release low toxicity ions, such as magnesium oxide NPs, are not inflammogenic because the ions that are released are low in toxicity. The ζ potential is a BED for insoluble NPs because the charge on the surface of the particle can be exposed in the acidic milieu of the phagolysosome, interacting with the lysosomal membrane leading to loss of integrity. The oxidative potential of NPs remains one of the BED that has been most explored, and the ability of NPs to cause oxidative stress in cells is a key factor in determining cell toxicity, inflammogenicity, and oxidative DNA adduct formation. The final BED discussed here is high aspect ratio, where long fibers or nanoplatelets cause inflammation and its consequences. This arises as a consequence of the paradoxically small aerodynamic diameter of fibers or thin platelets, enabling them to deposit beyond the ciliated airways where their extended dimensions prevents them being fully phagocytosed by macrophages, leading to frustrated phagocytosis. While knowledge is accumulating on the BED, there is still a great deal of research yet to be done and challenges faced in utilizing this important parameter, possibly the most important one for nanotoxicology.

The Biologically Effective Dose (BED) in Toxicology

Dose is central to toxicology, and the concept of dose for particles can actually be considered more deeply in terms of what is driving the response. Specifically, particles while often measured in terms of mass do not exert their effect as a consequence simply of their mass. The term used to describe the actual component of the total dose that drives adverse effects is the biologically effective dose (BED). We here define the particle BED as "the entity within any mass dose of particles that drives a critical pathophysiogically relevant form of toxicity in tissue", for example, inflammation, genotoxicity, or cellular proliferation. The BED is useful concept for several reasons (Figure 1 and Table 1). Any given particle sample might have more than one BED such as the presence of polyaromatic hydrocarbons (PAHs) as well as reactive transition metals in PM_{10}, and indeed, the BED might drive more than one response, for example, a local

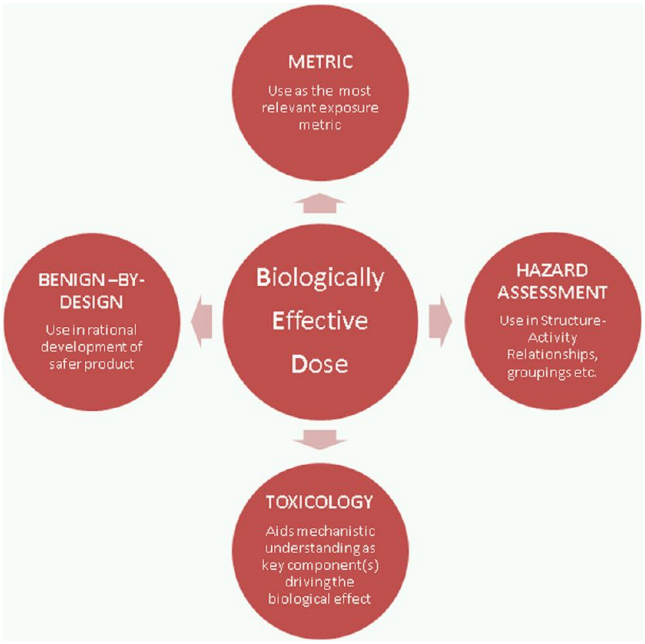

FIGURE 1. The uses and importance of the biologically effective dose.

response at the portal of entry and a more distal response in a target organ that it affects directly by translocation or indirectly by release of mediators at the portal of entry. Therefore in considering any BED, we need to define the response of interest and in terms of this Account, we confine ourselves to inflammation as the response of interest since it has multiple roles to play in particle-induced health effects and pathology.

It is notable that, despite knowledge of the BED and the obvious benefit of measuring the BED as a better guide for risk management, neither the quartz nor fiber BED, which are known, is the metric used in exposure measurement/risk assessment; the mass and fiber number are the metrics used for quartz and fibers respectively. The reason behind this simply reflects the challenges in measurement of particle exposure other than mass, particularly using real-time measurement systems. While there are improvements in this area, such as being able to measure surface area (often based on algorithms rather than direct measurement), certain factors such as particle reactivity or biopersistence are unlikely to ever be integrated into real-time exposure metrics.

