Review Article

Should Experimental Chemists Be Doing More to Help Evaluate the Toxicological Potential of Nanoparticles?

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More general testing of nanoparticles (NP) for properties that are amenable towards biological activity, and thus potentially conducive to nanotoxicity, should be conducted on a broader scale by experimental chemists to help assess the pernicious threat that NP may present to human health or to the environment. For example, evaluation by measuring NP-biomolecule bioaffinity using techniques such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) is advocated, thereby echoing a similar call to computational and theoretical chemists to expand their studies and make at least part of their work, where feasible, relevant to nanotoxicity.

The materials peculiar and intrinsic to the nanotechnology industry are often composed of particles with at least one dimension in the range 1–100 nm, thereby designating these materials as nanoparticles (NP). NP are used for all manners of applications [1–4]: for example, in the chemical industry as catalysts, in medicine as drug delivery devices and imaging agents, and in a wide range of consumer products such as car tires, sports equipment, and even personal care products such as sunscreens and cosmetics. Indeed, the growth of the nanotechnology industry has been explosive, and the entire market is expected to realize a multitrillion dollar valuation by 2015 [1, 5, 6]. Thus, NP have the potential to become as ubiquitous and pervasive in the anthropogenic world—and as an inevitable consequence in the natural world too—as plastics are presently [7].

However, NP can be derived from a broad range of chemical compositions and are not related by anything other than the one physical attribute. Moreover, NP, relative to bulk material of the same chemical composition, possess unusual properties due to their size [1, 8, 9], which is the very essence of what makes them so useful and desirable in the first place. But these peculiar properties can also potentially give rise to unexpected deleterious health and safety effects [1, 3, 5, 10] as well as rendering the materials difficult to analyze [5, 11, 12] or even to characterize comprehensively, an aspect which is of

vital importance for valid and meaningful nanotoxicological assessments [1, 5, 11, 12]. Additionally, various NP formulations (i.e., NP of the same chemical composition but of differing size, geometry, aggregation, surface properties, and so forth or other attributes arising from the manner of their preparation) consequently can also have very different properties to each other [1, 8, 9] and thus too differing toxicological effects [1, 3, 8, 10, 11, 13]. Hence, the biological properties of a bulk material, however well described, do not provide a viable indication of the likely biological properties of NP of the same chemical composition. Take as an example, single-walled nanotubes (SWNT) [14], perhaps the quintessentially iconic NP [6, 15], in comparison to fullerene, C_{60} (Figure 1), both are allotropes of carbon and are often viewed as being “soot-like”, and therefore the two could have similar toxicological profiles or even that both are possibly innocuous [6]. Yet SWNT have been assessed as much more toxic [9] than fullerene, and they even appear to possess greater risk than 5-micron quartz [9, 16] with regards to pulmonary toxicity. SWNT are therefore a potentially dangerous material when airborne whilst fullerene has been demonstrated to be toxic in aqueous solution [17, 18]. A much discussed and investigated concern [4, 8, 10, 11, 15, 19–24] is the similarity, and hence possible similar disease-inducing properties, of SWNT and multi-walled nanotubes (MWNT) to asbestos fibers due to

FIGURE 1: Fullerene, C_{60} , (a) and a single-walled nanotube (SWNT, (b)). Both are allotropes of carbon but have substantially different biological properties.

their small diameter, fibrous nature, and inertness leading to biopersistence. Several investigations revealed models which exhibited the hallmarks of damage associated with disease and one study which even induced mesothelioma in mice using MWNT [24].

Thus, it is of considerable concern the threat that NP may pose to human health [1, 3, 5, 25] due to their increasing use in personal care products and in medical applications, as well as their widespread manufacture and use generally. Indeed, humans are becoming ever more exposed at an intimate level to NP. Over the course of the full life cycle of NP, there are a number of opportunities for human exposure to NP. At the manufacturing stage there is an opportunity for direct exposure to workers through accidents or occupational exposure, as well as accidental or deliberate—either legal or negligent—release of NP into the environment. At the consumer stage, exposure to NP in products may occur either through intended use of the product, accidents or accidental damage or noncompliant usage, irrespective of whether the NP are fully contained, for example, in products such as car tires or sports equipment, or “free” to provide direct exposure, for example, when present in personal care products. Finally, disposal of the products after the consumer has finished with them may also lead to release of NP into the environment or to widespread human exposure through dumping or attempted destruction by combustion or other means, irrespective of whether this is done in compliance with legal guidance or as a consequence of negligent practice. Therefore, the nature of the NP—and any possible changes to its constitution in terms of particle size, aggregation, surface coatings, and so forth, thereby resulting in significant changes to its original formulation—must be considered over the full course of the life cycle [5, 25], and the manner in which exposure can occur can vary widely over this time period, hence compounding profoundly the evaluation of the toxicological dangers of NP and increasing the challenge even further of the evaluation of the potential risks that NP pose.

