

# Nanobiomonitoring and Surveillance

## Opportunities to Confirm the Protection of Nanomaterial Workers

BY MARTIN D. BARRIE, JOHN BAKER, MARK D. HOOVER,  
AND CHARLES L. GERACI



Over the last decade, advances in nanotechnology and in the many industries that apply it have led to increases in the number of workers and consumers potentially exposed to engineered nanomaterials (ENMs) and their aggregates and agglomerates. Among the industries where nanotechnology is prominent are the manufacturing of surfaces and coatings, electronics, medical and healthcare products, food, clothing, cosmetics, building materials, and household items.

Detailed health risk characterizations from exposures to ENMs are limited. However, some evidence for the potential adverse health effects associated with such exposures has been derived from air pollution epidemiological investigations of fine and ultrafine particulate matter and from studies in biological systems involving cells, tissues, and laboratory animals. That growing body of work supports the industrial hygiene paradigm to anticipate and recognize, evaluate, and control and confirm the protection of workers from nanomaterial-associated risks.

The use of biological monitoring to assess nanomaterial exposure and effect, also called nanobiomonitoring, offers an additional opportunity to evaluate, control, and confirm worker protection. Two critical challenges are how to use nanobiomonitoring to understand the progression from exposure to response (Figure 1) and how to apply biological markers

(biomarkers) of exposure and biomarkers of effect in the surveillance of nanomaterial-related hazards, exposures, and medical responses (Figure 2).

### **BIOLOGICAL MONITORING**

In 1980, at a meeting jointly sponsored by the European Economic Community (EEC), NIOSH, and OSHA, biological monitoring was defined as “the measurement and assessment of agents or their metabolites either in tissues, secretions, excreta, expired air or any combination of these to evaluate exposure and health risk compared to an appropriate reference.” For the purpose of protecting nanotechnology workers and consumers, the term “nanobiomonitoring” can be used for the application of biological monitoring in nanotechnology.

Biomarkers of exposure and biomarkers of effect generally refer to specific objective, quantitative measurements of a medical state that reflect an interaction between a chemical or physical agent and a biological system. Biomarkers of exposure generally involve the measurement of the chemical or agent and its metabolites that reflect an internal target dose. Biomarkers of effect generally reflect cellular or biological changes or other measurable adverse effects. The use of biomonitoring aids the assessment of health risk.

### **Use of Biomarkers in Industrial Hygiene**

Industrial hygienists are no strangers to the use of biomarkers for assessing the exposure to, and effects of, hazardous substances. ACGIH has published detailed documentation supporting the use of “Biological Exposure Indices” (BEI) as guidance values for assessing biological monitoring results. Examples of BEI include the measurement of carboxyhemoglobin (COHb) in blood and carbon monoxide in exhaled breath to assess exposure to carbon monoxide, and dichloromethane in urine to assess exposure to dichloromethane (methylene chloride). Since exposure to dichloromethane can result in elevated levels of COHb, the documentation for these two BEI indicates the degree to which toxicology and metabolism of chemicals must be considered in selecting biological determinants of exposure and effect. Most BEI are based on the correlation of exposure at the Threshold Limit Value (that is, on biomarkers of exposure), but some—lead, for example—are based on the development of an adverse health effect (biomarker of effect).

### **Insights from Experimental Studies**

One of the main concerns for the use of ENMs is their high surface catalytic activity, which may pose environmental and health hazards upon release. Because some nanomaterials may be able to pass through the cell membranes, they have the potential to directly interact