At the level of individual cells, the BED of various nanoparticles (NP) is becoming better understood as discussed below. The BED is synonymous with particle physicochemical characteristics that are relevant for structure/toxicity (activity) relationships (STR). This is because structures defined in STRs drive the response and therefore are ideally the

TABLE 1. Details on How the BED Can Be Used for Hazard Identification, Risk Management, Predictive Toxicology, and Benign-by-Design

use of BED	rationale
hazard identification	the BED allows prediction of the types of hazard outcome, for example, a highly oxidative particle is likely to cause oxidative DNA adducts; a fiber-shaped nanoparticle may pose a mesothelioma hazard
risk management	since the BED drives the adverse effect, measuring the BED as the exposure metric most closely measures the harmful exposure and so would allow the most effective risk management
predictive toxicology	determining the BED and its potency using toxicological approaches allows prediction of the type of hazard (hazard identification) and likely potency in causing pathogenic effects
benign-by-design	knowledge of the BED allows particles designers a structural target that they may address to produce a safer, less harmful particle

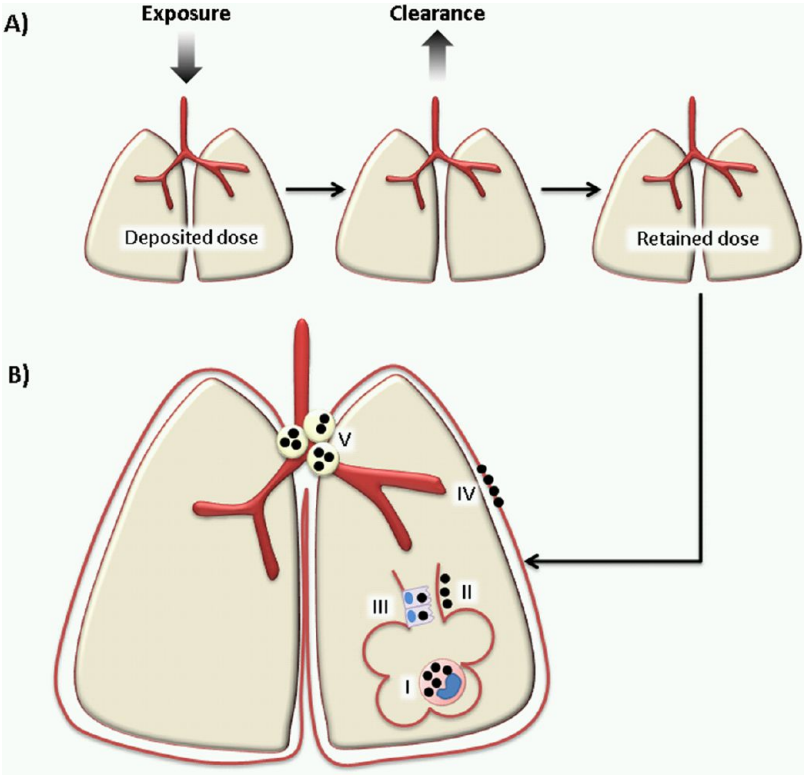

FIGURE 2. (A) The retained dose shown as the deposited dose minus the dose that is mechanically cleared and (B) the sequestration compartments in the lungs where the retained particle dose (black dots) can be found:-(I) alveolar macrophages; (II) interstitium in terminal airways; (III) epithelial cells; (IV) the parietal pleura; (V) mediastinal lymph nodes. In addition, if lesions arise, particles can be trapped in the developing lesions.

BED for a specific pathophysiologically relevant response(s). The BED therefore is crucial and can be useful in a number of ways (Figure 1) that aid hazard identification, risk management, predictive toxicology (i.e., *in silico* approaches), and benign-by-design to allow the informed development of safer nanomaterials. More detailed examples of these uses are given in Table 1.

Retention Compartments Where the BED Is Applied

It is informative to consider where the BED is applied. The lung is a complex organ, and the delivery of a particle dose is a complex interplay related to the anatomy of the lung, the flow of air, and the aerodynamic size and composition of the particles, all of which govern how much and where an inhaled dose of particles deposits in the lung. A major part of the "ineffective" dose is the fraction that is cleared by mechanical clearance (Figure 2A) to leave the retained dose. The retained dose distributes into various compartments over time where residence can be transient or more long-lived. Classically the clearance from the airways, propelled by the mucociliary escalator, is much faster than clearance from beyond the ciliated airways, which depends on macrophage action. Depending on the particle in question and its BED, there can be an effect in these compartments that contributes to pathogenicity. While low toxicity particles generally cause minimal effects at plausible doses, pathogenic particles can have various pro-inflammatory, mitogenic, genotoxic, or other pathophysiogically relevant effects. The compartments are shown in Figure 2B, and the

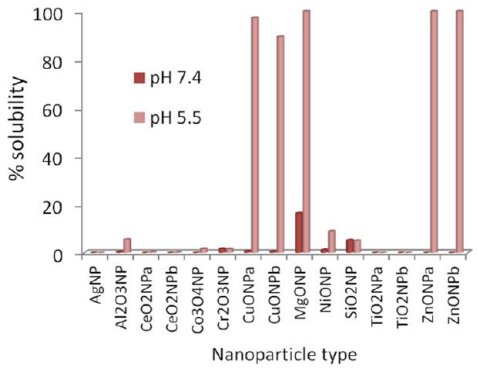

FIGURE 3. Solubility (%) of a panel of metal/metal-oxide NPs in Gamble's solution at pH 7.4 and 5.5; data are shown as mean; all SEM were <10% of the mean. Same composition NPs from different sources are labeled with a and b subscripts such as CeO_2NP_a and CeO_2NP_b. Solubility test was carried out three times. Data were redrawn from ref 1.

effects of the particle BED in these compartments give responses that provide the pathological nuances that are seen with different particles and that we would anticipate with different NPs.

