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Nanotoxicity assessment: all small talk?

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This volume of *Advanced Drug Delivery Reviews* is dedicated to issues in nanomaterials toxicity assessment and understanding. In the past decades, materials engineered into nanotechnology have moved from an exotic research pursuit to inclusion in hundreds of mainstream consumer products with nanomaterials markets exceeding billions of dollars annually. Many medical innovations under assessment now claim benefits from nanotechnology. Drug delivery systems by their very nature are part of these international pursuits with several nanophase formulations approved for human use, and dozens more nano-therapeutics in clinical trials. With this nano-phase invasion of new materials and products into nearly every aspect of life comes increasing calls for prudent assessment of new safety and exposure risks: published health assessments and toxicological studies of nanosystems are growing at exponential rates annually. It is often argued that human exposure to environmental non-engineered, natural sources of nanomaterials is by far and away the most significant exposure to nanomaterials, persisting throughout the human presence on earth. Certainly, the human physiome must have developed tolerance to constant nanoparticulate exposure from diverse natural sources. By contrast, deliberate exposure to human-made nanosystems is a relatively recent phenomenon with no histories yet substantial enough to provide clear or broad-sweeping safety or hazard assessments. One argument in this regard is that synthetic nanomaterials with specific engineered properties (i.e., applied surface coatings, specifically controlled size ranges, dopants, drug inclusion, optical, redox, and electronic properties) concentrated within products as a single species, and distributed into the ecosystem (i.e., disposal into the food chain and ecosystem) are unique to humans at this time. Human tissues and organs have not witnessed such materials or properties. Certainly, in this case of nano-medicines and therapeutics, humans have no evolutionary experience with nano-engineered systems in acute dosing regimens. These arguments about possible “exotic nanomaterials toxicity” have brought about a flurry of concerns from particulate toxicologists in defense of humans as both public stakeholders of substantial government speculations in this enterprise, and possible unwitting victims of adverse nanotechnology impacts.[1,2]

Engineered nanomaterials have all the traits that should raise eyebrows with regard to health assessments of any particulate: novelty in both form and function, unique chemistry and physics by design, complex interactions with biological and environmental milieu, biopersistence (both organismal and within the food chain), ready dispersibility and possible bioaccumulation, tissue penetration, and/or irreversible biochemical and materials activities. These types of properties have history in case studies of toxicities resulting from newly introduced substances. [3] Like these case studies, nano-engineered materials are already in consumer markets, and in some cases, the risks of such exposure personally and environmentally are unknown or poorly understood. The global compendia of nanomaterials safety data to date raise few

certainties either pro or con. One can find almost any result published for a given nanomaterial: from “overt toxicity” to “no observable toxicity”.[4–7] Hence, a classic “cup is either half-full or half-empty” analogy with regard to nanotechnology’s promise exists where exaggerated commercial benefit forecasts and motivations are offset by equally extreme, doomsday adverse human health impact scenarios. Public health policy mandates and responses are inconsistent: few are willing to risk political or policy careers influenced by diverse stakeholders without compelling evidence. Enter nanotoxicology.[8]