Since the size of NP can be commensurate with biomolecules, it raises the prospect that NP can interact with biomolecules in ways not normally envisaged [1, 5]. Particularly relevant are the association of NP with genetic material such as RNA and DNA as well as proteins [5], either by general agglomeration or aggregation or by specific ligand—protein

binding. NP-genetic material interactions have obvious implications for the disruption of genetic processes whilst NP-protein interactions are of concern as they can potentially inhibit biological processes. It has been established that NP within a biological environment can acquire a coating or “corona” of proteins [5] which can not only render NP more water soluble but which can make them more bioaccessible by facilitating their transport around the organism and even to gain entry into cells [1, 5]. Since the binding of NP to proteins can also increase their water solubility even though the NP themselves may be quite insoluble, this can also lead to the subsequent transport of the NP through the biosphere in addition to in vivo transportation.

Along with the grave concerns for human health, it simply has to be accepted that compounds generated by the commercial activities of man infiltrate the environment which ultimately results in their ubiquitous and insidious presence [3, 4, 7]. Hence, the wholesale presence of NP in the environment must be an anticipated consequence if, as expected, NP follow the way of plastics in production terms [7] and other large-scale pollutants such as heavy metals, PAHs, and pharmaceuticals, as a result of NP being produced on a massive industrial scale. One misconception is that, upon entry into the environment, NP may simply “settle out” as part of the sludge and innocuously “disappear” if they are not composed of material that is intrinsically poisonous in any form. This is not altogether without some rationale given the presence of naturally occurring NP in the environment [4, 17], for example, various components of soot, clays, volcanic outputs, forest fire debris, and so forth. There is also a measure of complacency [6] stemming from the perception that NP are not “intrinsically” new or toxic if they have been manufactured from familiar material or material that has been tested previously as bulk material and found to be benign, though this perception is diminishing of late. The essence of the problem, however, is the sheer volume [4, 26] and variety of material that is likely to be produced in the forthcoming years and subsequently introduced into the environment and the ensuing questions [27] of bioaccessibility, bioavailability, biopersistence, and bioaccumulation.

Whilst legitimate concerns and debate regarding the biological effects of NP have been ongoing, nonetheless, there is still considerable consternation that not enough is being

done to assess the dangers that NP may present even though testing has advanced considerably from the very limited amount that was conducted only a decade ago [4, 9]. Numerous toxicological studies have been conducted thus far, and studies are continually expanding, and there are now even specialized journals focused on nanotoxicology. There are additional concerns also that regulation and legislation may be lagging [13] or not appropriate [28]. All this attention, though, may be deceptive given the pace of development and production with the demand that each new NP formulation and any subsequent modification over the course of the life cycle need to be assessed. The nature of NP and the complexities involved have been well reviewed and discussed with respect to their potential deleterious effects for human health and the environment at large (e.g., [1, 3–6, 8–10, 13, 15, 19, 25, 29, 30]). Regulation of the occupational health and safety issues of NP and the necessary legislation, or concern over the lack thereof, have also been well reviewed and discussed [1, 3–5, 8, 12, 13, 25, 28].

However, if the challenges for the safe handling of nanotechnology set out in the Five Grand Challenges enunciated by Maynard et al. [3] are to be met (specifically, the second of the Five Grand Challenges, namely, develop, and validating methods to evaluate the toxicity of engineered nanomaterials), and similarly for the three challenges outlined for the new toxicology of nanoscale materials [5] (specifically the third concerning biointeractions), then more should be done. Although unquestionably unmatched otherwise, the problems with whole-animal assays are the amount of time and money usually required to conduct the work. Also, there is the problem for non-specialists to perform the experiments. With so many NP and various formulations being produced, the challenge at hand is how to manage the evaluation of so many new products as it quickly becomes logistically prohibitive to subject all NP formulations to biological and toxicological tests [20, 29]. Since biological and toxicological studies are expensive, require large amounts of effort and time, and are ethically demanding [1], there is an innate need for some amount of additional testing to help alleviate this burgeoning demand [3, 29, 30]. Hence the call, for example, for cell culture testing as a means to provide rapid screening of potential hazardous NP [9, 29, 30] as a means to then focus or prioritize the need for further whole-animal testing [1]. For example, measuring oxidative stress levels or injury in cell cultures have been proffered as one potential means for simple and rapid testing [1, 29, 31]. However, what are lacking are bioaffinity studies given the aforementioned propensity for some NP to interact with biomolecules. The possible shortfall in this aspect can be potentially ameliorated by experimental chemists as they have at their disposal instruments of ever-increasing sophistication able to test for bioaffinity to therefore address this deficiency. Indeed, it is now becoming increasingly possible to assess biomolecular affinities by techniques commonly available and familiar to mainstream chemistry due to advancements in technical design and method development, ever more so given that noncovalent interactions with biomolecules which may only be intrinsically weak can give rise to sizable effects given the huge surface area-to-mass ratio of NP. Familiar

instrumentation includes techniques such as nuclear magnetic resonance (NMR) and mass spectroscopy (MS).