Biologically Effective Doses of Nanoparticles

The BED, as discussed above, gets to the heart of how any particle dose drives response. Within any mass dose of particles that elicits a toxic response, there must be one or more entities that are the actual drivers, and these, by definition, are the BED(s). They can take the form of one or more physicochemical characteristics associated with the particles and can be soluble or ionic species released from the particle or characteristics integral to the particle surface or the particle shape. Below we outline exemplar BEDs that have been described for various nanoparticle types.

Soluble Toxins Released from NPs. Some NPs will undergo a degree of solubility (ionization) in biological fluids. Using metal oxide NPs, we have seen measurable but limited solubility in water or neutral conditions[1] (Figure 3). However, in the acidic conditions typical of phagolysosomes and lysosomes, NPs show variable solubility (Figure 3); copper oxide NPs, magnesium oxide NPs, and zinc oxide NPs showed rapid, complete dissolution in acid conditions (pH 5.5), while other NPs showed minimal dissolution (Figure 3).

The neutral-soluble fraction may be important for particles bathed in lung-lining fluid or the fluid of the interstitium, which have a pH around neutrality. For example, the concentration of soluble ions from the nickel oxide NP, zinc oxide NP, and copper oxide NP suspensions in neutral saline ranged from 5 to 12 ppm.

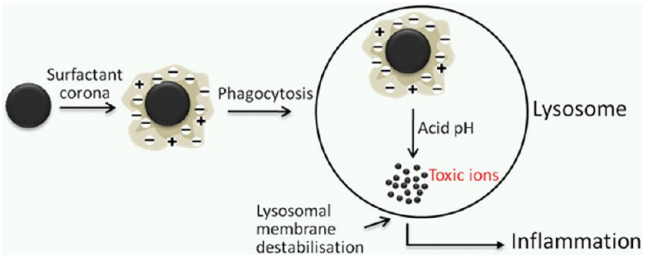

FIGURE 4. The mode of action of high-solubility NPs composed of material that ionizes to toxic ions in causing inflammation. High-solubility NPs deposit in the neutral environment of the lung lining and first develop a corona from the lung surfactant into which they deposit. They are then taken up into macrophage phagolysosomes, which have an acidic pH. In the acidic phagolysosomal milieu, dissolution to toxic ions is greatly accelerated compared with dissolution rate at neutrality and the toxic ions accumulate to a high concentration. This causes lysosomal membrane destabilization and inflammation. See ref 1 for more details.

Treatment of the alveolar type II epithelial cell line, A549 with the neutral-soluble fraction of zinc oxide NPs and copper oxide NPs increased interleukin (IL)-8 via activator protein (AP)-1 or nuclear factor κB (NF-κB) activation while that with nickel oxide NPs did not. Chelation of ions in the neutral-soluble fraction of zinc oxide NPs and copper oxide NPs abolished the effect demonstrating the role of the ions as the BED for the inflammatory reaction in these cells, and in a rat lung instillation model, low concentrations of neutral-soluble fractions had a role in recruiting neutrophils to the lung.[2]

The acid-soluble fraction is relevant because of the compartmentation of particles into macrophage phagolysosomes, which typically have a pH of around 5.5 and dissolution commonly occurs much faster in this acidic environment. For example, zinc oxide NPs produced an acute eosinophilic inflammation in a rat instillation model,[2] and instillation of the relevant concentration of soluble zinc ions produced the same magnitude of eosinophilic inflammation as the whole NP, suggesting that the BED for eosinophil recruitment was zinc ions and not the NP *per se*. On the other hand, despite being highly soluble at pH 5.5, magnesium oxide NPs did not elicit inflammation in the rat instillation model.[1] This is because Mg^{2+} is a nontoxic ion and may even protect against cell injury.[3] Therefore, the relative toxicity of the compositional ions of high-solubility NPs is the principal parameter dictating the toxicity of these NPs and its toxic ions can be the BED (Figure 4).

