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NEWS AND OPINIONS

The role of biological monitoring in nano-safety

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Summary The ability to predict and then mitigate potential health effects is crucial for sustainability of nanotechnology, yet approaches to testing must evolve to provide both specificity and efficiency whilst reducing the burden of animal testing. To provide risk assessment with adequate information, a complementary strategy relying on the use of exposure biomarkers can be envisaged as part of a holistic approach to supporting effective risks management systems. Candidate biomarkers should be further validated in controlled human studies. This article discusses the meaning and role of biomonitoring as key component of a comprehensive toolbox of nanosafety.

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Nano-objects, including their aggregates and agglomerates (NOAA) [1,2] are increasingly being brought to the commercial market as a key to industrial innovation in many fields. Increased availability and use of NOAA brings with it innovative applications but also the possibility of personal exposure with potentially unwanted health effects; a key concern for the long-term sustainability of nanotechnology. Engineered nano materials (ENM) have not yet been reported to cause health effects in humans; however, there is accumulating evidence from animal studies supporting the concern that exposure to some nanomaterials could be harmful. As a result, ensuring the safety of nano-enabled products is seen as a crucial element in full exploitation of the benefits of nanotechnology [3,4].

The existing framework for risk assessment (RA) of ENM has substantial limitations in supporting the health impact assessment of this new class of chemicals. The ''Strategic Research Agenda towards Safe and Sustainable Nanomaterial and Nanotechnology Innovations'' [5] released by the *NanoSafety Cluster* — an EU Commission initiative to maximize the synergies between the existing projects addressing all aspects of nanosafety — recognizes that the conventional RA framework may fail to fully estimate the risks from ENM due to methodological limitations and knowledge uncertainties.

Accurate assessment of health risk of ENM requires information on target organs of ENM toxicity and knowledge of relevant endpoints gathered from studies in which exposures have been well characterized. Screening strategies consisting of *in vitro* and *in vivo* assays have been developed for increasing the rate of hazard identification [6—8]. To support this, high-throughput and high-content screening [7] along with the alternative test strategies [8] have been envisaged as powerful tools to expedite the pace of hazard identification and RA. The data generated by these approaches will certainly improve safety assessment, yet the complex and multi-faceted nature of events occurring at the nano-bio interfaces means that the full replacement of *in vivo* assessment is not yet possible. Moreover, the nature of these interactions is complex, changeable and the biological behaviour of ENM is not yet predictable solely on the basis of size, shape and surface chemistry, since these inherent properties are subject to change in response to biological environment. Therefore, toxicological testing should be seen as a spectrum where the further we get from humans by using increasingly simplified models, more caution should be exercised in the interpretation of findings. To overcome uncertainties due to the inherent limitations of simplified models, a complementary strategy facing the need to assess the health impact of nanotechnology in those potentially exposed is proposed [4,9] and a key component of this is the application of biological monitoring (BM).

BM is used in occupational and environmental health to identify potential hazards of new and emerging chemicals and monitor groups at risk of health outcomes. The main objectives of BM [10] are: (i) assessment of individual or group exposure; (ii) identification of early (preferably specific) effects which are indicative of an actual or potential health effects, and, ultimately, (iii) assessment of health risk to exposed subjects.

Biomarkers are measurable or observable parameters that reveal designated events in a biological system and are useful in assessing the effects or exposure to harmful substances, especially where there are low or intermittent levels of exposures, mixtures of toxicants that may act synergistically, or exposure resulting in disease with long latency period. Since biomarkers are ideally directly related to the adverse effects which one attempts to prevent, the use of BM represents an essential tool in health RA [10]. However, a major challenge is the specificity of biomarkers along with the health significance of the observed changes.

The issue of (nano)specificity of biomarkers is challenging because the reduction in size of particles to the nanoscale is believed to drive new and unpredictable effects meaning that a robust knowledge of toxicological properties of certain nanoparticles is not yet apparent and may not be confidently inferred from bulk particles of the same composition. However, evidence thus far from conventional particles, suggests similar paradigms of particle and nanoparticles toxicity [11] such as the role of shape, surface area, presence of reactive transition metals. Therefore within the hierarchy of biomarker development (Fig. 1), an efficient approach is to draw exploratory and candidate biomarkers from other fields of particle toxicology, in particular air pollution studies. This is because in real exposure scenarios, ENM share some characteristics with combustion-derived ultrafine particles and these similarities could drive the knowledge-based data to select high value biomarkers [12]. Biomarkers of exposure and effect are potentially available because a panel of parameters indicative of local or systemic inflammation and oxidative damage has been validated in air pollution studies [13,14]. Limited field studies have applied such biomarkers in exposed workers [15—17] yet some of these suffer from a lack of quantitative exposure assessment and characterization to both ENM and environmental background which limit the validation of biomarkers against exposure levels.