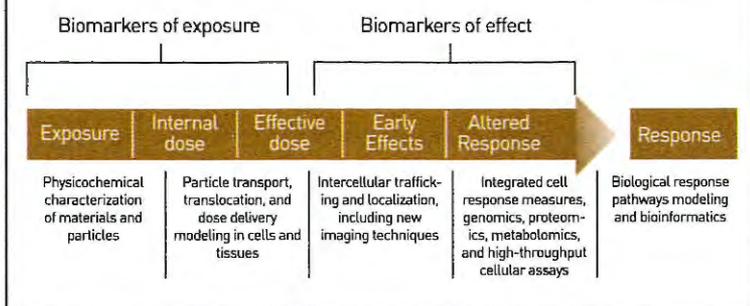
with cellular components and cause adverse biological effects. Experimental studies conducted thus far on animals *in vivo* and mammalian cell systems *in vitro* have demonstrated cytotoxic and genotoxic effects following exposure to some types of ENMs. One study found that exposure of rodents to single- and multi-walled carbon nanotubes by inhalation and intra-tracheal instillation resulted in cardiovascular and pulmonary effects involving chronic and acute inflammation, collagen deposition, granuloma formation, and lung fibrosis. Toxicity at systemic, immunological, dermal, and developmental levels has been demonstrated in animal models after various modes of nanoparticle exposure (airway, oral, topical, intravenous, intraperitoneal, and subcutaneous injection). For example, following exposure in mice and rats, single-walled carbon nanotubes were found distributed in most of the organs, including the brain, indicating that the nanomaterials can easily pass through the blood-brain barrier. Among the organs, retention of single- and multi-walled carbon nanotubes and nano-titanium dioxide in the lungs was the most common observation related to particle distribution.

Adverse biological effects caused by nanomaterial exposure have been demonstrated using animals and mammalian cell systems. What remain largely obscure are toxicological and mechanistic data regarding human exposure kinetics, dose metrics, the extent of nanomaterial uptake, cellular fate after uptake, and the fate of nanoparticles within the cellular compartments. These knowledge gaps make it difficult to accurately predict the adverse effects on human health.

Recent investigations have identified various biomarkers that implicate potential adverse health outcomes from nanomaterial exposures. A recent assessment of fifteen epidemiologic studies of workers handling nanomaterials found reports of increased markers of biological effects associated with nanomaterial exposures. These included increased systemic inflammation and pulmonary effect markers in sputum, increased pulmonary effect markers in serum, and increased markers of oxidation on nucleic acids, lipids, and proteins.

Elevated biomarkers of oxidative damage of nucleic acids and proteins have also been observed in the exhaled breath condensate of workers with relatively high levels of exposure to titanium dioxide nanoparticles. Oxidative stress is considered one of the mechanisms by which nanomaterials can cause genotoxicity. Workers exposed to multi-walled carbon nanotubes were found to have elevated pro-fibrotic inflammatory mediators in their serum and sputum samples, compared to controls, and silver nanoparticles have been reported to produce concentration-dependent cytotoxicity and genotoxicity in the human lymphoblast TK6 cell micronucleus assay.

**Figure 1:** The role of biomonitoring in the progression from exposure to a hazard to the resulting biological response. Adapted from "Human Biomonitoring of Engineered Nanoparticles: An Appraisal of Critical Issues and Potential Biomarkers," *Journal of Nanomaterials* (June 2012).



### MEDICAL SCREENING AND HAZARD SURVEILLANCE

Occupational health surveillance can be used to track illness or change in a biological function over time, identify and characterize trends, and define the magnitude and scope of exposure and response. Surveillance can also inform attempts to identify causal associations between exposure and health outcomes for the development of prevention strategies. Integral parts of occupational health surveillance are hazard identifications and surveillance and medical screening. Medical screening focuses on the early diagnosis and treatment of exposed individuals. Identifying and characterizing the exposure and health data over time can lead to implementation of strategies for prevention (substitution, engineering or administrative controls, training).

Figure 1 identifies the role of biomarkers of exposure and biomarkers of effect in understanding the progression from exposure to health response. The surveillance paradigm presented in Figure 2 integrates the use of biomarkers of exposure and biomarkers of effect with hazard, exposure, and medical surveillance; these tools are used to confirm worker protection and enable early identification and mitigation of potential adverse health outcomes resulting from exposure. A surveillance program that integrates biomarkers of exposure and effect should consider various physical characteristics of ENMs and biological activities associated with nanomaterial exposures. These elements include the physicochemical characterization of the nanomaterial, models of particle transport and translocation, cellular uptake, and biological response pathways.

### Interim Guidance from NIOSH

When publishing Current Intelligence Bulletin 60, "Interim Guidance for Medical Screening and Hazard Surveillance for Workers Potentially Exposed to Engineered Nanoparticles," NIOSH noted that insufficient scientific and medical evidence existed to recommend

specific medical screening for the broad categories of materials that are considered ENMs. Instead, NIOSH recommended providing the same screening and surveillance, if any, that would be recommended for the "bulk" or "parent" material. NIOSH also discussed the need for continued *in vivo* and *in vitro* toxicological research to identify candidate biomarkers that could be used in medical screening. Importantly, the testing (assays) for these biomarkers as part of a surveillance program should have the requisite sensitivity, specificity, and positive predictive values for viability.