In addition to degree of solubility impacting on the BED, solubility also is a factor in biopersistence. Dissolution to *harmless ions* is likely to be nonharmful since there is no

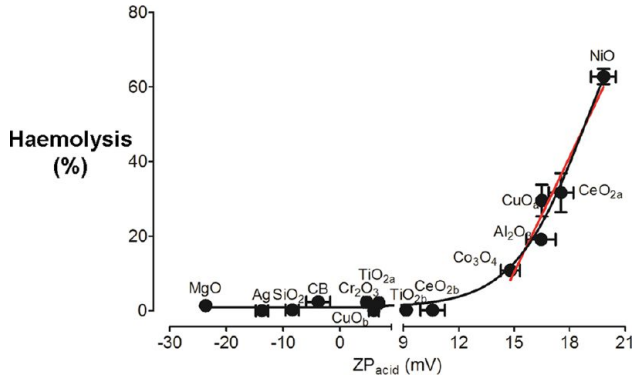

FIGURE 5. Effect of the ζP_{acid} value on the ability of any NP sample to cause lysis of erythrocyte membranes. Redrawn from ref 4.

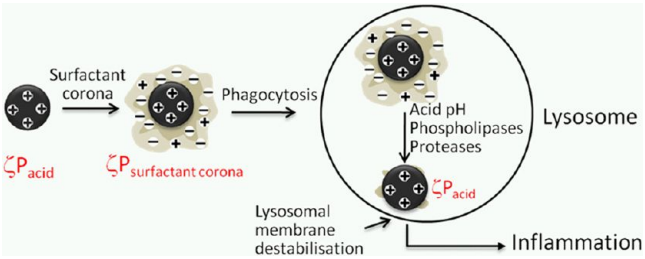

FIGURE 6. The hypothesized mechanism of inflammation caused by low solubility, high ζP_{acid} NPs. The high ζP_{acid} of NP is changed to $\zeta P_{surfactant\text{-}corona}$. This corona, however, can be digested by the enzymes and the acidic conditions of lysosomal fluid, which can restore the high ζP_{acid}, which is inflammogenic via destabilization of lysosomal membrane. Redrawn from ref 4.

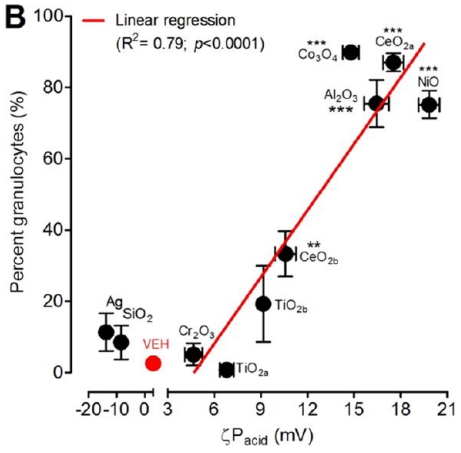

FIGURE 7. Relationship between ζP_{acid} and lung inflammogenicity. Plot of ζP_{acid} versus percentage of total granulocytes in the BAL. NPs having negative ζP_{acid} were not associated with inflammation but NPs having positive ζP_{acid} had a significant linear correlation with the percentage of total granulocytes in the BAL (Pearson correlation test, $R^2 = 0.86$, $p = 0.0009$, 95% confidence interval = 0.64−0.99. Used with permission from ref 4. Copyright 2012 Society of Toxicology.

build-up of particles; however, dissolution to *harmful ions* would lead to pathogenic effects. The consequences of slow or partial dissolution are more difficult to predict and would depend on the toxicity of the ions and of the residuum of the particle that is retained.

ζ-Potential. The electric potential created between the charged groups associated with the surface of a particle and the suspension medium is the ζ potential (ζP) and provides information concerning the surface charge of particles. The ζP, as might be anticipated, shows dynamic changes depending on the pH of the medium and the adsorption of protein to form the corona.[4] Subsequently most metal oxide NPs have a negative ζP in PBS (pH 7.4), a predominantly positive ζP in acidic physiological saline (pH 5.6) (ζP_{acid}), and a slightly negative ζP when there is a corona of proteins ($\zeta P_{surfactant\text{-}corona}$) or lung lining fluid. The basis for advancing ζP as a BED is that when NPs are phagocytosed by alveolar macrophages the proteolytic enzymes and acidic pH (pH 5.6)[5] found in the phagolysosome may strip off the corona and reveal the naked surface of the particle[4] restoring the precoronal ζP_{acid}. If the restored ζP_{acid} of the NP has a high positive value, it has the potential to bind to and damage membranes, as shown by ability to lyse erythrocyte membranes as a model membrane target (Figure 5).

We suggest that the same type of enhanced interaction between NPs with a high positive ζP_{acid} and the internal face of the lysosomal membrane could cause destabilization of the lysosomes, which can trigger inflammation or cell death (Figure 6).

Thus the ζP_{acid} represents a BED for some NPs, and this is supported by the finding that the ζP_{acid} of metal/metal-oxide NPs showed a linear correlation with the ability of a range of NP samples to cause acute lung inflammation. This was shown by instilling a panel of metal/metal oxide NPs into rat lungs at equal surface area dose and plotting the ζP_{acid} with the extent of the inflammation.[4] There was a clear threshold ζP_{acid} level triggering both inflammation (Figure 7) and the membranolytic effect (Figure 6) that was around +12 mV[4] (Figure 7). Therefore for some NPs the ζP_{acid} represents the BED, but this may be quite site-specific since it requires an acidic and hydrolytic environment such as the macrophage phagolysosome for its full expression.