Many current nanosystems under development comprise nanoparticles as fundamental building blocks. Specialized nanoparticle chemistries (e.g., metals, ceramics, and carbon allotropes) are produced in metric tons annually for commercial ventures. Interestingly, more and more size- and treatment- dependent properties are reported to distinguish nanomaterials of a given chemistry. Gold nanoparticles unreactive to catalytic reactions in the 40-nm size regime are found to be very reactive chemical catalysts in the Au_{55} cluster size due to distinguishing oxide chemistry.[9] While virgin carbon nanophases (e.g., C60, multi-wall and single-wall nanotubes) are not readily water-dispersible or stable as colloids without aggregation, many adsorbates and etching processes alter this nanomaterial surface to provide aqueous stability. However, most of these important modifications used both *in vitro* and *in vivo* for this popular carbon-based materials set are not reported nor distinguished. Nonetheless, these may very well be critical for understanding their bioavailability and behaviors in aqueous biological test systems. The development of modern particle toxicology, mostly focused on respiratory adverse events historically, has dominated environmental health and is heavily influencing the current thinking for nanotoxicology as well. Nanomaterials size overlap with well-studied inhalable ultrafine particles (diameter <0.1 μm) sourced from urban air pollution provides a convenient, immediately accessible comparison and some initial basis for toxicity concerns. Ultrafine particles from air pollution are long-known to increase morbidity and mortality from pulmonary and cardiovascular causes with both long-term and immediate effects.[10–13] Moreover, inhaled ultrafine (nanometer range) particles in rodents distribute beyond the lung and can cause both pulmonary and systemic inflammation and promote blood coagulation within minutes to days of exposure.[14–16] To date, human studies, limited to acute exposures, measuring both pulmonary and systemic inflammatory endpoints, have been inconsistent, perhaps in part attributable to variable particle sources.[17–20] Nanomaterials toxicology is being developed within a known model historically addressing the toxicology of metal fumes, radioactive and nuisance dusts, rat lung particle overloads, silica, asbestos and synthetic fibers, and more recently air pollution particles.[21,22] Given the confounding aggregation phenomena known for these nano-systems under “real” conditions,[23] atmospheric chemistry, advanced colloid science, and even virology will contribute to understanding and distinguishing nanoparticle toxicology in model *in vitro* and *in vivo* systems.

Significantly, toxicological information compiled for nanomaterials must also consider actual human exposure levels. Any particulate material, nano-sized or larger, is expected to produce adverse effects at high enough doses in a given dosing context. Unfortunately, dosing or exposure amounts for various different nanomaterials applied in toxicological studies both *in vitro* and *in vivo* are frequently unrealistically excessive for most any plausible exposure scenario. Dosing is also delivered to model experimental systems as a bolus (i.e., unrealistically high dosing rate), with very limited relevance to actual human exposures except in certain extreme cases, some certainly including bolus nanomedicine dosing. In fact, drug delivery systems constitute a special case of nanomaterials exposure where drug vehicle and therapeutic chemistry and characterization data are well-documented, and dosing is carefully controlled in both formulation and administration. This represents an opportunity to exploit in tool-kit development for safety and efficacy testing.

A third general concern with model nanomaterials studies is the lack of ability to control or even know the physical state of the nanomaterial introduced or residing within environmental or biological model systems.[23] Many nanomaterials must use surface modification with coatings or surfactants to impart aqueous solution-phase stability or deliberately alter bioavailability. However, not all aqueous solutions with these nano-materials are comparable. Colloidal stability has always been complex when multiple surfactants are present. Presence of food, environmental or serum proteins, lipids, fatty acids, fungal or bacterial remnants ubiquitous to real samples will alter nano-phase material physical presentation to model test systems,[24] complicating and confounding both the measurement and interpretation of mechanisms in the resulting host reactivity, toxicology, or, in the case of nanomedicines, therapy. That these solution-phase aggregation differences might produce rather profound contrasting results reported in the literature for similar nanomaterials remains an open issue. This also has important implications for behaviors of nano-phase drug delivery systems *in vivo*.