As with conventional particles, the nanoparticle surface forms the point of contact with cells therefore surface area and surface chemistry are important determinants of nanoparticle interaction with biological systems [18,19] and toxicity [20]. However the chemical identity of particles can be further modified after contact with biological fluids such as lung lining fluid or blood due to binding of biomolecules, such as proteins and lipids, to form a corona that interacts with biological systems [21]. Moreover, besides the inherent properties of the material, the capacity of ENM to act as vectors for the transport of other toxic chemicals to sensitive tissues (*Trojan horse effects*) should be considered [22]. Such alterations in surface characteristics are an important experimental consideration yet toxicologists often try to understand the toxicological profile of ENM by challenging their experimental systems with clean — but not naked — particles which may not be truly representative of particle—cell interactions in a real-life scenario. This latter point adds to the artificiality of laboratory based testing yet the issue of truly representing real life scenarios is challenging. When considering the harmful effects of particles in occupational/environmental settings, these are consistently associated with a multiplicity of factors, including changes occurring at the surface of nanoparticles. Agglomeration/aggregation of nanoparticles in contact with biological media represent a critical issue in laboratory experiments affecting the actual dose delivered to the system (*in vitro*) [23] and the dose at the target sites (*in vivo*).

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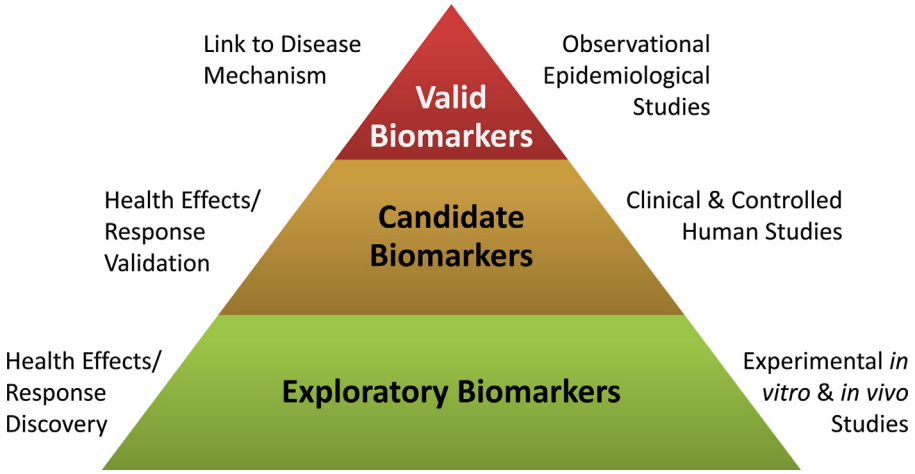

Figure 1 Layout of biomarkers research as condition of the responsible development of nanotechnologies and safety of workers exposed to engineered nanomaterials (ENM). With the advancement of the knowledge about the interaction between ENM and biological systems, the relevance of the toxicology data to humans should be considered. Advances in ''-omic'' techniques and systems toxicology can provide information on whether specific biological pathways are activated/perturbed, thus identifying fingerprints and nano-specific endpoints. Controlled human studies considering exposure data are needed to assess the validity of candidate biomarkers which can be further applied in observational studies aimed at assessing their predictivity towards relevant health outcomes. Validated biomarkers will enable the progression of knowledge about potential risks associated to ENM and will support the implementation of consistent occupational limit values as a key component of an effective risks management system.

This issue of particle modification and the subsequent effects this may have on toxicity/mode of action creates further difficulties in isolating truly specific biomarkers. Taken with the observation that there is no step change in toxicity at the 100 nm nano-definition threshold and little support for truly nano-specific mode of action in toxicity, it is evident that there are considerable hurdles in nanoparticle-specific biomarkers. Candidate biomarkers of exposure should consider the unusual properties of ENM as compared to chemicals, such as the ability to translocate from the route of entry or perhaps evade uptake, accumulate in different regions of the body, or change their chemical identity upon the interaction with biomolecules [24].

Overall it is evident that predicting health effects resulting from exposure to ENM is difficult due to their heterogeneity as well as lack of available tests. Given this uncertainty, a complementary approach of BM in high exposure/risk populations (linked with exposure monitoring) could provide important support and validation of laboratory analysis making the holistic use of hypothesis driven laboratory analysis and BM a pragmatic solution.

Another important tool is systems toxicology (ST) which attempts to model the (patho)physiological interactions with substances using computational tools. This could also be a means for the selection of biomarkers by providing information on dynamic interactions with ENM and by assessing whether specific biological pathways are activated/perturbed by specific ENM, thus identifying nano-specific fingerprints [25].

Due to the impracticability of tracking changes in the chemical identity during their life-cycle, the RA of specific nanoparticles cannot be based solely on laboratory science or predictive modelling, but should be supported by observational studies in occupationally exposed workers. Carrying out strictly controlled epidemiological studies is recognized as the main requirement for the assessment of biomarker validity whilst at the same time bridging the gap between laboratory research and the real world [9,26]. From epidemiological data, *biologically plausible* statistical associations can be found that, taken together with experimental data, suggest causation or simple association between exposure and health effects. Human studies, although ethically sensitive, are needed to assess the validity of candidate biomarkers which can be then assessed for their ability to predict health effects. These validated biomarkers will advance the knowledge about potential risks associated with exposure and to assess the effectiveness of remediation strategies (*e.g. safety-by-design*) [22,27]. While future studies should address the (nano)specificity of biomarkers, the priority is to assess whether alteration in biomarker baseline values occur in groups of exposed workers as compared to a control group and if there is a relationship between modification of the biomarker(s) and adverse human health effects. Overall, it is clear that biomarkers and biological monitoring need to be used widely in the toolbox of nanosafety to support laboratory testing and risk assessment.

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