Oxidative Potential. Throughout the particle toxicology literature, there are numerous instances where pathogenic particles possess oxidative potential and cause their effects via the final common pathway of oxidative stress. For example, in the case of quartz, the elicitation of oxidative stress and the formation of damaging hydrogen bonds between the silanol groups at the quartz surface and the cell membrane is a key driver of oxidative stress and ensuing

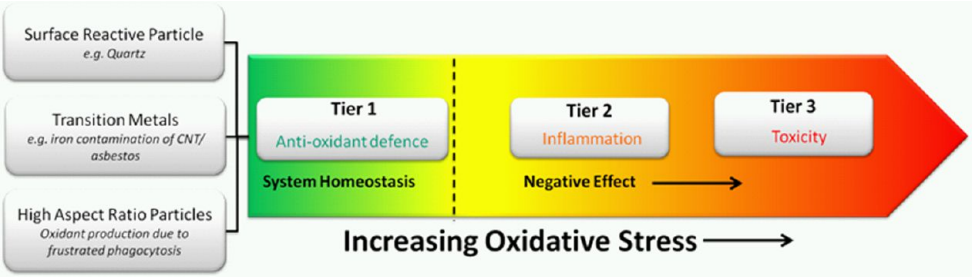

FIGURE 8. The relationship of biologically effective dose to the driving of oxidative stress and graded cellular responses. Adapted from ref 22.

inflammation.[6] It should however be noted that in some cells nonoxidative pro-inflammatory mechanisms also prevail.[7] In some studies, NP oxidative activity measured directly by chemical methods can be related to their ability to cause inflammation,[8] but other studies have not found this simple relationship[9]

Taking another example of a different NP, the generation of oxidative stress in cells by exposure to carbon nanotubes has been described by several research groups.[10] The oxidative potential of carbon nanotubes has been ascribed to the transition metal catalysts remaining after production, which can be a source of free radicals.[11] {However for longer fibers of carbon nanotubes, the length of the fibers and not iron is the BED (see below).}

Nel and co-workers[12]suggested a hierarchy of response to particle-induced oxidative stress. Within this model, the normal physiological levels of oxidative stress are dealt with by the cell by gene expression for antioxidants but intermediate levels of oxidative stress may cause a more severe responses such as inflammation (e.g., via activation of NF-κB), while high levels of oxidative stress cause cell death (Figure 8).

As shown in Figure 8, several BEDs can have oxidative potential and so exert their effect through oxidative stress. Examples include phagocytosis of long fibers driving the release of superoxide radicals due to activation of the NADPH oxidase system.[13] Another example of BED driving oxidative stress is the photocatalytic activity of titanium dioxide under UV light.

High Aspect Ratio Particles. Nanofibers. The geometric shape/structure of particles has long been accepted as a crucial factor in enhancing toxicity. The role of fiber length/aspect ratio first became apparent because of asbestos, which caused a worldwide epidemic of lung cancer and fibrosis, as well as pleural diseases such as mesothelioma, pleural fibrosis, and pleural plaques. The correlation of adverse health effects and fiber structure is a robust structure–toxicity relationship encapsulated in the fiber pathogenicity paradigm (FPP), which defines the features that define the fiber BED[14] (Figure 9).

Particles/fibers that deposit beyond the ciliated airways are cleared via alveolar macrophages, and this relies on complete phagocytic uptake. However longer fibers cannot readily be completely taken up by macrophages, which results in frustrated phagocytosis (Figure 10). This incomplete or frustrated phagocytosis is pro-inflammatory[14] as shown in Figure 11.

As well as being pro-inflammatory, the sheer bulk of a long fiber(s) inside a macrophage is likely to be an obstacle to the normal process of motility leading to accumulation of longer fibers in the lower respiratory tract,[15] recruitment of inflammatory cells, secretion of pro-inflammatory mediators, and generation of reactive oxygen species (see below)[14] The clearance rate of longer fibers is also impacted by the biopersistence of the deposited fibers. Nonbiopersistent long fibers can break into smaller fragments and be cleared via macrophages whereas biopersistent long fibers remain in the lower respiratory tract, and some may translocate to the pleural space surrounding the lungs.[14] The generality of the translocation of particles and fibers from the lungs to the pleural space has been demonstrated, and liquid and particles in the pleural space drain to mediastinal lymph nodes by drainage through stomata on the parietal pleura.[14] With a diameter <10 μm,[16] the parietal pleura therefore acts like a sieve, retaining longer fibers at the parietal pleura where they can cause pathogenic responses. There is therefore a good mechanistic basis for biopersistent long fibers as the BED of long fibers for pleural responses.