MS possesses the advantages of great sensitivity, excellent molecular weight discrimination, relative low cost, and speed. Bioaffinity analyses using MS are starting to be applied more widely [32–35], and this is likely to become ever more so with the development of cold-spray ionization (CSI) methodology [36] for the analysis of weak associations [37], particularly for nanoscale structures [36]. The well-known limitations of MS, which can often be easily circumvented, include aspects such as the fact that mixtures can be cumbersome to handle without unique masses, so generally the assistance of separation methods (e.g., chromatography) are required, or, preferentially, samples are prepared individually with respect to the analytes. Of most concern is that artifacts can readily be introduced and signal lost due to the effects of sample preparation, the ionization method in use, and the behavior of the resultant ions. But clearly the potential is there to apply MS further to NP—biomolecular associations.

The strong advantages of NMR are the unique characterization and probing of different species; hence, even mixtures can be easily dealt with—and ever more so when applying sophisticated techniques or in tandem with chromatography. NMR is also very applicable to dynamic equilibria phenomena, thus making the technique readily amenable to bioaffinity analysis. The application of NMR to bioaffinity analysis is well advanced, and there is a wealth of NMR techniques that have been developed for such purposes [38–40] with many experiments in suitable cases to choose from, for example, saturation transfer, waterLOGSY, trNOE, T_2 , T_1 , diffusion, and so forth. The well-known limitation of NMR, sensitivity, is an aspect that is under constant improvement with persistent gains in technology from ever-increasing operating field strengths and cold probe technology.

However, in contrast to compounds isolated from plants or animals which are routinely bioassayed, it is not a standard procedure generally to measure affinities of various compounds to biomolecules by instrumental means. Since NP are on the verge of adopting a wholesale presence in our world, chemists should perhaps feel obliged to do all they can to preclude repeating many of the past and present catastrophes that occurred have and are occurring with wholesale environmental pollutants such as heavy metals, PAHs, pharmaceuticals, CFCs, plastics, asbestos, and pesticides such as DDT. To evaluate the toxicological potential of NP is not a simple task because of the immense number of NP formulations being produced and likely to be produced in the future resulting in the proverbial “infinite” number of samples to be tested and which can only necessarily therefore be tackled by a collective effort. Since it is quite normal for bioactivity tests to be performed for extracts of natural origin, isolated natural products, and even synthesized compounds, the argument therefore is that consideration should be given to whether bioaffinity testing should become a more widely adopted practice for NP as a means of gaining additional data on their potential hazardous properties as a matter of course in normal reporting rather than being limited only to specific toxicological reports. Thus, perhaps experimental chemists could contribute and address the challenges as part of their work. This

suggestion takes a cue from Barnard [10] who has called on computational and theoretical chemists to do more, and if possible, render aspects of their work relevant to nanotoxicity where feasible.

A wealth of results generated in this way across a broad spectrum of systems will clearly be amenable to analysis and evaluation through enhanced data mining capabilities. Indeed, not only can searches be made for negative attributes, but positive aspects for human health can also be investigated; for example, NP have been noted to target mitochondria and thereby initiate programmed cell death and as such represent a potential cancer chemotherapy therapy [1], and the potential of nanomaterials as antimicrobial agents is also an area of endeavor [41]. A generalized screening using chemical instrumentation is readily foreseeable for these applications of NP as well as providing a means to investigate the modes of action.

The concern regarding the danger that NP represent to our health and the environment in general is, of course, a problem of considerable magnitude, and, apart from immediate toxicity, there are also the concerns of long-term biopersistence and the consequences of chronic exposure. With regards to the debilitating legacies of the last century, ozone depletion, massive industrial pollution, global climate change, habitat loss, and so forth, is it possible to preclude nanotechnology from leaving a similar calamitous legacy? Thus far we have managed to avoid the problems and disasters of certain past technologies and industries, though nanotechnology has somewhat lacked guidance and cohesion with respect to regulation and environmental health and safety assessments despite the many calls for such [4, 8]. Thus the question, echoing Barnard's call [10], is posed; should experimental chemists be doing more to help evaluate the toxicological potential of NP to help alleviate the dilemma society faces with respect to NP in assessing the threat that they may present to human health or to the environment.

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