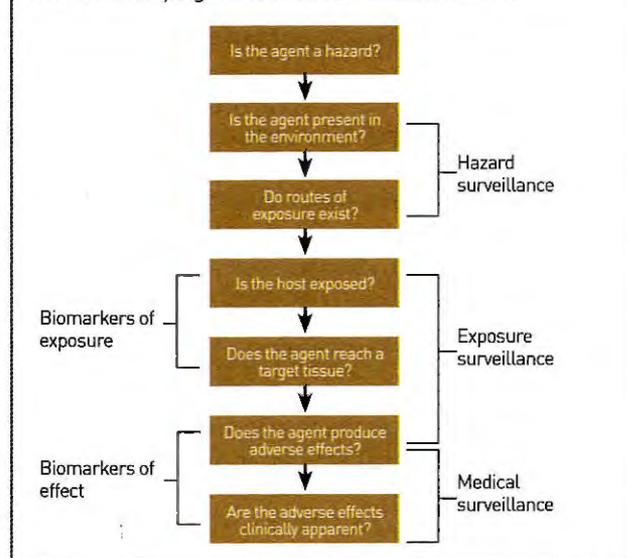
### Fine and Ultrafine Titanium Dioxide

Current Intelligence Bulletin 63, "Occupational Exposure to Titanium Dioxide," reviewed various mechanisms of toxicity related to exposure to fine or ultra-fine titanium dioxide. In that review, NIOSH referred to the previously mentioned "Interim Guidance for Medical Screening" in lieu of specific medical screening or surveillance of biomarkers for titanium dioxide. Although NIOSH noted that pulmonary inflammation was an exposure-related health effect that may act as a precursor for tumor initiation, it was concluded that pulmonary inflammation in itself could not be used as a specific biomarker for lung cancer.

### Carbon Nanotubes and Nanofibers

Current Intelligence Bulletin 65, "Occupational Exposure to Carbon Nanotubes and Nanofibers," reviewed various mechanisms of toxicity related to these nanoparticles. NIOSH recommended establishing a baseline medical and

**Figure 2:** The roles of biomarkers of exposure and biomarkers of effect in the surveillance of industrial hygiene hazard, exposure, and medical response. Adapted from "Public Health Surveillance: A Tool for Targeting and Monitoring Interventions" in *Disease Control Priorities in Developing Countries*, 2nd edition (2006).



occupational history and performing spirometry and chest X-ray as an initial step for medical surveillance, and investigating abnormal findings according to the recommendations of a qualified medical professional. No specific biomarkers were recommended, although one approach to setting a Recommended Exposure Limit considered (among other factors) the role of nanoparticle-specific surface area in pulmonary inflammation measured as percent neutrophils in bronchoalveolar fluid.

### **OSHA Guidance on Biomarkers for Other Exposures**

OSHA has included the consideration of biomarkers in several substance-specific health standards. For example, the OSHA standard for occupational exposure to lead requires employers to make available biological monitoring in the form of blood sampling and analysis for lead and zinc protoporphyrin levels to covered employees. Additional OSHA standards that include requirements for medical screening and surveillance can be found at <http://bit.ly/oshasurveillance>.

### **Challenges for Occupational Health Surveillance**

As production and use of ENMs increase, occupational health surveillance, potentially incorporating the use of nanobiomonitoring, will remain critically important in safeguarding human health. Innovative studies using well-characterized ENMs and state-of-the-art techniques with consideration for appropriate routes of exposure in appropriate human cell systems are required to critically evaluate the biological effects of nanomaterials on human health. Such studies are likely to identify novel organ/cell type-specific biomarkers.

Discovery of novel biomarkers specific for ENM exposure will not only assist in monitoring human exposure to ENMs but will also aid in predicting the short- and long-term adverse biological effects on human health. Application of the components of occupational health surveillance, including hazard and medical surveillance, is critically important in helping to define the magnitude and scope of occupational health risks among those exposed to ENMs.