The similarities between asbestos and manufactured nanofibers, including nanotubes, -rods, and -wires, has engendered concern as to their potential to cause similar hazards. Following initial data supporting the hypothesis that nanofibers conformed to the FPP in the peritoneal cavity,[17] we went on to test for length-dependent fiber pathogenicity after direct exposure of the mesothelial lining of the pleural space using a panel of high aspect ratio

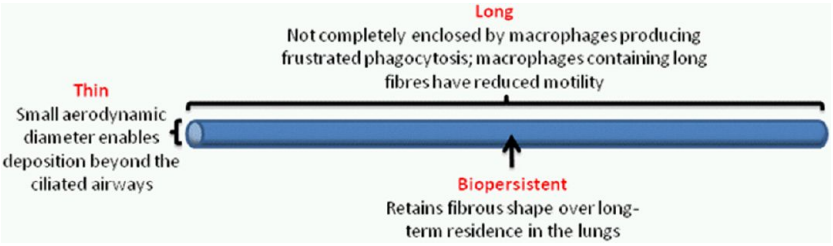

FIGURE 9. The three structural elements that determine the toxicity of fibers; see text for details.

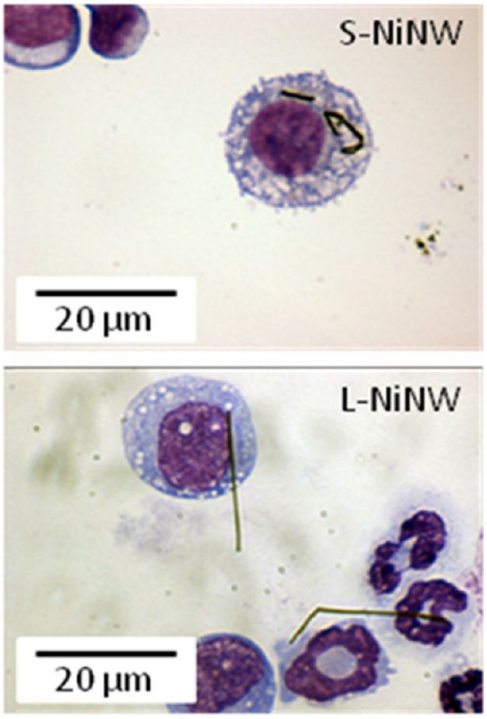

FIGURE 10. Macrophages that have phagocytosed short nickel nanowires (S-NiNW, upper image) and long nickel nanowires (L-NiNW, lower image). With short nanowires, the fibers can be clearly seen completely enclosed within the cell (upper image, white arrow) while the phagocytosis of long fibers produces the classical picture of frustrated phagocytosis with the fiber protruding from the cell surface (lower image, black arrow). Image modified from ref 23.

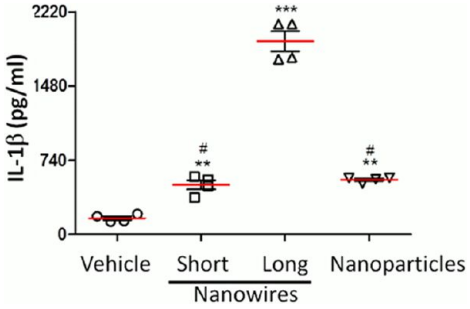

FIGURE 11. Pro-inflammatory effects, as production of the cytokine IL-1β by THP-1 macrophages exposed to vehicle, long or short nickel nanowires, or nickel nanoparticles. Note the clear specific effect of the long nanowires in stimulating IL-1β release.

nanoparticles (HARN) samples.[18] Only long nanofibers and long asbestos fibers elicited sustained inflammation in the pleural space and extensive lesion formation and fibrosis along the parietal pleura. These studies support the contention that long, biopersistent nanofibers represent the BED for this type of nanomaterial. Most recently, we have addressed the threshold fiber length for the onset of fiber pathogenicity in the pleural space using silver nanowires and nickel oxide nanowires with tightly sized length categories. After direct intrapleural injection, we were able to identify a clear cutoff value of 5 μm for acute long fiber effects in the pleural space.[19] We are therefore able to further delineate the nanofiber BED as biopersistent fibers longer then 5 μm.[18]