In this volume of *Advanced Drug Delivery Reviews*, these issues related to nanotoxicity assessment are addressed from diverse, needed perspectives. An article from the FDA (Sadrieh) introduces the regulatory challenges of in preclinical safety assessments of engineered nanoparticles. This sets the stage for the types of data required and methods best suited to provide those data for required safety and efficacy testing of FDA-regulated products, and also sets benchmarks of some utility for other related nanomaterials risk assessment testing. As the FDA does not regulate science or technology – only products intended for human use – validated pre-clinical methodologies are important as a broad class of tests. The article submission from the Nanotechnology Characterization Laboratory (an analytical facility of the USA National Cancer Institute) describes an important characterization challenge: interaction of nanomaterials with plasma proteins that affect their physical states and behaviors in biological milieu and resulting possible influence on their therapeutic mechanisms. Protein-nanoparticle binding is a particularly difficult and significant component of understanding nanomaterials fates in biological systems.[24] The interactions between many proteins in blood, tissues, and disease sites remain a major issue since most analytical methods do not have requisite sensitivity to distinguish nanomaterials properties *in situ* or in real-time in physiological fluids [23] (i.e., blood plasma contains up to 70 mg/mL of soluble proteins of >2000 different types and physical chemistries). *Ex situ* or sample-processed desiccated analytical methods (e.g., electron microscopies, vacuum-based surface analysis) have tendencies to produce particle aggregation artifacts from the dry-down procedure where liquid surface tension forces far exceed inter-particle forces. Many technical challenges surround new tool-kit development for nanomaterials *in vivo* or simulated *in vivo* environments *in vitro* that might produce reliable and predictive data for actual *in vivo* responses, including nanomedicine therapeutic values and hazard assessments. This *in vitro* characterization issue is identified and further developed in the article by Jones and Grainger that profiles various *in vitro* characterization methods used for nanomaterials assays to date. From basic surface analytical profiling of nanomaterials as-supplied or made, to contamination common to most any laboratory environment, protein, oxidation, and cell-based assays, literally hundreds of methods for *in vitro* characterization are available, almost all without any standards or validation. Few comparisons have been performed using well-known model systems. Hence, a major obstacle to the nanotherapeutics field, also for the FDA's scrutiny of other nanomaterials technologies, has been to establish a common, accepted framework for nanomaterials analysis that spans pre-biologics characterization and quality control features for nanomaterials classes and their pre-clinical assessments *in vivo*. This article serves to tie in with the first two articles in identifying and justifying approaches and proper directions to establish standard operating protocols for nanomaterials biological characterization assays.

The last three articles in this volume focus on *in vivo* nanomaterials characterization. The most commonly observed failure in the biomaterials translational development spectrum in general is the noted disconnect between *in vitro* and *in vivo* performance that fails to predict actual host response. This is likely true for nanomaterials deployed *in vivo* as well, including imaging agents and drug delivery systems. Hence, developing high-throughput, sensitive, reliable, and predictive *in vivo* preclinical screening models to elucidate aspects of biocompatibility and bioperformance for nanomaterials is critical. The article from Forrest describes efforts to connect nanomaterials physicochemical properties to observed *in vivo* toxicity in animal models. Beyond non-invasive direct imaging methods (i.e., chemiluminescence, radio-imaging, MRI), few approaches are sensitive or available to provide direct information on *in vivo* fate of nanomaterials. *Ex situ* methods (histology, blood analyses, biopsies) can have difficulties locating nanomaterials within the relevant sample biology without preparation artifacts. The article from Jin describes the use of magnetic iron oxide nanoparticles for gathering information *in vivo* based on the ability of these particles to be imaged using MRI methods. This information is commonly used for early-state disease detection, but this approach can also be useful for monitoring non-disease particle collection points, *in vivo* nanomaterials biodistributions, and pharmacological/toxicological data. The last contribution by Furgeson to this volume provides a new approach to *in vivo* toxicity screening using the zebrafish *in vivo* model already well-known to geneticists. Use of these rapidly growing, well-characterized fish, their embryos, and their knock-out genetic models in live multi-well plate assays provides a rapid screening tool for mutagenic and toxic properties of nanomaterials and nanotherapeutics. This also provides possible avenues to mechanistic data given the relative ease by which these fish grow and reproduce, the convenience of rapid growth and harvest, capabilities to manipulate nanomaterial exposure conditions, properties and dosing, access to full genomic data banks and previous soluble drug screening methods. It is anticipated that the live zebrafish model will provide a needed scientific bridge between overly simplified *in vitro* assays and *in vivo* rodent models in the classic translational characterization efforts for nanomaterials in biological milieu. Taken together, these articles in this issue provide a thorough description of both issues and approaches in the nanomaterials toxicity challenge that is so relevant to adeptly addressing the “cup half-empty, half-full” controversy facing the nanotechnology field currently.

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