### **EARLY DETECTION**

Biomarkers of exposure and effect are important components of nanobiomonitoring and surveillance efforts that are intended to provide for early detection of adverse health effects potentially associated with nanomaterial exposures. The development and application of nanobiomonitoring will potentially allow for the development of early intervention strategies to limit or mitigate exposures. However, given the heterogeneity of nanomaterials' physical and chemical properties, a major challenge will be to identify and validate biomarkers of

exposure and effect that apply to all nanomaterials, routes of exposure, and health endpoints. Ethical considerations and issues surrounding informed consent, confidentiality, and potential stigma and discrimination based on surveillance results should also be considered. ●

---

**MARTIN D. BARRIE, PhD, JD**, is senior scientist in epidemiology at Oak Ridge Associated Universities. He can be reached at (865) 576-6250 or [martin.barrie@orau.org](mailto:martin.barrie@orau.org).

**JOHN BAKER, CIH**, is a senior project manager at Bureau Veritas. He can be reached at (281) 832-2894 or [john.baker@us.bureauveritas.com](mailto:john.baker@us.bureauveritas.com).

**MARK D. HOOVER, PhD, CHP, CIH**, is co-director of the NIOSH Center for Direct Reading and Sensor Technologies. He can be reached at (304) 285-6374 or [mhoover1@cdc.gov](mailto:mhoover1@cdc.gov).

**CHARLES L. GERACI, PhD, CIH**, is associate director for nanotechnology and emerging materials at NIOSH and co-manager of the Nanotechnology Research Center. He can be reached at (513) 533-8339 or [cgeraci@cdc.gov](mailto:cgeraci@cdc.gov).

## **RESOURCES**

- Annals of Occupational Hygiene*: "Industrial Production and Professional Application of Manufactured Nanomaterials-Enabled End Products in Dutch Industries: Potential for Exposure" (April 2013).
- Assessment of Toxic Agents at the Workplace: Roles of Ambient and Biological Monitoring*. Proceedings of the International Seminar held in Luxembourg, December 8-12, 1980 (1984).
- Disease Control Priorities in Developing Countries*, 2nd edition: Chapter 53, "Public Health Surveillance: A Tool for Targeting and Monitoring Interventions," <http://bit.ly/publichealthsurveillance> (PDF, 2006).
- International Journal of Environmental Research and Public Health*: "Combined Toxic Exposures and Human Health: Biomarkers of Exposure and Effect" (March 2011).
- International Journal of Nanomedicine*: "Blood-brain Barrier Transport Studies, Aggregation, and Molecular Dynamics Simulation of Multiwalled Carbon Nanotube Functionalized with Fluorescein Isothiocyanate" (March 2015).
- Journal of Breath Research*: "Oxidative Stress Markers Are Elevated in Exhaled Breath Condensate of Workers Exposed to Nanoparticles during Iron Oxide Pigment Production" (February 2016).
- Journal of Hazardous Materials*: "Airborne Engineered Nanomaterials in the Workplace—A Review of Release and Worker Exposure during Nanomaterial Production and Handling Processes" (January 2017).
- Journal of Nanomaterials*: "Human Biomonitoring of Engineered Nanoparticles: An Appraisal of Critical Issues and Potential Biomarkers" (June 2012).
- Journal of Nanoparticle Research*: "Assessing the First Wave of Epidemiological Studies of Nanomaterial Workers" (October 2015).
- Journal of Occupational and Environmental Medicine*: "General Principles of Medical Surveillance: Implications for Workers Potentially Exposed to Nanomaterials" (June 2011).
- Journal of Toxicology and Environmental Health, Part B: Critical Reviews*: "Inhalation Exposure to Carbon Nanotubes (CNT) and Carbon Nanofibers (CNF): Methodology and Dosimetry" (September 2015).
- Mutation Research*: "Genotoxicity of Silver Nanoparticles Evaluated Using the Ames Test and In Vitro Micronucleus Assay" (2012).
- NIOSH: "Current Intelligence Bulletin 60: Interim Guidance for Medical Screening and Hazard Surveillance for Workers Potentially Exposed to Engineered Nanoparticles" (February 2009).
- NIOSH: "Current Intelligence Bulletin 63: Occupational Exposure to Titanium Dioxide" (April 2011).
- NIOSH: "Current Intelligence Bulletin 65: Occupational Exposure to Carbon Nanotubes and Nanofibers" (April 2013).
- Toxicology and Applied Pharmacology*: "Fibrosis Biomarkers in Workers Exposed to MWCNTs" (2016).