Nanoplatelets. Recently high aspect ratio materials made of graphene in the form of nanoplatelets are attracting substantial scientific interest. Like fibers, the platelet shape could pose unusual risk to the lungs and the pleural space after inhalation due to their aerodynamic properties. Very thin sheets with a high aspect can have a low aerodynamic diameter (D_{ae}) compared with their geometric size. The respirable size for humans cuts off at around an D_{ae} of 5 μm.[20] We calculated the aerodynamic diameter of a commercial form of graphene nanoplatelets (GNPs), which could be up to 30 μm across, and we found the 30 μm diameter nanoplatelets to possess an D_{ae} of 3.3 μm, well inside the respirable range (Table 2). Such a particle can therefore deposit beyond the ciliated airways where the macrophages will fail to fully engulf them, producing frustrated phagocytosis[21] and inflammation. We reported that respirable-sized but physically large and extended graphene nanoplatelets were able to cause inflammation in the lung and the pleural space after pharyngeal aspiration and direct intrapleural injection.[21] The inflammatory response was accompanied by frustrated phagocytosis of pulmonary and pleural macrophage observeable in vitro (Figure 12), and macrophage cells treated with respirable GNPs in vitro produced increased cytokine levels compared with a

TABLE 2. Range of Sizes of Nanoplatelets in the GNP Sample as Their Projected Diameter[a] and the Calculated Aerodynamic Diameter, Clearly Showing That the Nanoplatelets Having a Very Large Projected Diameter even up to 30μm Are Still Respirable

projected diameter [μm]	aerodynamic diameter (μm)
5	1.3
10	1.9
15	2.3
20	2.7
25	3
30	3.3

[a]The diameter of a circle having the same area as the particle.

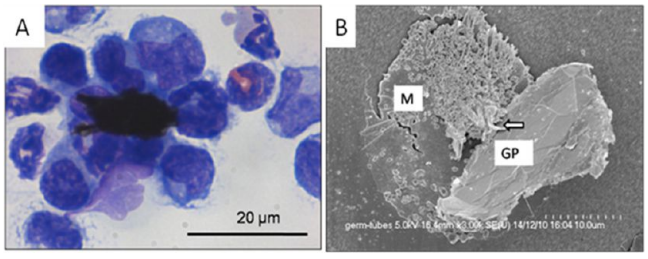

FIGURE 12. (A) Light micrograph of macrophages lavaged from the pleural space following instillation of graphene nanoplatelets. Note accumulation of cells around a large but respirable nanoplatelet with several cells showing frustrated phagocytosis as they attempt to enclose the same nanoplatelet. (B) Scanning electron microscope image of a macrophage (M) in culture failing to fully enclose a large yet respirable graphene nanoplatelet (GP) producing classical frustrated phagocytosis; arrow shows the attempt of the macrophage membrane to enclose the nanoplatelet.

nanoparticle carbon black control (Figure 13). Thus, for nanoplatelets, large extended thin particles with D_{ae} are a BED.

Collectively our data with nanofibers and nanoplatelets highlights the shape of high aspect nanomaterials as a BED, the driver for in vivo toxicity. Both nanofibers and nanoplatelets with nanoscale diameter or thickness can be very large in one or two dimensions but possess a low D_{ae}, which allows them to deposit in the lower respiratory tract. Therefore the fibrous and platelet habit can be seen as key BEDs if the material of which they are composed is biopersistent.

Benefits and Challenges of the BED. Despite the accumulated knowledge regarding the various BEDs that NPs possess and their roles in driving pathological processes, their remain major gaps and challenges in their use. In any single type of NP numerous factors may contribute to the BED making for a compound BED that could be the sum of particle shape, composition, soluble toxins, etc. Furthermore different BEDs may also impact on different pathways with differing potencies and may also be affected by aggregation in air and potential disaggregation in the lungs. As a hypothetical example, a long thin nanofiber may have a

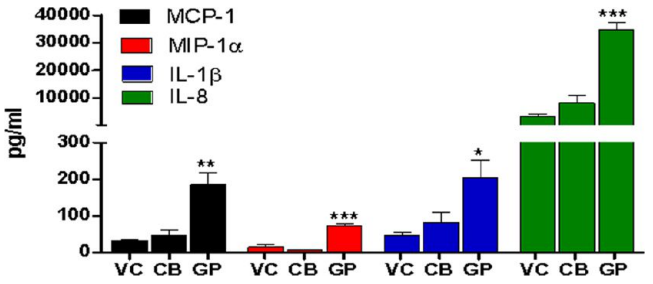

FIGURE 13. Release of four cytokines from THP-1 cells treated with vehicle (VC), carbon black (CB), and graphene nanoplatelets (GP) Adapted from ref 21. Note that GP produces a consistent and significant increase in release of the four cytokines compared with vehicle or nanoparticulate carbon black, a compact nanoform of graphene.

BED as a consequence of high aspect, but if it is composed of a reactive material, it may have ζ potential type BED and may also release soluble ions that have a soluble ion type of BED. Adoption of a metric based on such a complex BED seems to pose almost insuperable difficulties and so merely knowing the BED, if the BED is compound and complex, does not necessarily offer a solution to the issue of basing the metric on the BED. However some particles are likely to have a single BED, for example, surface area in the low-toxicity, low-solubility materials, and these pose a clearer target.

Conclusion

Knowledge of the BED is of great value for a variety of reasons in considering the hazard of any nanoparticle (Figure 1). By considering a particle not just as a single entity, but as a sum or sums of the attributes that drive toxicological response in a biological systems, one can understand the root drivers of toxicity, which opens the way to a number of key applications (Figure 1). Data is continuing to accumulate on the nanoparticle hazard and with increased knowledge of the toxicological profile/biological interactions of nanoparticles and the physicochemical properties, nanoparticles may be grouped into categories with a similar BED for hazard evaluation purposes. Other potential BEDs have been listed,[21] but firm evidence for their role in toxicological processes and the development of adverse effects and disease remain to be demonstrated. Other BEDs no doubt will become apparent as more data accumulates, but we do not consider that there will be a very large number, just as the variety of BEDs for conventional pathological particles is quite limited.

BIOGRAPHICAL INFORMATION

Ken Donaldson was born 1950 in St Andrews, Scotland, and attended the University of Stirling, obtaining a B.Sc. Hons. (First Class) in Biology (1978), and University of Edinburgh, obtaining a

Ph.D. (1982) and D.Sc. (1994). He was Head of the Cell Biology Unit of the Institute of Occupational Medicine, Edinburgh (1984−1992) and Professor of Pathobiology at Edinburgh Napier University (1992−2002) and is presently Professor of Respiratory Toxicology in the University of Edinburgh. He has published over 330 peer-reviewed papers, reviews, and book chapters on the toxicology of all the major types of pathogenic particles.

Anja Schinwald was born in 1987 in Hallein, Austria. She received a BA.rer.nat and a MA.rer.nat (First class) from Paris Lodron University of Salzburg/Johannes Kepler University of Linz in Molecular Biosciences (2010). Currently she is in her third year of a Ph.D. program at the University of Edinburgh focusing on high aspect ratio nanomaterials in relation to the asbestos pathogenicity paradigm.

Fiona Murphy was born in 1984 in Galway, Ireland, and attended the National University of Ireland, Galway, obtaining a B.Sc. (Hons) in Biotechnology (2007), and University of Edinburgh, obtaining an M.Sc. in Life Sciences (2008). She carried out her Ph.D. studies in the University of Edinburgh assessing the potential pathogenicity of carbon nanotubes in the pleural cavity. She is currently employed as Career Development Fellow by the MRC Toxicology Unit in Leicester, U.K., where her interests are focused on the molecular mechanisms involved in the development and progression of malignant mesothelioma.

Wan-Seob Cho was born in 1977 in Korea and attended the Seoul National University obtaining a B.Sc. in Veterinary Medicine (2000), M.Sc. (2002), and Ph.D. (2004) in Veterinary Pathology. He was a Scientific Officer in the Korea Food and Drug Administration (2002−2008) and a postdoctoral fellow at the University of Edinburgh (2008−2011). He took up the post of Assistant Professor of Toxicology in Dong-A University, Korea, in 2011. He has published over 50 peer-reviewed papers and book chapters on toxicology and his current interests are structure−toxicity relationships of metal/metal-oxide nanoparticles.

Rodger Duffin was born 1975 in County Antrim, Northern Ireland, and attended Edinburgh Napier University, obtaining a B.Sc. Hons (First Class) in Biomedicine with Toxicology (1997), and University of Edinburgh, obtaining a Ph.D. (2001). After postdoctoral training abroad, he returned to the University of Edinburgh Centre for Inflammation Research and became an MRC Senior Fellow in Respiratory Medicine. In 2012, he became Deputy Director of Research in CXR Biosciences, Dundee. He has published over 90 peer-reviewed papers, reviews, and book chapters on the toxicology of pathogenic particles. His current research interests are focused on nanotoxicology and investigative and mechanistic toxicology towards drug development.

Lang Tran was born in 1958 in Saigon. He has worked as a Mathematical Modeller and Toxicologist at the Institute of Occupational Medicine in Edinburgh since 1990. He has published extensively in Nanotoxicology and is currently the coordinator of a number of large EU FP7 projects.

Craig Poland was born in Aberdeen, Scotland, in 1980 and undertook a B.Sc. (Hons) degree in cell biology and pathology at the University of St Andrews (2002) followed by an M.Sc. in Biomedical Sciences at Edinburgh Napier University (2005) and Ph.D. in the toxicology of nanoparticles (2009) at the University of Edinburgh. In 2010, he moved to the Institute of Occupational Medicine as a research toxicologist where his focus is on assessment of nanomaterial hazard and risk. His overall research interest lies in understanding particle/biological interactions and how this can better inform safe particle design and inform risk assessment to protect worker and consumer health.

FOOTNOTES

The authors declare no competing financial interest.

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